UNIVERSIDADE FEDERAL DA PARAÍBA

CENTRO DE CIÊNCIAS DA SAÚDE

PROGRAMA DE PRODUTOS NATURAIS E SINTÉTICOS BIOATIVOS

DANILO DUARTE DE ASSIS GADELHA

AVALIAÇÃO DOS EFEITOS DA SUPLEMENTAÇÃO COM *Lactobacillus acidophilus* LA – 05 NOS PARÂMETROS CARDIOVASCULARES DE ANIMAIS HIPERTENSOS

JOÃO PESSOA – PB
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Tese apresentada ao PROGRAMA DE PÓS-GRADUAÇÃO EM PRODUTOS NATURAIS E SINTÉTICOS BIOATIVOS do Centro de Ciências da Saúde da Universidade Federal da Paraíba, como requisito para obtenção do título de DOUTOR EM PRODUTOS NATURAIS E SINTÉTICOS BIOATIVOS, área de concentração: FARMACOLOGIA.

ORIENTADORA: PROF.ª DR.ª SANDRA RODRIGUES MASCARENHAS

COORIENTADORA: PROF.ªDR.ªMARIA DO SOCORRO DE FRANÇA FALCÃO

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Ata da 354ª (trecentésima quinquagésima quarta) Tese de Doutorado do aluno do Programa de Pós-Graduação em Produtos Naturais e Sintéticos Bioativos **Danilo Duarte de Assis Gadelha**, candidato ao Título de "Doutor" em Produtos Naturais e Sintéticos Bioativos na área de concentração Farmacologia.

Às oito horas e trinta minutos (08h30) do dia treze de fevereiro do ano dois mil e vinte e três (13/02/2023), em ambiente virtual de videoconferência através do aplicativo Google Meet, link: https://meet.google.com/rcj-xjqx-qjn, reuniram-se em caráter de Solenidade Pública os membros da Comissão designada para examinar o aluno **Danilo Duarte de Assis Gadelha**, candidato ao Título de "DOUTOR" em Produtos Naturais e Sintéticos Bioativos na área de concentração Farmacologia. Foram componentes da Banca Examinadora os pesquisadores Thyago Moreira de Queiroz, Ph.D em Farmacologia, Ian Porto Gurgel Amaral, Ph.D em Biologia, Marianna Vieira Sobral, Ph.D em Farmacologia, Margareth de Fátima Formiga Melo Diniz, Ph.D em Farmacologia, Maria do Socorro de França Falcão, Ph.D em Farmacologia e Sandra Rodrigues Mascarenhas, Ph.D em Fisiologia. Sendo o primeiro, integrante do corpo docente da Universidade Federal de Pernambuco e os demais, integrantes do corpo docente da Universidade Federal da Paraíba. Dando início aos trabalhos, a Presidente da Banca, professora Sandra Rodrigues Mascarenhas, após declarar os objetivos da reunião, apresentou o candidato **Danilo Duarte de Assis Gadelha**, a quem concedeu a palavra para que dissertasse oral e sucintamente sobre o tema apresentado e intitulado "Avaliação dos efeitos da suplementação com *Lactobacillus acidophilus* (LA – 05) nos parâmetros cardiovasculares de animais hipertensos". Após discorrer sobre o referido tema durante cerca de cinquenta minutos, o candidato foi arguido pelos Examinadores na forma Regimental. Em seguida, passou a comissão, em caráter secreto, a proceder à avaliação e julgamento do trabalho, concluindo por atribuir-lhe o conceito **APROVADO**. Em face da aprovação, declarou a Presidente, achar-se o examinado **Danilo Duarte de Assis Gadelha**, legalmente habilitado a receber o Título de "DOUTOR" em Produtos Naturais e Sintéticos Bioativos, na Área de Concentração Farmacologia, cabendo a Universidade Federal da Paraíba, providências, como de direito, a expedição do Diploma que o mesmo faz jus. Nada mais havendo a tratar, foi lavrada a presente ata que é abaixo assinada pelos membros da Comissão e pelo(a) discente.

Prof.ª Dr.ª Sandra Rodrigues Mascarenhas (Orientadora)

Prof.ª Dr.ª Maria do Socorro de França Falcão (Coorientadora)

Prof. Dr. Thyago Moreira de Queiroz (Examinador)

Prof. Dr. Ian Porto Gurgel Amaral (Examinador)

Prof.ª Dr.ª Marianna Vieira Sobral (Examinadora)

Prof.ª Dr.ª Margareth de Fátima Formiga Melo Diniz (Examinadora)

Danilo Duarte de Assis Gadelha (Discente)

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“Lamentar uma dor passada, no presente, é criar outra dor e sofrer novamente.”

William Shakespeare

Aos meus pais e irmãos, a quem me referencio na tentativa de ser uma pessoa melhor todos os dias.

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Agradeço, inicialmente, ao **Deus** da minha fé, que sem Ele meu espírito não teria alimento e força para sustentar as dores da carne e suportar todas as adversidades encontradas ao longo do caminho.

À minha família de sangue: **mãe**, **pai** e **irmãos**a quem também dedico essa luta por sempre acompanharem de perto, com ênfase à minha mãe, que dentre todos, foi a que mais teve acesso nessa jornada. Serei eternamente grato.

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Obrigado, mil vezes, obrigado

Avaliação dos efeitos da suplementação com *Lactobacillus acidophilus* LA – 05 nos parâmetros cardiovasculares de animais hipertensos. Gadelha, D. A. Tese apresentada ao Programa De Pós-Graduação em Produtos Naturais e Sintéticos Bioativos. CCS/UFPB. 2023.

Resumo

A disbiose intestinal é caracterizada pelo desequilíbrio na microbiota intestinal e tem sido relacionada à hipertensão arterial. Benefícios na modulação da pressão arterial são observados após mudanças nos componentes dietéticos visando a modulação da microbiota com uso de probióticos. Probióticos são definidos como microrganismos vivos que, quando administrados em quantidades adequadas, beneficiam a saúde congênita do hospedeiro. O gênero *Lactobacillus* possui efeitos importantes no sistema cardiovascular por diminuir a pressão arterial de humanos e animais espontaneamente hipertensos, além de promover uma melhora na disbiose. Com isso, nosso objetivo foi avaliar os efeitos induzidos pela suplementação com *Lactobacillus acidophilus* LA-5 no sistema cardiovascular de ratos espontaneamente hipertensos. Para isso, foram utilizados ratos espontaneamente hipertensos (SHR) e Wistar Kyoto (WKY). Os animais foram divididos em três grupos: 1. Normotenso controle (WKY), 2. Hipertensos (SHR), e 3. Hipertensos + LA5 (SHR+LA5). Após instalação da hipertensão, os animais receberam diariamente a suspensão de *Lactobacillus acidophilus* (10^9 log CFU/animal) por um período de 8 semanas via oral. A implantação de cateteres em direção a aorta abdominal e a veia cava inferior foi realizada para registro da pressão arterial e frequência cardíaca, avaliação do barorreflexo e função autonômica dos animais. Em seguida, os animais foram eutanasiados e o plasma foi coletado para avaliação do estresse oxidativo. A artéria mesentérica superior foi coletada para ensaios de reatividade vascular. Os valores foram expressos como média ± erro padrão da média (e.p.m). Diferenças foram consideradas significativas quando $p<0,05$. O programa utilizado foi o GraphPad Prisma versão 8.00®. Como resultados, pudemos observar que a suplementação com LA-5 por 8 semanas via oral reduziu a pressão arterial diastólica do grupo SHR+LA5 quando comparados os animais do grupo SHR (122,0±4,3 *versus* 153,0±6,7 mmHg, $p<0,05$), porém não houve diferença na pressão arterial sistólica, tampouco na pressão arterial média. A suplementação com LA-5 não alterou a frequência cardíaca dos animais. Com relação a atividade autonômica, o grupo SHR+LA5 apresentou diminuição do componente LF (componente oscilatório da pressão arterial de baixa frequência relacionado com atividade simpática), indicativo da atividade simpática, quando comparado com o grupo SHR (7,38±0,22 *versus* 9,5±0,75%, $p<0,05$). O componente HF (componente oscilatório da pressão arterial de alta frequência relacionado com atividade parassimpática) e a relação LF/HF (balanço simpatovagal) não sofreram alterações. O barorreflexo foi avaliado de maneira induzida, com auxílio de substâncias vasoativas, e de maneira espontânea. A suplementação com LA-5 por 8 semanas via oral foi eficaz em aumentar a sensibilidade do barorreflexo induzido quando comparados os grupos SHR+LA5 e SHR (-2,48±0,32 *versus* -0,94±0,09 bpm.mmHg^{-1}, $p<0,05$), como também melhorou a sensibilidade do barorreflexo espontâneo (0,77±0,1 *versus* 0,44±0,04 ms.mmHg^{-1}, $p<0,05$). A suplementação com LA-5 também foi capaz de reduzir os níveis de malondialdeído quando comparado ao grupo SHR (33,78±161 *versus* 60,45±2,89 nmol/mL, $p<0,05$, respectivamente). No que diz respeito à reatividade vascular, o grupo SHR apresentou um prejuízo no perfil de relaxamento induzido pela acetilcolina com redução do efeito máximo comparado ao grupo WKY (71,35±9,37 *versus* 94,18±3,32%, $p<0,05$) e a suplementação com LA-5 foi capaz de melhorar o relaxamento (SHR+LA5: 111,23±6,45%, $p<0,05$). Curiosamente, o LA-5 melhorou o efeito relaxante máximo induzido pelo nitroprussiato de sódio quando comparado aos grupos SHR e SHR+LA5 (134,40±4,02 *versus* 106,41±5,52%, $p<0,05$). A suplementação não apresentou impacto no perfil de contração induzido pela fenilefrina ($E_{máx}$ -WKY: 100,28±9; SHR: 113,17±12,9; e SHR+LA5: 97,47±12,5%). Em conjunto, os achados apontam o *L. acidophilus* LA-5 como um potencial adjuvante no manejo da hipertensão, uma vez que promoveu uma redução da pressão arterial diastólica, aliada à redução da hiperatividade simpática, à melhorana sensibilidade do barorreflexo e da disfunção endotelial e a redução do estresse oxidativo.

Palavras-chave: barorreflexo, disfunção endotelial, estresse oxidativo, hipertensão, probiótico.

Evaluation of the effects of supplementation with *Lactobacillus acidophilus* LA – 05 on the cardiovascular parameters of hypertensive animals. Gadelha, D. A. Thesis presented to the Graduate Program in Natural and Synthetic Bioactive Products. CCS/UFPB. 2023.

Abstract

Intestinal dysbiosis is characterized by an imbalance in the intestinal microbiota and has been related to hypertension. Benefits in blood pressure modulation are observed after changes in dietary components aimed at modulating the microbiota with the use of probiotics. Probiotics are defined as “live microorganisms which, when administered in adequate amounts, benefit the congenital health of the host”. The genus *Lactobacillus* has important effects on the cardiovascular system by decreasing blood pressure in spontaneously hypertensive humans and animals, in addition to promoting an improvement in dysbiosis. Therefore, our objective was to evaluate the effects induced by supplementation with *Lactobacillus acidophilus* LA-5 on the cardiovascular system of spontaneously hypertensive rats (SHR). For this, spontaneously hypertensive and Wistar Kyoto (WKY) rats were used. The animals were divided into three groups: 1. Normotensive control (WKY), 2. Hypertensive (SHR), and 3. Hypertensive + LA5 (SHR+LA5). After the onset of hypertension, the animals received daily the suspension of Lactobacillus acidophilus (109 log CFU/animal) for a period of 8 weeks orally. The implantation of catheters towards the abdominal aorta and inferior vena cava was performed to record blood pressure and heart rate, baroreflex assessment and autonomic function of the animals. Then, the animals were euthanized and plasma was collected for assessment of oxidative stress. The superior mesenteric artery was collected for vascular reactivity assays. Values are expressed as mean ± standard error of mean (s.e.m). Differences were considered significant when $p<0.05$. The program used was GraphPad Prism version 8.00®. As a result, we could observe that oral supplementation with LA-5 for 8 weeks reduced the diastolic blood pressure of the SHR+LA5 group when compared to SHR group (122.0±4.3 *versus* 153.0±6.7 mmHg, $p<0.05$), but there was no difference in systolic blood pressure in mean blood pressure. LA-5 supplementation did not alter the heart rate of the animals. Regarding autonomic activity, the SHR+LA5 group showed a decrease in the LF (low-frequency oscillatory component of blood pressure related to sympathetic activity) component, indicative of sympathetic activity, when compared with the SHR group (7.38±0.22 *versus* 9.5±0.75%, p<0. 05). The HF (oscillatory component of high-frequency blood pressure related to parasympathetic activity) component and the LF/HF (sympathovagal balance) ratio did not change. The baroreflex was assessed in an induced manner, with the aid of vasoactive substances, and spontaneously. Oral supplementation with LA-5 for 8 weeks was effective in improving the sensitivity of the induced baroreflex when comparing the SHR+LA5 and SHR groups (-2.48±0.32 *versus* -0.94±0.09 bpm.mmHg^{-1}, $p<0.05$), but also improved the sensitivity of the spontaneous baroreflex (0.77±0.1 *versus* 0.44±0.04 ms.mmHg^{-1}, $p<0.05$). LA-5 supplementation was also able to reduce malondialdehyde levels when comparing the SHR+LA5 and SHR groups (33.78±161 *versus* 60.45±2.89 nmol/mL, $p<0.05$, respectively). With regard to vascular reactivity, the SHR group showed an impairment in the relaxation profile induced by acetylcholine with a reduction in the maximum effect compared to the WKY group (71.35±9.37 *versus* 94.18±3.32%, $p<0.05$) and LA-5 supplementation was able to improve relaxation (SHR+LA5: 111.23±6.45%, $p<0.05$). Interestingly, LA-5 improved the maximum relaxing effect induced by sodium nitroprusside when compared to the SHR and SHR+LA5 groups (134.40±4.02% *versus* 106.41±5.52, $p<0.05$). Supplementation had no impact on the contraction profile induced by phenylephrine (Emax - WKY: 100.28±9; SHR: 113.17±12.9; and SHR+LA5: 97.47±12.5%). Together, the findings point to *L. acidophilus* LA-5 as a potential adjuvant in the management of hypertension, since it promoted a reduction in diastolic blood pressure, combined with a reduction in sympathetic hyperactivity, an improvement in baroreflex sensitivity and endothelial dysfunction and the reduction of oxidative stress.

Keywords: baroreflex, endothelial dysfunction, hypertension,oxidative stress, probiotics.

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Ang II	Angiotensina II
AT_1R	Isoforma 1 do receptor da angiotensina
CEUA	Comitê de Ética no Uso de Animais
CVLM	Bulbo ventrolateral caudal
DAG	Diacilglicerol
DC	Débito cardíaco
DCVs	Doenças cardiovasculares
ECA	Enzima conversora de angiotensina
EROs	Espécies reativas de oxigênio
FC	Frequência cardíaca
FEN	Fenilefrina
GPCR	Receptor acoplado à proteína do tipo G
HA	Hipertensão arterial
HF	*High frequency*
IP	Intervalo de pulso
IP_3	Trifosfato de inositol
LF	*Low frequency*
MDA	Malondialdeído
NA	Núcleo ambíguo
NO	Óxido nítrico
NPS	Nitropussiato de sódio
NTS	Núcleo do trato solitário
PA	Pressão arterial
PAM	Pressão arterial média
PAD	Pressão arterial sistólica
PAS	Pressão arterial diastólica
PAP	Pressão arterial de pulso
PIP_2	Bisfosfato de Inositol
PKC	Proteína cinase C
PLC	Fosfolipase C
PVN	Núcleo paraventricular do hipotálamo

RVPT	Resistência vascular periférica total
RVLM	Bulbo ventrolateral rostral
SFO	Órgão subfornical
SNC	Sistema nervoso central
SRAA	Sistema renina-angiotensina-aldosterona
TBA	Ácido tiobarbitúrico
VS	Volume sistólico

Sumário

Introdução

1. Introdução

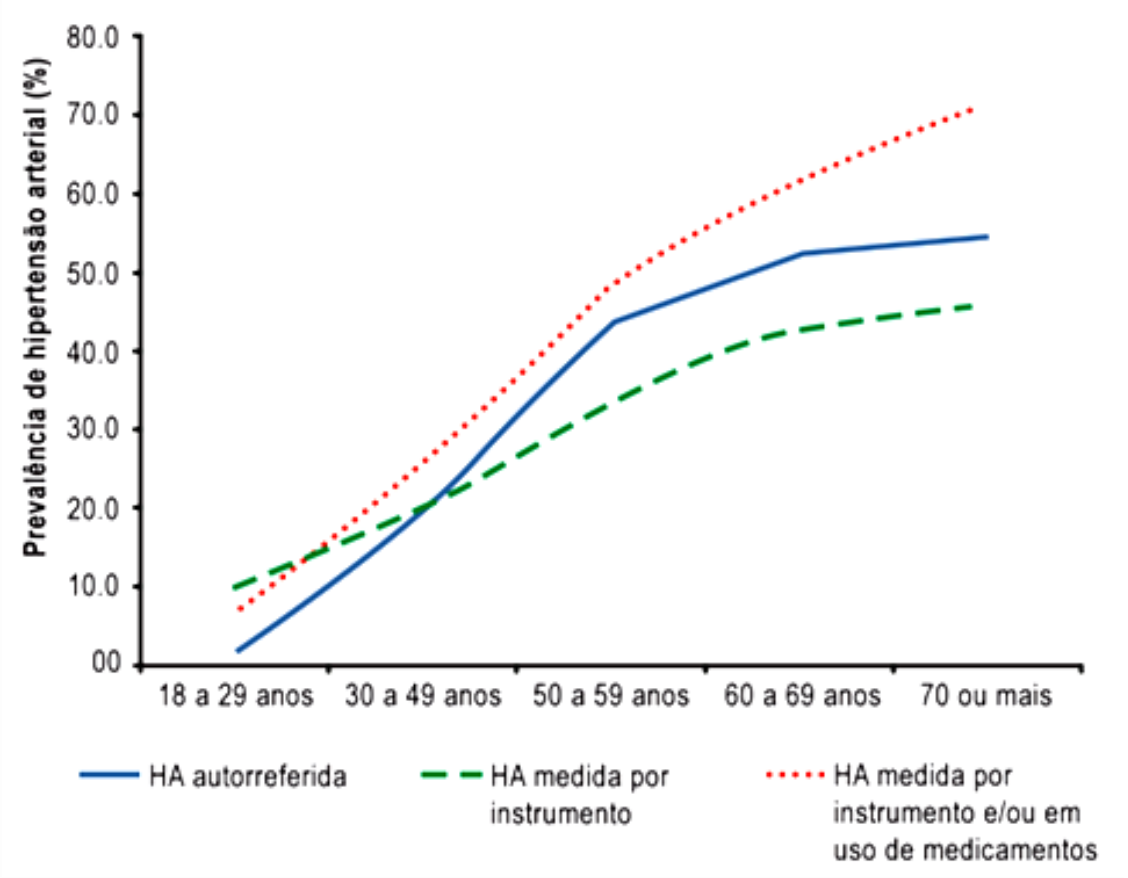
A hipertensão arterial (HA) é uma condição clínica crônica grave sendo considerada o principal fator de risco relacionado àsdoenças cardiovasculares (DCVs), doenças cerebrovasculares e doença renal crônica, aumentando tanto a morbidade quanto a mortalidade precoces (Lawes; Hoorn; Rodgers, 2008; Singh; Mensah; Bakris, 2010; Wilkins*et al.*, 2010; Chobanian, 2011; Forouzanfar *et al*., 2017). Portanto, a HA configura um importante problema de saúde públicae o seu controletem grande impactono estadode saúdeda população (Marques *et al*., 2015).

A HA é definida por valores de pressão arterial sistólica igual ou superior a 140 mmHg e/ou pressão arterialdiastólica igual ou superior a 90 mmHg (Diretrizes Brasileiras de Hipertensão Arterial, 2020).A classificação da hipertensão consiste em primária – também chamada de essencial – e secundária. Caracteriza-se uma hipertensão primária quando sua etiologia é desconhecida, sendo associada ao estilo de vida do indivíduo e fatores como obesidade, envelhecimento, diabetes e/ou alcoolismo. Estima-se que 90 a 95% dos indivíduos hipertensos são portadores da HA primária. Já a hipertensão secundária caracteriza-se pela determinação do seu surgimento, podendo ser relacionada a dano ou mau funcionamento em órgãos importantes para o controle da pressão arterial, a exemplo dos rins (Chobanian *et al*., 2003; Simplicio *et al.*, 2016). Haja vista a relação do estilo de vida com o desenvolvimento da hipertensão, é importante a manutenção de práticas saudáveis para a prevenção da HA, além de outras desordens cardiovasculares. Todavia, ainda que o quadro hipertensivo seja presente mesmo com a mudança de hábitos, tratamentos medicamentos estão disponíveis para auxiliar o ajuste pressórico.

Estima-se que a HA afete mais de 1,5 bilhão de pessoas ao redor do mundo (Organização Mundail de Saúde, 2013). No Brasil, em 2017, foram registrados 1.312.663 óbitos, sendo 27,3% devido a DCV. Essas doenças representaram 22,6% das mortes prematuras no Brasil (entre 30 e 69 anos de idade). Com relação à HA, a prevalência foi maior entre homens, além de, como esperado, aumentou com a idade por todos os critérios, chegando a 71,7% para os indivíduos acima de 70 anos (Figura 1). Em uma década (2008

a 2017) foram estimadas 667.184 mortes atribuídas somente à HA (Brasil, 2020). Embora ainda haja um debate sobre a fisiopatologia da HA, sabe-se que é uma condição clínica complexa e multifatorial, que combina fatores genéticos, alterações neuro-hormonais, alterações em estruturas vasculares e processos inflamatórios (Artom; Vecchié; Pende, 2016).

Figura 1 – Prevalência populacional de hipertensão arterial segundo diferentes critérios diagnósticos, em adultos com 18 anos de idade ou superior, ambos os sexos, por faixa etária (Brasil, 2013).



Fonte: Nilson *et al.*, 2020.

1.1 A Pressão Arterial e seus mecanismos de controle

Conceitualmente, pressão arterial (PA) é definida como a força (pressão) que o sangue exerce nas paredes dos vasos sanguíneos durante o bombeamento rítmico do coração. Na homeostase, a pressão no sistema cardiovascular garante a perfusão adequada de sangue por todos os órgãos com entrega de oxigênio e nutrientes para as células (Vasques *et al.*, 1997).

Matematicamente, a PA pode ser determinada pelo produto das variações do débito cardíaco (DC) e resistência vascular periférica total (RVPT) (Equação 1). O DC corresponde ao volume de sangue bombeado pelo coração a cada minuto, sendo expresso como produto do volume sistólico (VS, em mL) pela frequência cardíaca (FC, em bpm); e a RVPT resulta do tônus vascular (Wess; Eglen; Gautam, 2007).

Equação 1 – Fórmula geral da pressão arterial. ΔPA, variação da pressão arterial; ΔDC, variação do débito cardíaco; RVPT, resistência vascular periférica total; VS, volume sistólico e FC, frequência cardíaca.

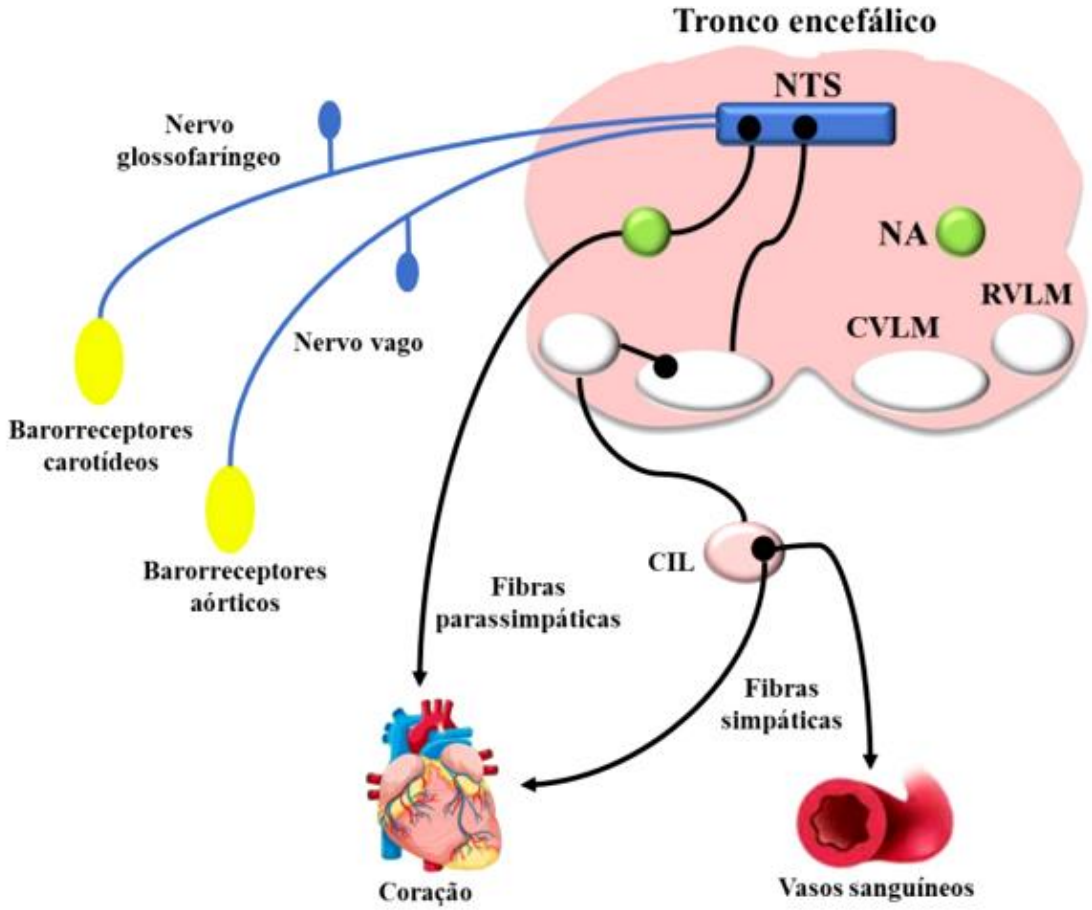
$$\Delta PA = \Delta DC \times RVPT, \qquad \text{onde } DC = VS \times FC$$

O controle da PA se dá por diferentes mecanismos regulatórios que atuam de forma integrada para proporcionar uma perfusão tecidual adequada sob diferentes circunstâncias (Duke-Novakovski; Carr, 2015). Alterações nesses mecanismos de regulação podem levar a distúrbios fisiológicos como a HA (Cain; Khalil, 2002).De forma geral, a PA é controlada por ações integradas de mecanismos que agem em curto e longo prazo, dos sistemas neural, cardiovascular, renal e endócrino (Campagnole-Santos; Haibara, 2001; Chopra, Baby, Jacob, 2011). Dentre os principais mecanismos envolvidos na regulação da PA, destaca-se o controle neural em curto prazo, desempenhado pelos reflexos cardiovasculares, tendo como principal representante o reflexo barorreceptor; e o controle renal em longo prazo, incluindo especialmente o Sistema Renina-Angiotensina-Aldosterona (SRAA).

O barorreflexo (Figura 2) ocorre por meio de mecanorreceptores, chamados de barorreceptores. Estes são sensíveis ao estiramento ocasionado pelo aumento da PA no vaso. Os barorreceptores localizam-se na parede do arco aórtico (barorreceptores aórticos) e na bifurcação do seio carotídeo (barorreceptores carotídeos). Eles são responsáveis por monitorar alterações na PA a cada batimento cardíaco e promove mudanças na PA e FC de forma a compensar essas variações. Estruturalmente, as terminações dos barorreceptores compõem a camada adventícia dos vasos juntamente com colágeno e elastina. Os barorreceptores possuem canais iônicos

mecanossensíveis que mudam de conformação quando estimulados pelo estiramento dos vasos, desta maneira o potencial de ação é desencadeado e os sinais aferentes são enviados em conjunto ao Sistema Nervoso Central (SNC) por meio do nervo depressor aórtico e pelo nervo do seio carotídeo, os quais se associam ao nervo vago e ao nervo glossofaríngeo, respectivamente (Krieger, 1964; Irigoyen *et al.*, 2001; Tu, Zhang, Li, 2019). Ambos formam sinapses com neurônios do Núcleo do Trato Solitário (NTS), um centro integrador localizado no bulbo. A partir do NTS, partem duas vias distintas, formadas pelas vias parassimpato-excitatória e simpato-inibitória, propiciando a capacidade do barorreflexo de agir sobre situações de aumento ou diminuição de PA. Na via parassimpato-excitatória, neurônios do NTS enviam projeções para o Núcleo Ambíguo (NA) que possui neurônios pré-ganglionares parassimpáticos. Na via simpato-inibitória, neurônios do NTS projetam-se para a região caudal ventrolateral do bulbo (CVLM), estimulando neurônios GABAérgicos que enviam projeções inibitórias para a região rostral ventrolateral do bulbo (RVLM), nessa encontram-se neurônios pré-autonômicos simpáticos com atividade marcapasso, os quais uma vez inibidos pelo CVLM, reduz a atividade simpática de forma geral (Correa *et al.*, 2008; Botelho-Ono *et al.*, 2011; Braga *et al.*, 2012). Em resposta ao aumento de PA, há prevalência da função autonômica parassimpática pela ativação da via parassimpato-excitatória e redução da função autonômica simpática pela via simpato-inibitória, com isso é observada redução na FC; redução do DC; redução da força de contração (inotropismo negativo) e da resistência vascular; esses eventos em conjunto culminam na diminuição da PA. Por outro lado, em resposta à redução da PA, os barorreceptores são menos ativados, há uma parassimpato-inibição associada à simpato-excitação. Nesse novo contexto ocorre o aumento da FC, do DC, aumento da força de contração (inotropismo positivo) e da resistência vascular, resultando na elevação da PA.

Figura 2 – Estrutura funcional do barorreflexo. O esquema representa o funcionamento do barorreflexo, suas aferências e eferências, regiões centrais de controle da pressão arterial e alguns órgãos alvo deste reflexo, como o coração e os vasos sanguíneos. NTS, núcleo do trato solitário; NA, núcleo ambíguo; CVLM, bulbo ventrolateral caudal; RVLM, bulbo ventrolateral rostral; CIL, coluna intermédio lateral.



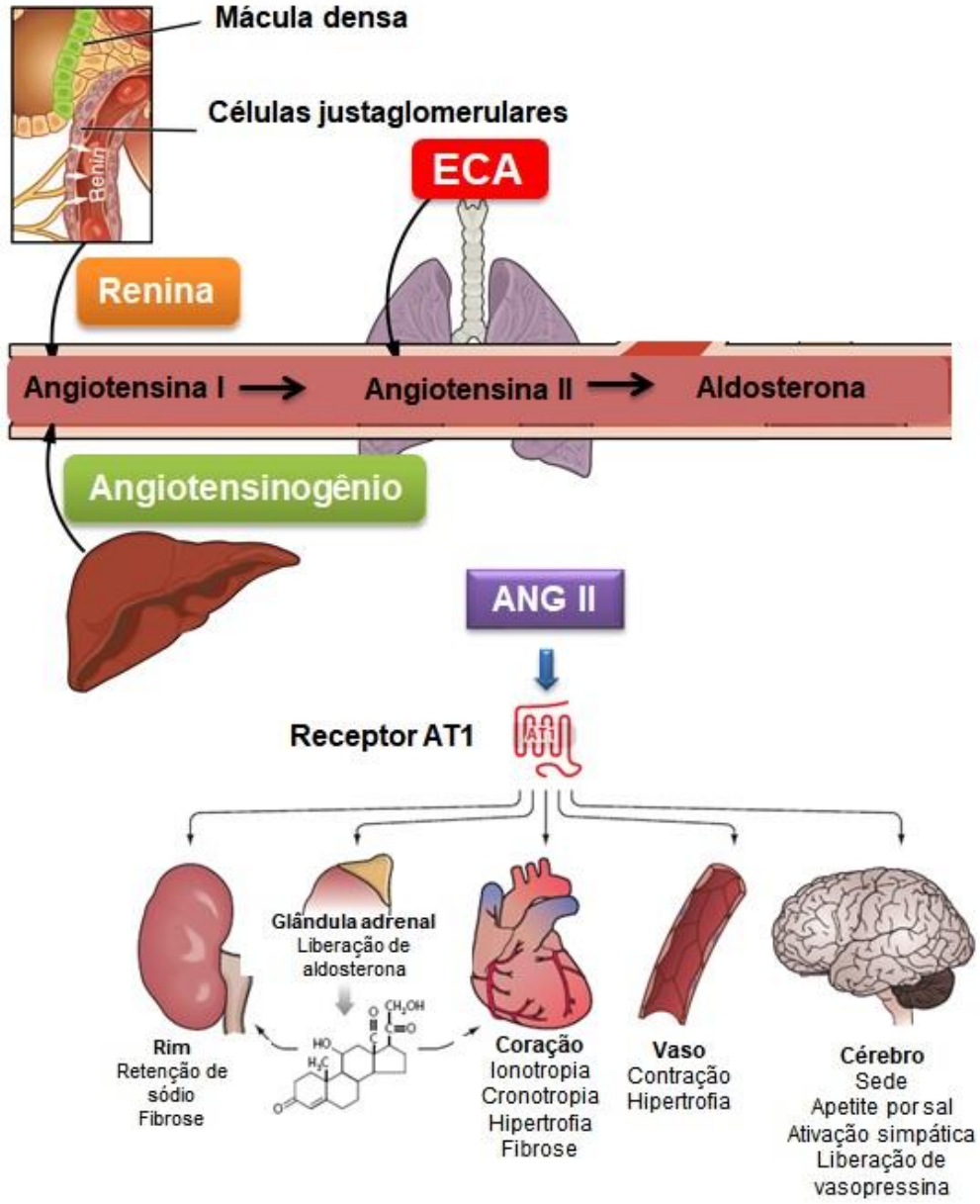
Situação da PA	Simpático	Parassimpático	Resposta
Aumento	Diminuição	Aumento	Redução da PA
Diminuição	Aumento	Diminuição	Elevação da PA

Fonte: Adaptado de Donaughy *et al.,* 2002 e Vasquez *et al.,* 1997.

No controle da PA em longo prazo, os rins exercem uma importante influência pela ativação do SRAA (Figura 3), bem como impacta diretamente a regulação do volume extracelular. Nesse sistema, o aparelho justaglomerular localizado nos néfrons e formado por células da mácula densa, células mesangiais e células justaglomerulares, atua na modulação da PA através de vias hormonais. As células justaglomerulares se encontram na parede das arteríolas aferentes e são células musculares especializadas que sintetizam,

armazenam e liberam renina após alguns estímulos como a redução da concentração de Na^+ e redução da perfusão renal devido à diminuição da PA sistêmica (Li, *et al*., 2017). A renina é uma enzima responsável pela clivagem de angiotensinogênio, um zimogênio produzido pelo fígado e liberado na corrente sanguínea, em angiotensina I, este decapeptídio, por sua vez, sofre ação da enzima conversora de angiotensina (ECA) transformando-se em angiotensina II (Ang II), um octapeptídeo, que na circulação promove efeitos, como por exemplo, a vasoconstrição, que culminam no aumento da PA (Campos, 2009).

Figura 3 – Sistema Renina-Angiotensina-Aldosterona. Esquema ilustrativo da formação da angiotensina II e sua ação em diversos alvos para controle da pressão arterial a longo prazo. ECA, enzima conversora de angiotensina; ANG II, angiotensina II; AT_1R, isoforma 1 do receptor da angiotensina.



Fonte: Adaptado de Bader *et al.*, 2010.

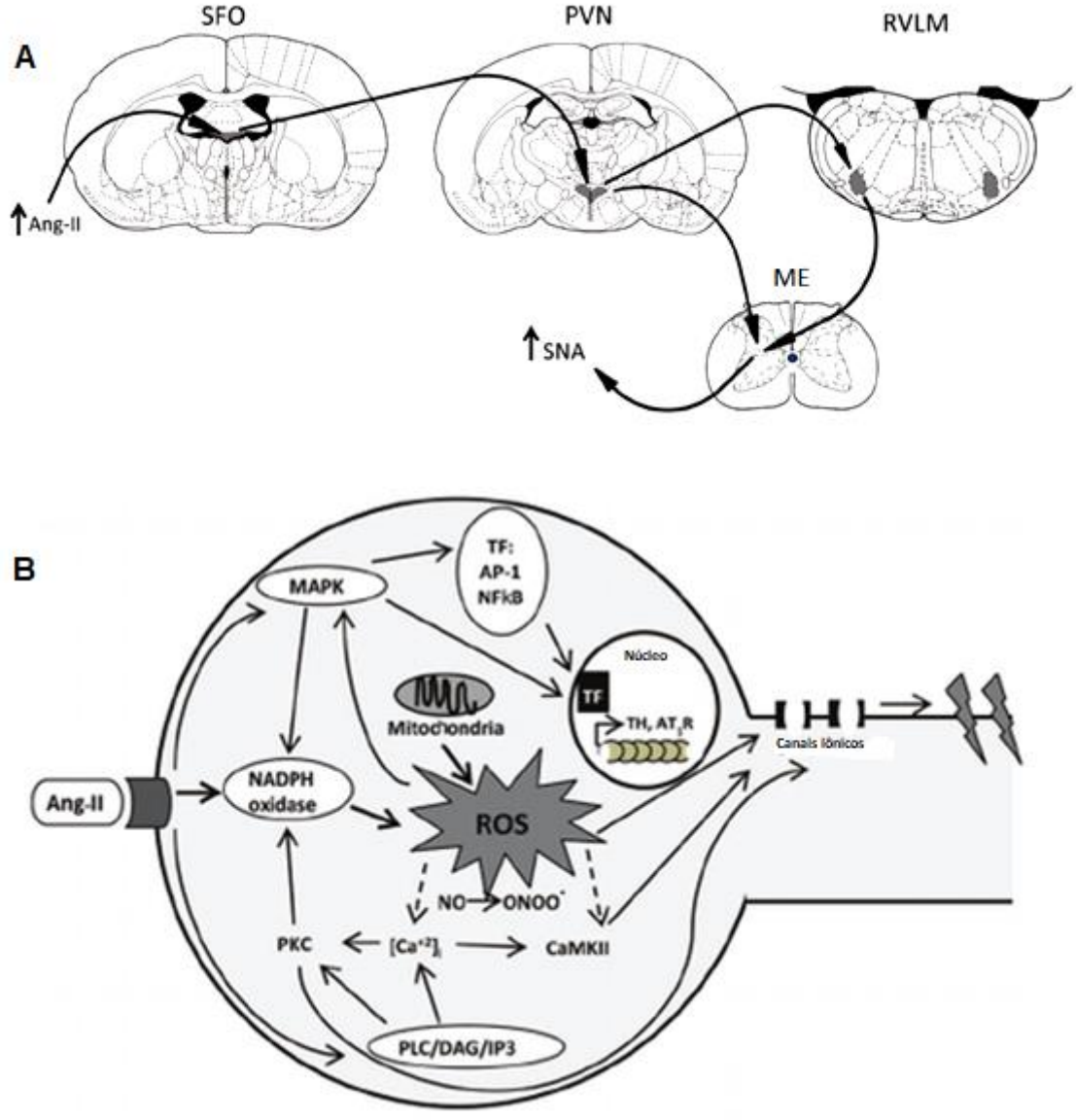
A Ang II interage, preferencialmente, com o receptor AT_1 (AT_1R) localizado nos vasos sanguíneos, coração, em regiões do SNC, dentre outras, promovendo efeitos como a elevação do tônus simpático, aumento da reabsorção de sódio e consequentemente de água, aumento da RVPT pela vasoconstrição e estresse oxidativo (Goldblatt, 1934; Werner *et al.*, 2008). Na interação da Ang II com AT_1R, um receptor acoplado a proteína $G_{q/11}$ (GPCR),

há a união dos dímeros da enzima fosfolipase C (PLC) colocando-a em sua forma ativa. A PLC é responsável pela hidrólise do bisfosfato de Inositol (PIP_2), um lipídeo de membrana, em trifosfato de Inositol (IP_3) e diacilglicerol (DAG), esses funcionam como segundos mensageiros elevando os níveis de cálcio intracelular, pela abertura dos canais para cálcio dependentes da proteína cinase C (PKC) (Horwitz *et al.*, 2003).

Muitos estudos têm relatado que a presença de Ang II e AT_1R em determinadas regiões do SNC, como o órgão subfornical (SFO), o núcleo paraventricular do hipotálamo (PVN) e o RVLM, está associada com a modulação de parâmetros cardiovasculares (Saavedra, 2005). No SNC, os neurônios e astrócitos expressam os componentes do SRAA, como a renina, angiotensinogênio, AT_1R e ECA (em particular no plexo corióideo e nas células endoteliais dos capilares), desta forma a Ang II também pode ser sintetizada centralmente (Kloet *et al.*, 2015, Xue *et al.*, 2016). Além disso, a Ang II circulante é capaz de acessar o SNC pelas regiões desprovidas de barreira hematoencefálica localizadas nos órgãos circunventriculares, estimulando sua produção central (Kloet *et al.*, 2015). Por outro lado, em algumas desordens cardiovasculares a permeabilidade da barreira hematoencefálica é prejudicada facilitando o acesso da Ang II circulante em alta concentração (Biancardi *et al.*, 2014). Nessas regiões, o acoplamento da Ang II ao AT_1R promove a geração de espécies reativas de oxigênio (EROs) decorrentes da ativação da enzima Nicotinamida Adenina Dinucleotídeo Fosfato (NADPH oxidase) pela fosforilação de suas subunidades promovida pela PKC, o que está relacionado em casos patológicos com hiperatividade simpática e disfunção do barorreflexo (Braga *et al.*, 2011).

Os radicais livres derivados do oxigênio desempenham um papel importante no desenvolvimento da hipertensão tanto a nível central pela disfunção do barorreflexo e influência na elevação da atividade simpática (Figura 4) (Braga *et al.*, 2011; 2012), quanto a nível periférico pela diminuição da biodisponilidade do óxido nítrico (NO), um vasoprotetor que atua controlando o tônus vascular (Dusse *et al.*, 2003; Harrison; Gongora, 2009; Tan *et al.*, 2017).

Figura 4 – Esquema ilustrativo do eixo SFO-PVN-RVLM. Representação do efeito da Ang II no SNC promovendo aumento da atividade simpática central e periférica. A - SFO, órgão subfornical; PVN, núcleo paraventricular; RVLM, medula ventro-lateral rostral; ME, medula espinhal; SNA, sistema nervoso autônomo. B – Ação da Ang II em um neurônio gerando aumento dos disparos elétricos e consequente aumento da atividade simpática.



Fonte: Adaptado de Braga *et al*., 2011.

Os diversos mecanismos de controle da PA lhe atribuem característica complexa e multifatorial. Descobrir quais falhas nesses sistemas de controle geram quadros hipertensivos pode ser complicado e mesmo com avanços nos estudos do entendimento da fisiopaologia e farmacoterapia da HA, intervenções estratégicas para reduzir ou prevenir a pressão arterial continuam

sendo um desafio. Estudos têm investigado os efeitos benéficos das alterações na pressão arterial devido uma mudança nos componentes dietéticos (Brito-Alves *et al.*, 2016).

As diretrizes atuais para o controle da hipertensão arterial propõem medidas de estilo de vida, incluindo abordagens dietéticas, quando apropriado, em todos os pacientes, incluindo aqueles que necessitam de tratamento medicamentoso. O objetivo dessas medidas de estilo de vida é reduzir a pressão arterial, controlar outros fatores de risco e reduzir o número ou as doses de medicamentos anti-hipertensivos (Mancia *et al.*, 2014).

1.2 Disbiose e Hipertensão

O trato gastrointestinal humano é um ecossistema complexo colonizado por trilhões de microrganismos, que juntamente com seus metabólitos constituem a microbiota intestinal (Ley *et al.*, 2006). A diversidade e estabilidade da microbiota intestinal e as interações microbianas e simbióticas regulam diversas funções fisiológicas no hospedeiro, incluindo a pressão arterial (Das e Nair 2019; Palmu *et al.*, 2020; Palmu *et al.*, 2021). Quando há um desequilíbrio na microbiota intestinal, com diminuição da diversidade e estabilidade da população microbiana e aumento da permeabilidade intestinal, ocorre o que chamamos de disbiose (Petersen e Round 2014). Vários estudos demonstraram que a disbiose intestinal está associada à HA (Muralitharan *et al.*, 2020; Silveira-Nunes *et al.*, 2020; Yang *et al.*, 2018). Em resumo, a disbiose pode promover hiperatividade simpática, inflamação sistêmica de baixo grau e disfunção endotelial, aumentando assim a PA (Ding *et al.*, 2014; Muralitharan *et al.*, 2020; Santisteban *et al.*, 2017; Toral *et al.*, 2019). Ao contrário, a HA também pode favorecer a disbiose, o que pode contribuir para a progressão do estado hipertensivo (Konopelski *et al.*, 2021; Qin *et al.*, 2018). Porém fortes evidências apontam que os componentes de uma dieta são capazes de modular a microbiota intestinal diminuindo a pressão sanguínea (Buettner *et al.*, 2007; Anhe *et al.*, 2015; Gomez-Guzman *et al.*, 2015; Grosso *et al.*, 2016).

Nesse contexto, terapias direcionadas à microbiota com probióticos foram propostas para tratamento ou prevenção da HA (Aoyagi *et al.*, 2017; Chi *et al.*, 2020; Qi*et al.*, 2020). Os probióticos são definidos como “microrganismos

vivos que, quando administrados em quantidades adequadas, beneficiam a saúde congênita do hospedeiro" (Hill *et al.*, 2014). Para segurança na utilização de probióticos, o Ministério da Saúde elenca requisitos em uma Resolução de Diretoria Colegiada (RDC nº 241/2018) que são necessários para que o microrganismo seja considerado um probiótico de fato, dentre eles ausência de registros de eventos adversos relevantes, obtidos a partir de estudos clínicos ou vigilância pós-uso, ausência de fatores de virulência e patogenicidade relevantes para a saúde humana, ausência de resistência potencialmente transferível a antibióticos relevantes para a saúde humana, ausência de produção de substâncias ou metabólitos que representem risco à saúde humana e susceptibilidade a, pelo menos, dois antibióticos. Entre as cepas potencialmente probióticas com propriedades anti-hipertensivas, aquelas pertencentes ao grupo *Lactobacillus* se destacam na modulação da PA. Na HA foi encontrada uma redução nos níveis de *Lactobacillus* no intestino (Palmu *et al.*, 2020; Razavi*et al.*, 2019) e a administração de algumas cepas de *Lactobacillus* melhorou a composição da microbiota intestinal e exerceu efeitos anti-hipertensivos em animais e humanos (Gadelha *et al.*, 2022).

Sabe-se também que os probióticos afetam a composição e a diversidade da microbiota intestinal (Cani *et al.*, 2009; Singh *et al*., 2013). Tem sido comumente observado que uma mudança no estado de saúde do hospedeiro foi acompanhada por uma mudança na microbiota intestinal(Yang *et al.*, 2015).

1.3 O gênero *Lactobacillus*

As bactérias do gênero *Lactobacillus* e *Bifidobacterium* são as mais utilizadas como probióticos, uma que têm sido isoladas de todas as porções do trato gastrintestinal de um humano saudável, além de possuírem efeitos benéficos na microbiota intestinal humana como efeitos antagônicos, competição e feitos imunológicos, promovendo uma melhora na resistência contra patógenos, evidenciando que o uso de microrganismos benéficos favorece os mecanismos de defesa naturais do hospedeiro (Dunne *et al.*, 1999; Puupponen-Pimia *et al.*, 2002).

Recentemente, o gênero *Lactobacillus* foi reclassificado devido à enorme diversidade genômica entre as mais de 260 espécies. Um total de 25 gêneros, incluindo o gênero alterado *Lactobacillus*, foram definidos com base em características fenotípicas, genotípicas e ecológicas (Salvetti *et al.,* 2018; Zheng *et al.*, 2020).Uma revisão publicada pelo nosso grupo de pesquisa compilou diversos estudos sobre variadas cepas do gênero *Lactobacillus* com propriedades anti-hipertensivas, demonstrando potenciais mecanismos de ação no sistema cardiovascular de animais e humanos. De modo reduzido, dentre os principais achados, a inibição da enzima conversora de angiotensina (ECA) em animais foi observada em cepas produtoras de peptídeos derivados da fermentação de alimentos, como a *Lactobacillus helveticus* LBK16H (Sipola *et al.*, 2002), *Lactiplantibacillus plantarum* TWK10 (Liu *et al.*, 2016) e *Lacticaseibacillus rhamnosus* EBD1 (Daliri *et al.*, 2019). Outros achados com efeitos relevantes no sistema cardiovascular foram catalogados para outras cepas, como a redução no estresse oxidativo de animais SHR quando administrado iogurte fermentado com *Lactiplantibacillus plantarum* Taj-Apis362 (Hussin *et al.*, 2020). Em humanos, a adição de *Lactobacillus casei* 01 em queijos foi capaz de inibir a ECA (Sperry *et al.,* 2018), além disso peptídeos provenientes da fermentação do leite por *Lactobacillus helveticus* reduziu a pressão arterial de hipertensos, provavelmente por efeito vasorrelaxante dependente de endotélio (Jauhiainen *et al*., 2007). Adicionalmente, o gênero Lactobacillus ainda apresentou outros efeitos no sistema cardiovascular, como redução da atividade simpática, melhora na produção de óxido nítrico, redução de marcadores inflamatórios (TNF-α, INF-β, IL-1β) e redução do estresse oxidativo (Oliveira *et al.*, 2021; Yuan *et al.,* 2022; Yap *et al.,* 2016).

Tratando mais especificamente do *Lactobacillus acidophilus*, poucos foram os estudos que avaliaram o efeito dessa espécie na hipertensão, Alguns trabalhos mostram um papel promissor da espécie *L. acidophillus* na pressão arterial de humanos quando associado a outros probióticos, como demonstrou Cicero (2021), ao usar um mix contendo *Lactiplantibacillus plantarum* PBS067, *Lactobacillus acidophilus* PBS066 e *Limosilactobacillus reuteri* PBS072 em uma fórmula simbiótica para pacientes com síndrome metabólica, os quais tiveram sua pressão arterial reduzida. Rezazadeh *et al.* (2021) demonstraram que um iogurte probiótico contendo *L. acidophilus* LA-05 e *Bifidobacterium lactis* Bb12

melhorou a sensibilidade à insulina, o estresse oxidativo e os níveis de ácido úrico em pacientes com síndrome metabólica. Moura *et al.* (2016) também demonstraram efeito antioxidante e melhora no perfil lipídico de ratos Wistar alimentados com sobremesa láctea contendo *L. acidophilus* LA-05. Todavia, quando o probiótico *L. acidophilus* foi utilizado para enriquecer alimentos, não foram observados efeitos na pressão arterial dos pacientes (Xavier-Santos *et al.*, 2018; Ivey *et al.*, 2015; Romão da Silva *et al.,* 2020). Ainda assim, o *Lactobacillus acidophilus* é amplamente reconhecido por ter efeitos probióticos, além de ser um dos microrganismos mais comumente sugeridos para uso dietético, sendo adicionado em várias matrizes alimentares (Sanders, 2003; Shah, 2007).

Com esses estudos foi possível observar que os principais efeitos encontrados com o *L. acidophilus* estavam relacionados a produtos derivados da fermentação, como peptídeos e ácidos graxos de cadeia curta e não com a utilização da cepa *in natura*. Não é possível descartar que os efeitos vistos são devido a processos fermentativos ocorridos no organismo dos animais, entretanto vale salientar que o modelo de suplementação adotada nesse trabalho não havia sido abordada com tanta ênfase na literatura até o momento.

O modelo animal escolhido para realização do trabalho possui como característica o desenvolvimento da hipertensão de acordo com a idade (Fazan Jr. *et al*., 2001). Os animais com hipertensão espontânea têm início no aumento dos níveis pressóricos a partir da 5ª semana de vida apresentando um nível de pressão considerado como hipertensão espontânea entre a 7ª e a 15ª semanas, atingindo um platô entre a 20a e 28a semanas, não havendo influência sexual nesse desenvolvimento (Yamori, 1984). A suplementação desses animais iniciou-se na 4ª semana de vida, o que caracteriza uma suplementação preventiva contra HA. Muito tem se discutido sobre as intervenções não farmacológicas para o tratamento e prevenção da hipertensão. Nesse contexto, corrigir as anormalidades dietéticas, o consumo excessivo de álcool, bem como a inatividade física que contribuem para a elevação da PA é uma abordagem fundamentalmente importante para a prevenção e o controle da hipertensão, seja de forma isolada ou em combinação com a terapia farmacológica.

Tendo em vista os efeitos benéficos do *L. acidophilus* relatados até o presente momento nossa hipótese é que uma suplementação com *Lactobacillus acidophilus* LA-05 seja capaz de melhorar parâmetros cardiovasculares de animais com hipertensão arterial. Portanto, o objetivo deste estudo foi investigar os efeitos induzidos pela suplementação com *L. acidophilus* LA-05 (LA5) sobre a modulação da PA, estresse oxidativo e reatividade vascular de ratos espontaneamente hipertensos, a fim de avaliar seu potencial como adjuvante no manejo da hipertensão arterial.

Objetivos

2.Objetivos

2.1 Objetivo geral

Avaliar os efeitos induzidos pela suplementação de *Lactobacillus acidophilus* LA-05 no sistema cardiovascular de ratos espontaneamente hipertensos.

2.2 Objetivos específicos

- Avaliar o efeito da suplementação com *Lactobacillus acidophilus* LA-05 na pressão arterial e frequência cardíaca de animais com hipertensão espontânea;
- Investigar os efeitos induzidos pela suplementação com *Lactobacillus acidophilus* LA-05 no barorreflexo de animais hipertensos;
- Estudar os efeitos da suplementação com *Lactobacillus acidophilus* LA-05 no controle autonômico da pressão arterial e frequência cardíaca;
- Investigar os efeitos da suplementação com *Lactobacillus acidophilus* LA-05 no estresse oxidativo em animais hipertensos;
- Avaliar os efeitos da suplementação com *Lactobacillus acidophilus* LA-05 sobre a reatividade vascular de animais hipertensos.

Metodologia

3. Metodologia

3.1 Animais

Foram utilizados ratos espontaneamente hipertensos (SHR) e Wistar Kyoto (WKY) com 04 semanas de idade, provenientes do biotério do Instituto de Pesquisas em Fármacos e Medicamentos (IPeFarM/ CEUA 4821160718). Estudos demonstram que ratos SHR iniciam o desenvolvimento da hipertensão a partir da 5ª semana e um nível considerado como hipertensão espontânea entre a 7ª e a 15ª semana, atingindo um platô entre a 20ª e 28ª semana, sem influência do sexo (Yamori, 1984). Os animais foram mantidos em gaiolas de polipropileno (4 animais/gaiola), com água filtrada e ração *ad libitum* (Labina, Purina Agribands). Foram mantidos em ciclo claro escuro de 12h e com temperatura (22 ± 2°C) e umidade controladas. Todos os protocolos e procedimentos experimentais foram submetidos à apreciação do Comitê de Ética no Uso de Animais (CEUA) da UFPB, sob número de protocolo 0004312.

3.2 Grupos experimentais

Os animais foram divididos em três grupos: 1. Normotenso Controle (WKY); 2. Hipertenso Controle (SHR); e 3. Hipertenso suplementado com a cepa *Lactobacillus acidophilus* LA-05 (SHR+LA-5). Os animais tiveram a suplementação iniciada após 04 semanas do nascimento e receberam diariamente a suspensão de *Lactobacillus acidophilus* (10^9 log CFU/animal) por um período de 8 semanas via oral mediante gavagem. A cepa foi cedida pela professora Drª. Marciane Magnani do Departamento de Nutrição da Universidade Federal da Paraíba.

3.3 Cepa teste e preparo do inóculo

Foi utilizada a cepa *Lactobacillus acidophilus* LA-05, comercializada pela Christian Hansen (Valinhos, Minas Gerais, Brasil). A cultura comercial liofilizada foi mantida sob congelamentoa -18 ºC e para utilização foi reidratada em caldo MRS e inoculada 24 horas a 37 ºC.

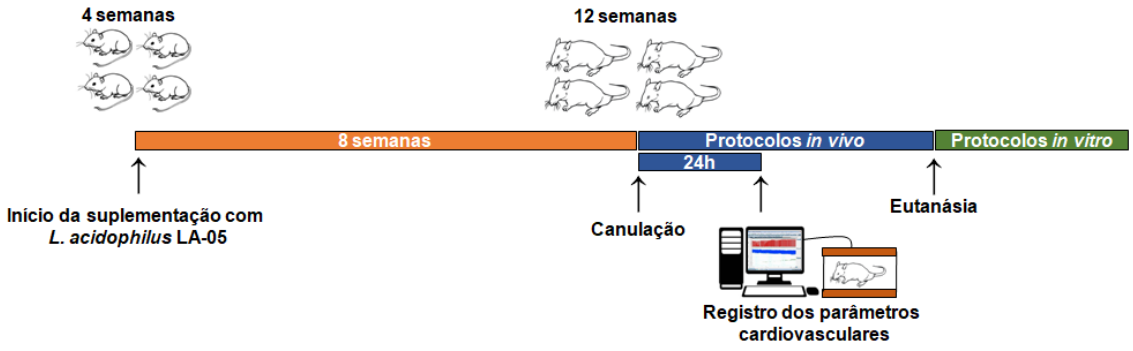
O inóculo da cepa foi obtido preparando a partir de suspensões em solução salina estéril a partir de culturas *overnigth* em caldo MRS (HiMedia, Mumbai, India). As células foram recolhidas por centrifugação (4500 ×*g*, 15 min, 4 ºC), lavadas duas vezes com solução salina estéril, ressuspensas e homogeneizadas utilizando um vórtex (30s) em solução salina para obter suspensões de DO (Densidade ótica) a 660 nm (DO_{660}) 1,0 correspondente a contagens de aproximadamente 9 log UFC/mL.

3.4 Protocolos *in vivo*

3.4.1 *Procedimento cirúrgico para registro da pressão arterial e frequência cardíaca em ratos não anestesiados*

Um dia antes dos registros de pressão arterial, os animais foram anestesiados com xilazina (10 mg/kg, i.p.) e cetamina (80 mg/Kg, i.p.) e em seguida foram implantados os cateteres de polietileno (PE_{10} conectado ao PE_{50}) na artéria e veia femoral, para registro da pressão arterial e infusão de drogas, respectivamente. Os cateteres foram exteriorizados subcutaneamente ao nível escapular para facilitar a conexão do cateter ao transdutor e em seguida os animais receberam uma injeção de cetoprofeno (5 mg/kg, i.p.) para prevenir processos inflamatórios. Após a cirurgia, os animais permaneceram em recuperação por 24 h até o início dos experimentos.

Figura 5 – Ilustração temporal da metodologia utilizada para suplementação dos animais hipertensos com *L. acidophilus* LA-05 seguida dos protocolos abordados *in vivo* e *in vitro.*



O registro da PA e da frequência cardíaca (FC) foi realizado 24 horas após o procedimento cirúrgico em animais não anestesiados por meio da conexão da cânula da arterial femoral com o transdutor mecanoelétrico de pressão, cujo sinal é devidamente amplificado (ML866/P, ADInstruments, Power Lab, Bella Vista, NSW, Australia), digitalizado por meio de uma interface analógico/digital e amostrado a 2000 Hz em um microcomputador equipado com um software apropriado (LabChartTM Pro, ADInstruments, Bella Vista, NSW, Austrália), para posterior análise. A pressão arterial média (PAM) e FC derivam da pressão arterial pulsátil (PAP) por meio deste sistema de aquisição.

Figura 6 - Ilustração do procedimento de implantação dos cateteres vasculares. A, identificação e dissecção da artéria e veia femoral, locais de inserção dos cateteres. B, exteriorização dos cateteres na região dorsal do animal.

3.4.2 *Avaliação do efeito da suplementação com Lactobacillusacidophilus*LA-05*no barorreflexo de animais*

Para avaliação do barorreflexo foram analisadas as alterações reflexas na FC induzidas por alterações transitórias na PA. Estas alterações foram induzidas por injeção intravenosa de fenilefrina (8 µg/Kg), agonista α1 adrenérgico; e nitroprussiato sódico (25 µg/Kg), doador de óxido nítrico; de maneira a se obter respostas pressoras e depressoras, respectivamente. Entre as injeções foi mantido um intervalo de no mínimo 15 minutos, para permitir que a PA e a FC retornassem aos valores basais. Para a determinação da

sensibilidade do barorreflexo adotamos a técnica do pico da resposta ao agente vasoativo (ΔFC/ΔPAM), foi construída uma reta e o *slope* da curva fornece o índice que representa a sensibilidade do reflexo, que foi comparado entre os grupos experimentais. Adicionalmente avaliou-se o barorreflexo espontâneo a partir do registro basal da PAM e FC dos animais, utilizando para isso o software Hemolab (Analyser versão 9.3).

3.4.3 *Avaliação dos efeitos da suplementação com LactobacillusacidophilusLA-05 no controle autonômico da pressão arterial e frequência cardíaca*

A atividade autonômica foi avaliada a partir da variabilidade da FC por análise espectral. A partir dos registros basais de PA e FC obtidos, sequências de valores consecutivos do intervalo sistólico foram extraídas de séries temporais de 4.000 a 4.500 batimentos cardíacos consecutivos (aproximadamente 10 minutos de registro contínuo da pressão arterial pulsátil), utilizando o módulo “variabilidade da frequência cardíaca - HRV” do LabChart 8.0.1 para o software Windows (ADInstrumentsPty Ltd, Austrália). A potência dos componentes oscilatórios obtidos dos ratos pertencentes aos grupos experimentais foi integrada nas bandas de baixa frequência (LF; 0,2–0,75 Hz) e alta frequência (HF; 0,75–2,5 Hz) e os resultados foram expressos em porcentagem (%). As bandas LF são representativas dos efeitos modulatórios da atividade simpática, enquanto as bandas HF estão associadas à modulação parassimpática ou respiratória. A proporção de potência LF para HF (relação LF/HF) foi usada para estimar o balanço simpatovagal (Shaffer; Ginsberg, 2017).

3.5 Protocolos *in vitro*

3.5.1 *Estudos de reatividade vascular*

3.5.1.1*Preparação dos anéis de artéria mesentérica superior isolada de ratos normotensos*

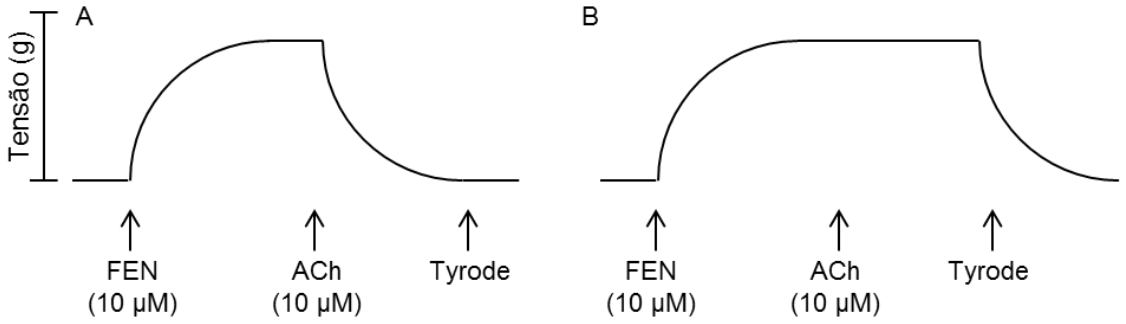
Ratos normotensos e hipertensos suplementados com LA5 por 8 semanas foram eutanasiados com o auxílio da guilhotina. Por meio de uma incisão na região ventral do animal, a artéria mesentérica superior foi identificada, removida e imediatamente colocada em solução Tyrode. Neste meio, a artéria foi dissecada e todo o tecido conjuntivo e adiposo foi removido com o cuidado de preservar a camada muscular e endotelial do vaso. Posteriormente, a artéria foi seccionada em anéis com 2-3 mm de comprimento e estes montados em duas hastes de aço, sendo uma considerada fixa e a outra conectada a um transdutor de força (PowerLab™, ADInstruments, MA, EUA) sensível a variações de tensões isométricas. Para a realização de alguns protocolos, removeu-se o endotélio vascular com fricção mecânica da parede interna do vaso contra as hastes de aço (COX *et al.*, 1989).

Os anéis obtidos foram transferidos para um banho de órgãos isolados, sendo imersos e mantidos em cubas (10 mL) contendo solução de Tyrode a 37 ºC e gaseificada com uma mistura de 95% de O_2 e 5% de CO_2 (carbogênio). Todos os anéis foram suspensos verticalmente por linhas de algodão fixadas ao transdutor de força e submetidos a uma tensão basal de aproximadamente 0,75 g por um período de 60 minutos (Furchgott; Zawadzki, 1980). Durante este período inicial, a solução de Tyrode foi trocada a cada 15 minutos com o objetivo de prevenir o acúmulo ou a interferência de metabólitos e a tensão basal foi ajustada quando necessário antes do início do experimento (Altura; Altura,1970).

Em todos os protocolos experimentais, após este período de estabilização, a viabilidade funcional do tecido muscular foi verificada através de uma contração induzida por fenilefrina (FEN, 10 µM), um agonista dos receptores α_1-adrenérgicos presentes no músculo liso vascular. Após esta indução, os anéis que obtiveram uma contração superior a 0,30g foram considerados funcionalmente viáveis.

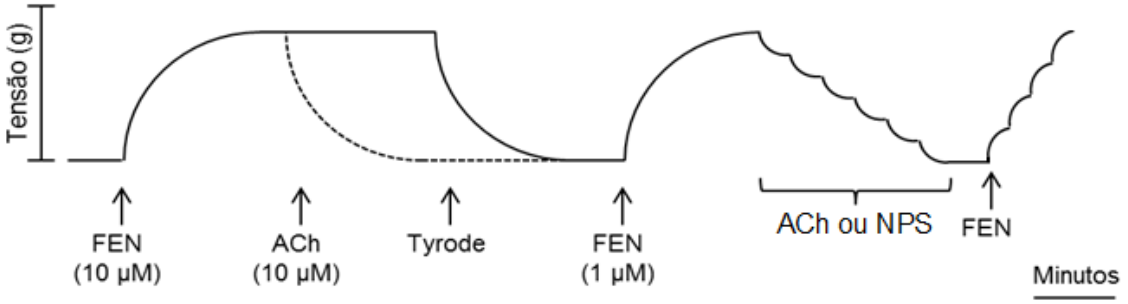
Posteriormente, com o intuito de avaliar a integridade das células endoteliais, verificou-se a presença do endotélio funcional através da adição de acetilcolina (ACh, 10 µM), um agonista dos receptores muscarínicos expressos no endotélio vascular (Furchgott; Zawadzki, 1980). Anéis com relaxamento superior a 80% foram considerados com endotélio funcional e anéis com relaxamento inferior a 20% foram considerados sem endotélio funcional.

Figura 7 – Esquema representativo do protocolo de verificação da viabilidade funcional do tecido muscular e endotelial vascular. (A) Anéis considerados com endotélio funcional. (B) Anéis considerados sem endotélio funcional.



Após um período de estabilização de 60 minutos e posterior verificação do endotélio funcional, foi induzida uma contração com FEN (10 µM). Logo após, concentrações crescentes do Ach (10^{-10}- 10^{-4} M), de maneira cumulativa, foiaplicada para a obtenção de uma curva concentração-resposta, em anéis com endotélio funcional e o Nitroprussiato de sódio (NPS) (10^{-12} – 10^{-5}M) em anéis sem endotélio funcional. O relaxamento foi expresso como percentagem reversa da contração induzida pela FEN.Adicionalmente foi realizada uma curva concentração resposta à FEN (10^{-10} – 10^{-4} M) sobre anéis submetidos a tensão basal. Após a obtenção das curvas concentração-resposta, foram calculados os valores de pD_2 (-log da CE_{50}) e $E_{máx}$ (efeito máximo).

Figura 8 – Esquema representando o protocolo para avaliação dos efeitos da suplementação com LA-5 sobre anéis de artéria mesentérica. Foram avaliados anéis com endotélio funcional (linha pontilhada) e anéis desprovidos de endotélio funcional (linha cheia).



3.5.2 *Avaliação do estresse oxidativo*

Após o registro de pressão arterial e frequência cardíaca, cerca de 2,5mL de sangue foicoletado centrifugado a 2000 g, 4º C, durante 15 min para obtenção do soro. Amostras foram coletadas para a dosagem das substâncias reativas ao ácido tiobarbitúrico (TBARS). A concentração de malondialdeído (MDA), um produto final da peroxidação lipídica, foimedida como indicador do estresse oxidativo. Nesse ensaio MDA reage com o ácido tiobarbitúrico para produzir um complexo vermelho. 400 µL de ácido perclórico (7%) foram adicionados a 250 µL da amostra, misturado e centrifugado a 600 g, 4º C, durante 20 minutos. O sobrenadante foi coletado, e adicionado a 400 µL do ácido tiobarbitúrico (0,6%), a mistura foi aquecida a 40ºC durante 1 hora e lida no espectrofotômetro a 532 nm. Uma curva padrão do MDA foi construída e os resultados foram expressos como mol de MDA/mL.

3.6. Análise estatística

Os valores foram expressos como média ± erro padrão da média (e.p.m). ANOVA *one-way* seguida de pós teste de Tuckey e ANOVA *two-way* seguida de pós teste de Boferroni foram utilizados. Diferenças foram consideradassignificativas quando $p<0,05$. O programa utilizado foi o *Graph Pad Prism* versão 8.00®.

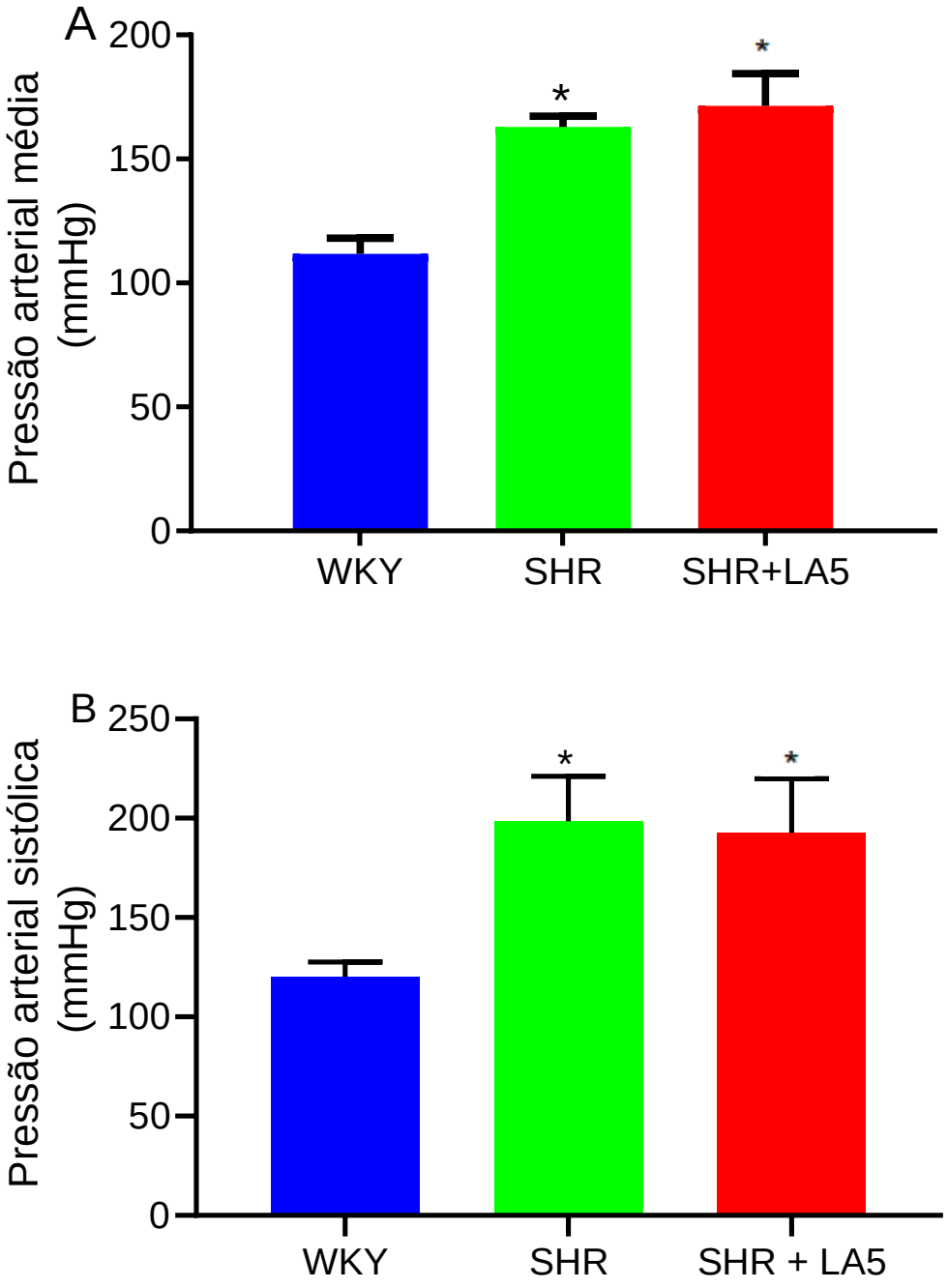
Resultados

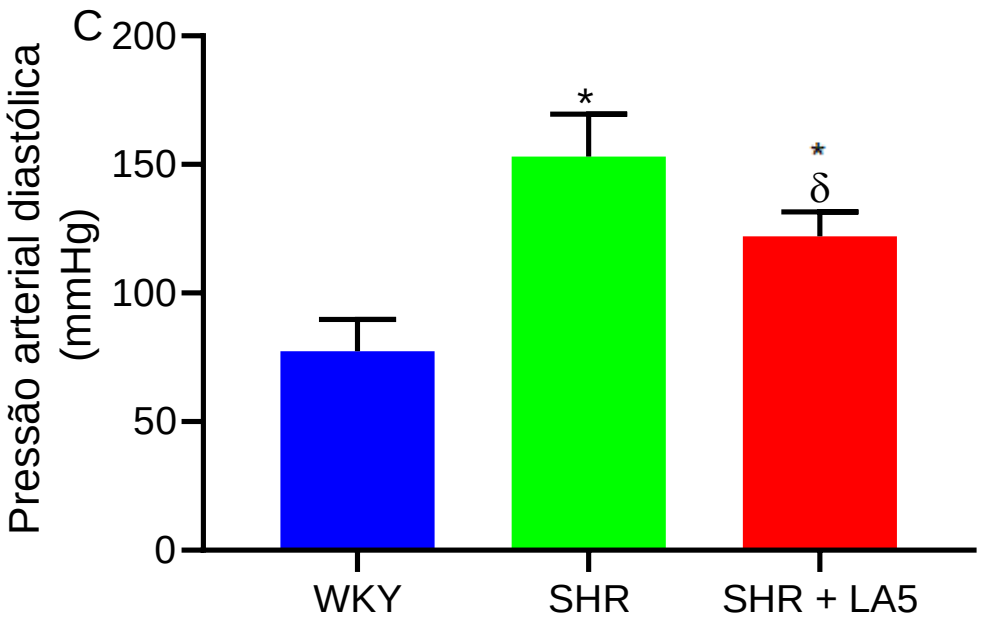
4. Resultados

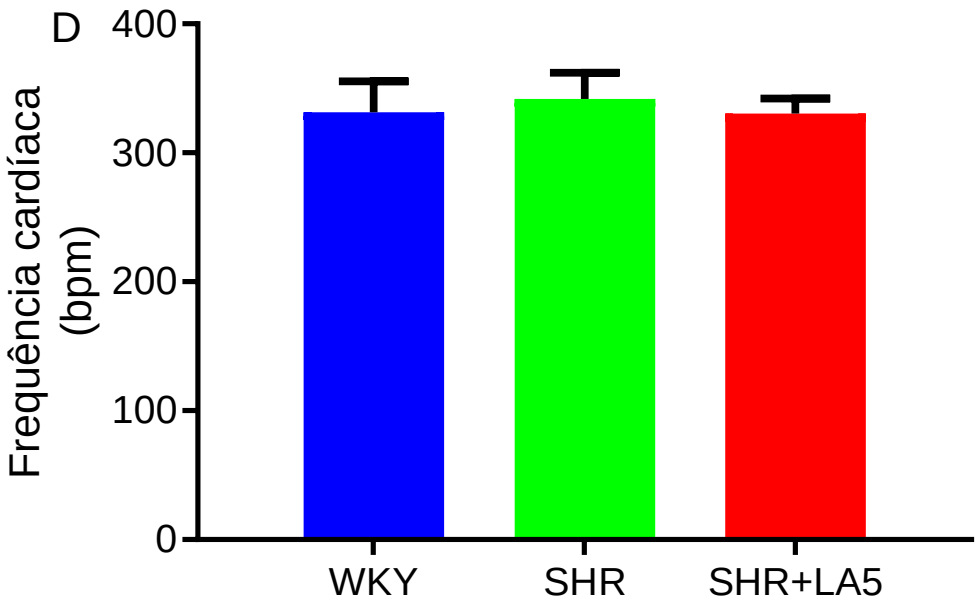
4.1 A suplementação com *L. acidophilus* LA-05 por 08 semanas não altera a pressão arterial média de animais com hipertensão espontânea

Ao final das 08 semanas, o grupo SHR apresentou níveis de pressão arterial média (PAM) mais elevados que o grupo WKY (162,8±4,3 mmHg *versus* 118,6±4,7 mmHg, respectivamente, $p<0,05$) (Gráfico 1A). A suplementação oral de LA5 por oito semanas não atenuou o aumento da PAM em SHR+LA5 (171,4±13 mmHg) (Gráfico 1A). Resultados semelhantes foram observados na pressão arterial sistólica, o grupo SHR apresentou níveis de Pressão Arterial Sistólica (PAS) mais elevados do que o grupo WKY (198,33±9,3 *versus* 120,1±3,0 mmHg, $p<0,05$), que não foi modificado pelo consumo de LA5 (192,6±12,2 mmHg) (Gráfico 1B). Por outro lado, os valores de Pressão Arterial Diastólica (PAD) foram maiores no grupo SHR em comparação com o grupo WKY (153,0±6,7 *versus* 77,3±5,0 mmHg, $p<0,05$) e a suplementação com LA5 reduziu significativamente a PAD no grupo SHR+LA5 (122,0±4,3 mmHg, $p<0,05$) (Gráfico 1C). A frequência cardíaca (FC) não foi significativamente diferente entre os grupos (WKY: 331,6±24 bpm; SHR: 341,8±20,3 bpm; e SHR+LA5: 330,4±11,8 bpm) (Gráfico 1D).

Gráfico 1 – Efeitos da suplementação com *L. acidophilus* LA-05 sobre PAM (A), PAS (B), PAD (C) e FC (D) de ratos espontaneamente hipertensos. Grupos: Controle normotenso (WKY, n=6); controle hipertenso (SHR, n=6) e SHR hipertenso suplementado com LA5 por 8 semanas via oral (SHR+LA5, n=5). Valores expressos em média ± erro padrão da média e p<0,05. **versus* WKY; δ *versus* SHR.




C
Pressão arterial diastólica (mmHg)
200
150
100
50
0
*
*
δ
WKY
SHR
SHR + LA5


D
Frequência cardíaca (bpm)
400
300
200
100
0
WKY
SHR
SHR+LA5

4.2 A suplementação com *L. acidophilus* LA-05 reduz a hiperatividade simpática de animais com hipertensão espontânea

O efeito da administração de LA5 por 8 semanas em ratos SHR na modulação autonômica foi analisado por análise espectral e é mostrado no Gráfico 2 e traçados representativos na Figura 6. Os espectros representativos (Figura 6) e o gráfico de grupo (2B) destacam o aumento esperado nas bandas LF em SHR em comparação com ratos WKY (9,5±0,75 *versus* 6,34±0,40%, respectivamente, $p<0,05$), caracterizando a hiperatividade simpática presente nesse modelo de hipertensão. A suplementação com LA5 diminuiu a magnitude das oscilações nas bandas LF no grupo SHR+LA5 (7,38±0,22%, $p<0,05$), refletindo a capacidade do probiótico em reduzir o tônus simpático. O componente HF e a relação LF/HF não foram diferentes entre os grupos (Gráficos 2C, D). Esses dados sugerem que o LA5 reduz a hiperatividade simpática presente em animais SHR, sem promover alterações significativas no equilíbrio simpatovagal.

Figura 9 – Avaliação do efeito da suplementação de LA5 por 8 semanas na análise espectral da variabilidade da frequência cardíaca em animais com hipertensão. Traçado representativo da análise espectral de um animal representativo por grupo.

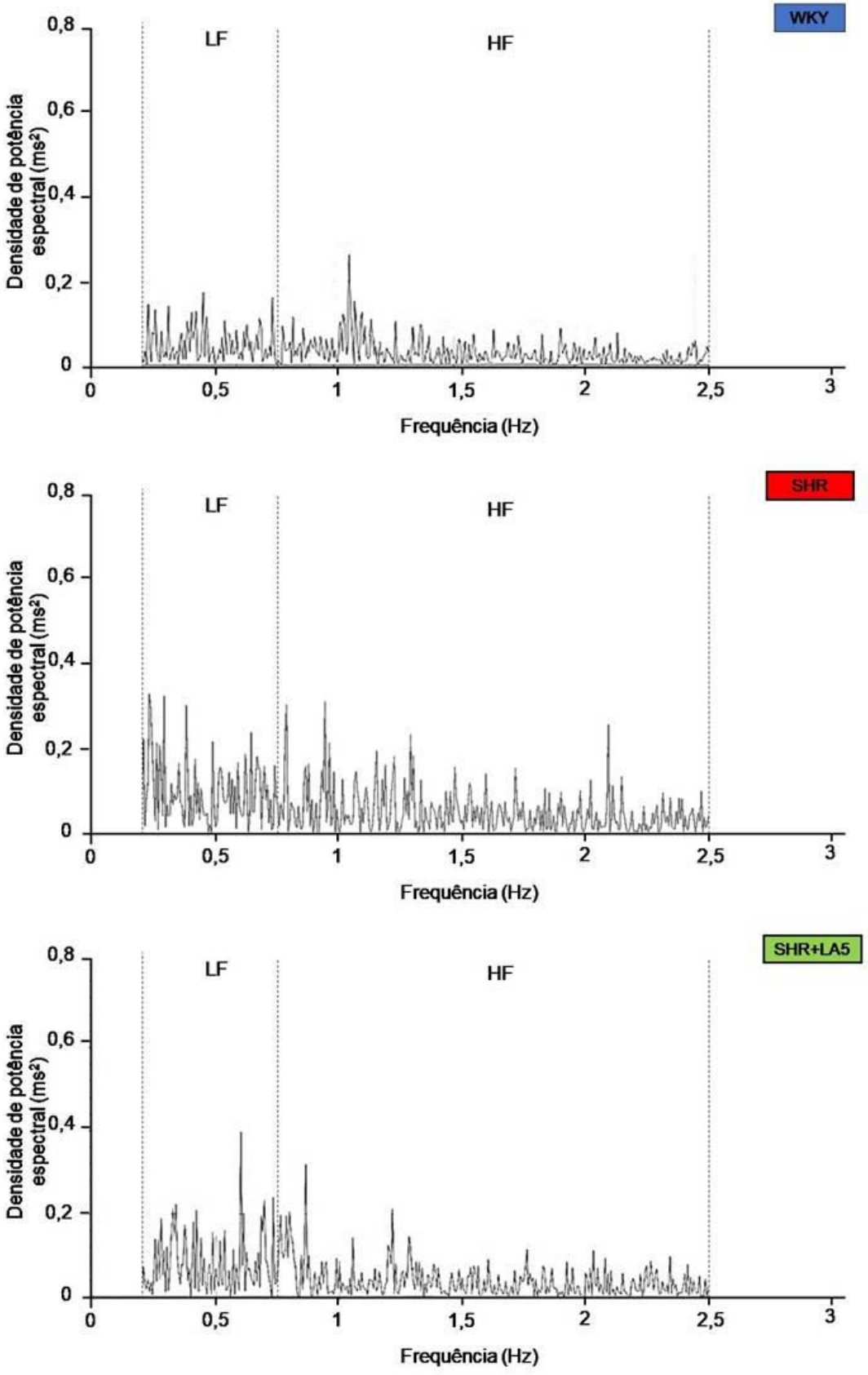
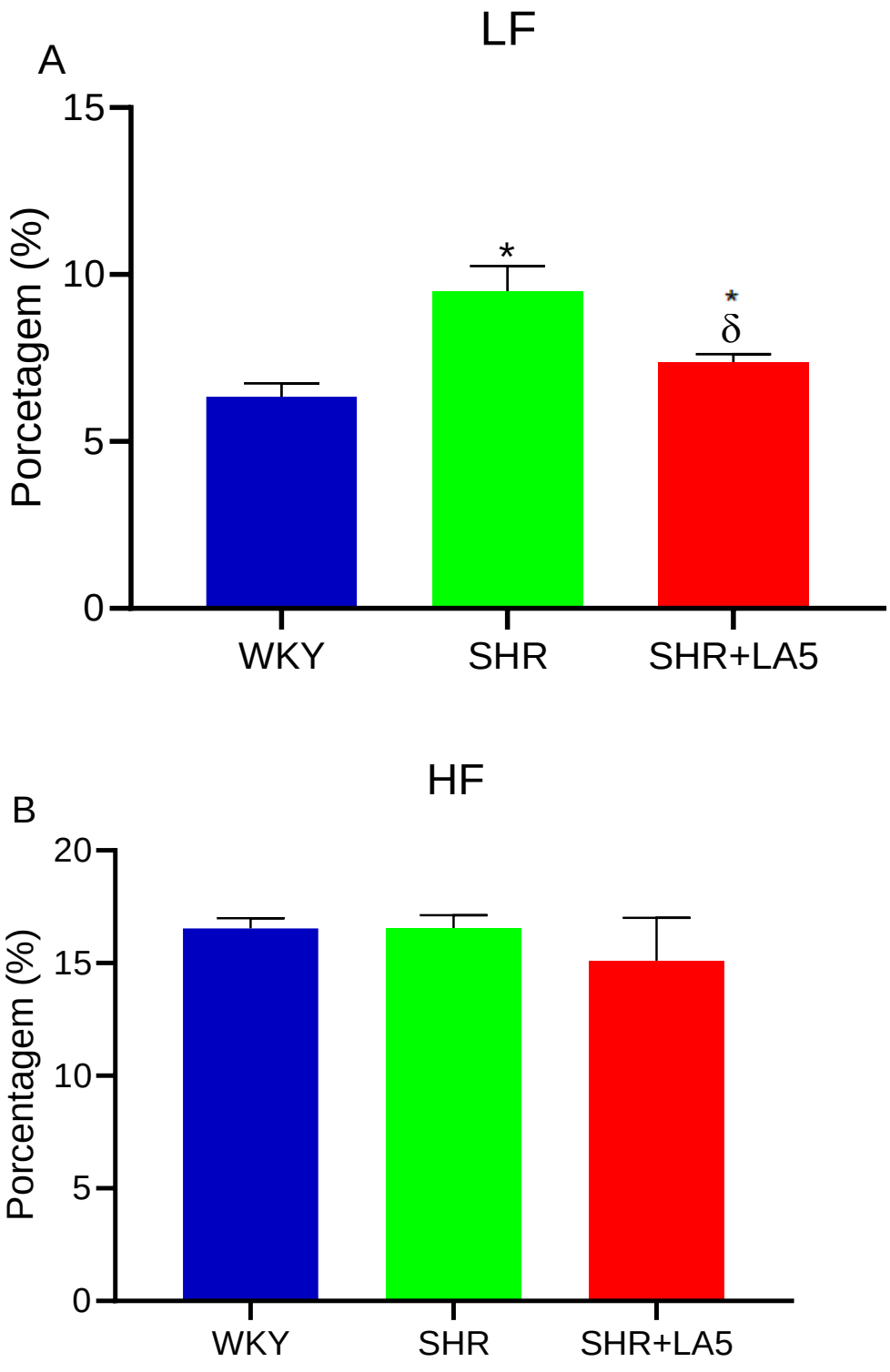
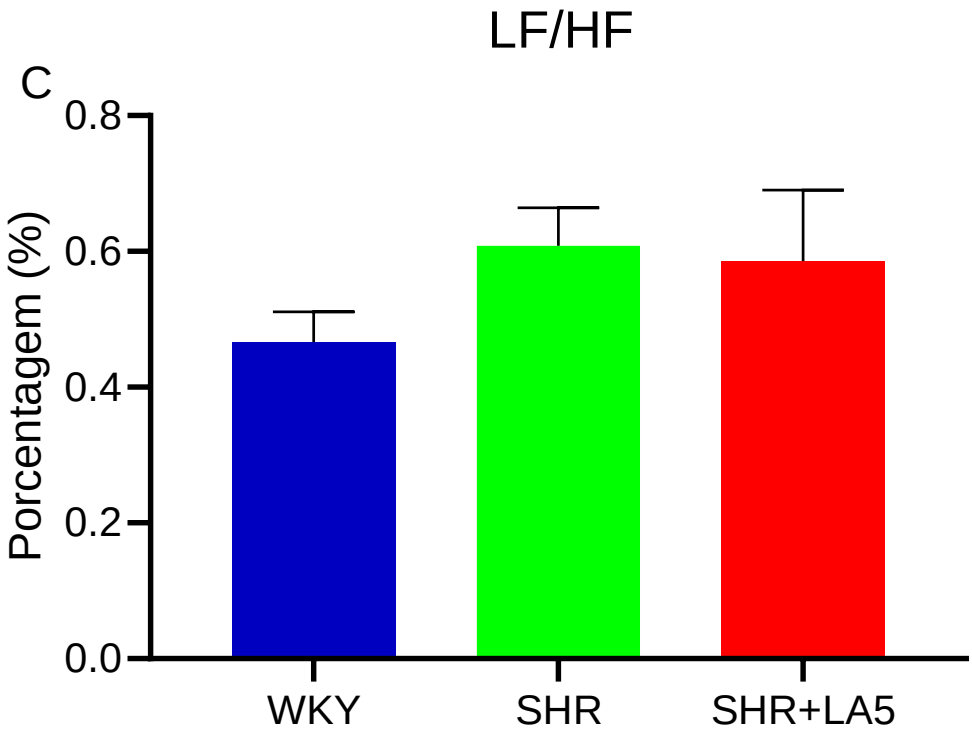


Gráfico 2 – Efeitos da suplementação com LA5 por 8 semanas na análise espectral da variabilidade da frequência cardíaca em animais com hipertensão. A – Baixa frequência (LF), B – Alta frequência (HF) e C – Equilíbrio de LF/HF. Grupos: Controle normotenso (WKY, n=6); controle hipertenso (SHR, n=6) e SHR hipertenso suplementado com LA5 por 8 semanas de suplementação oral com LA5 (SHR+LA5, n=5). Valores expressos em média ± erro padrão da média e p<0,05. * *versus* WKY; δ *versus* SHR.

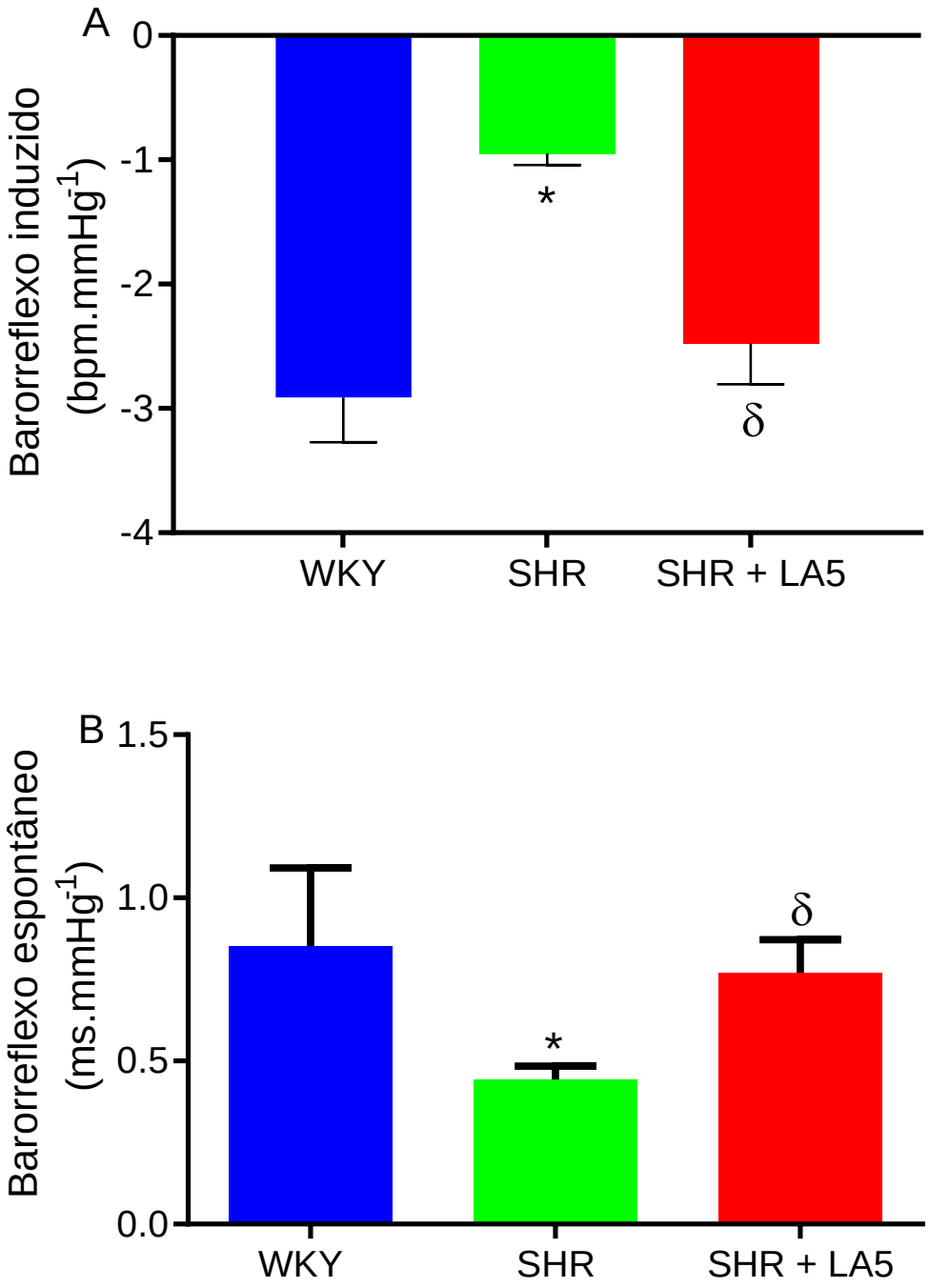



LF/HF
C
Porcentagem (%)
0.8
0.6
0.4
0.2
0.0
WKY
SHR
SHR+LA5

4.3 *L. acidophilus* LA-05 melhora a sensibilidade do barorreflexo de animais espontaneamente hipertensos

O grupo SHR apresentou redução na sensibilidade do barorreflexo (SBR) induzido quando comparado aos ratos WKY (-0,94±0,09 *versus* -2,91±0,35 bpm.mmHg^{-1}, respectivamente, p<0,05). A suplementação com LA5 foi capaz de prevenir o comprometimento da SBR em animais hipertensos, pois uma diferença significativa pode ser observada quando comparamos os grupos SHR+LA5 e SHR (-2,48±0,32 *versus* -0,94±0,09 bpm.mmHg^{-1}, p<0,05, Gráfico 3A, respectivamente). Adicionalmente, a sensibilidade do barorreflexo espontâneo foi significativamente prejudicado em SHR em comparação ao grupo WKY (0,44±0,04 *versus* 0,85±0,2 ms.mmHg^{-1}, respectivamente, p<0,05) e a suplementação com LA5 impediu a redução na sensibilidade do barorreflexo espontâneo (0,77± 0,1 ms.mmHg^{-1}, p<0,05, Gráfico 3B).

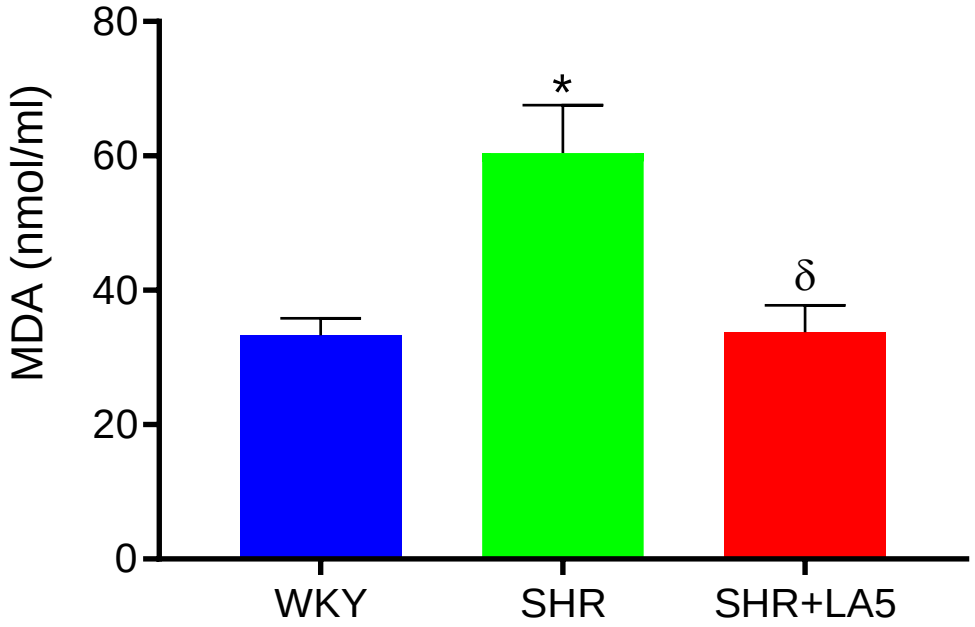
Gráfico 3 – Efeito da suplementação com *L. acidophilus* LA-05 na sensibilidade do barorreflexo de animais normotensos Wistar Kyoto (WKY) e espontaneamente hipertensos (SHR). A – Barorreflexo espontâneo; B – Barorreflexo induzido. Valores expressos como média ± erro padrão da média e $p<0,05$. WKY n=9; SHR n=4; e SHR+LA5 n=5. * *versus* WKY; δ *versus* SHR.



4.4 A suplementação com *L. acidophilus* LA-05 reduz o estresse oxidativo em animais com hipertensão espontânea

No Gráfico 4 é possível observar que ratos SHR apresentaram níveis séricos de MDA mais elevados quando comparados ao grupo WKY (60,45 ± 2,89 *versus* 33,26 ± 1,04 nmol/mL, $p<0,05$). A suplementação oral com LA5 por 8 semanas foi capaz de reduzir os níveis de MDA como observado nos SHR+LA5 comparados aos SHR controle (33,78 ± 1,61 *versus* 60,45 ± 2,89 nmol/mL, $p<0,05$). Esses resultados sugerem que a suplementação oral com LA-5 por 8 semanas é capaz de reduzir o estresse oxidativo em animais espontaneamente hipertensos.

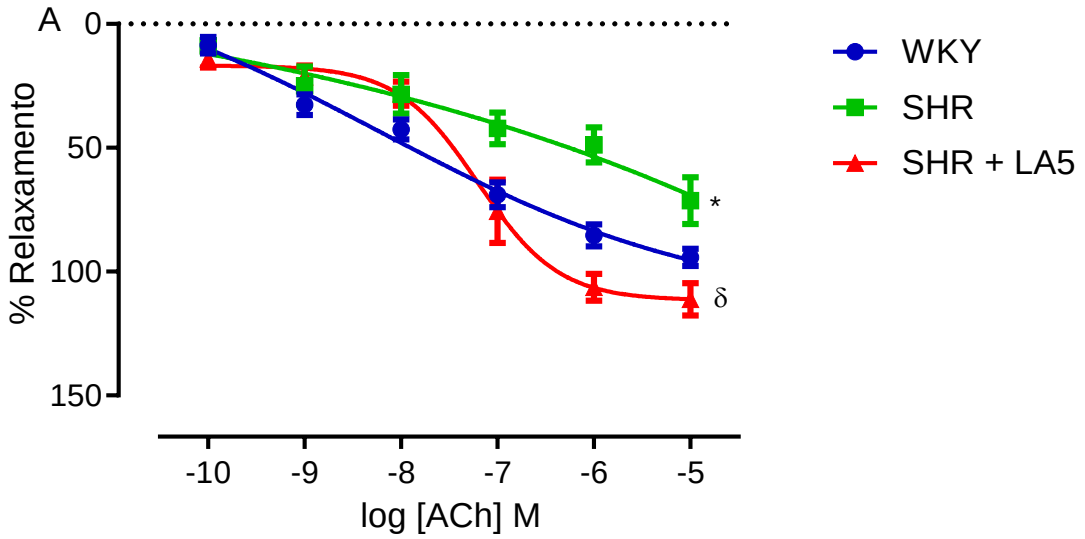
Gráfico 4 - Efeito da suplementação oral de *L. acidophilus* LA-05 por 8 semanas no soro de animais que receberam suplementação oral com LA5. Grupos: Controle normotenso (WKY, n=6); controle hipertenso (SHR, n=6) e SHR hipertenso suplementado com LA5 por 8 semanas de suplementados com LA5 (SHR+LA5, n=5). Valores expressos em média ± erro padrão da média e p<0,05. **versus* WKY; δ *versus* SHR.

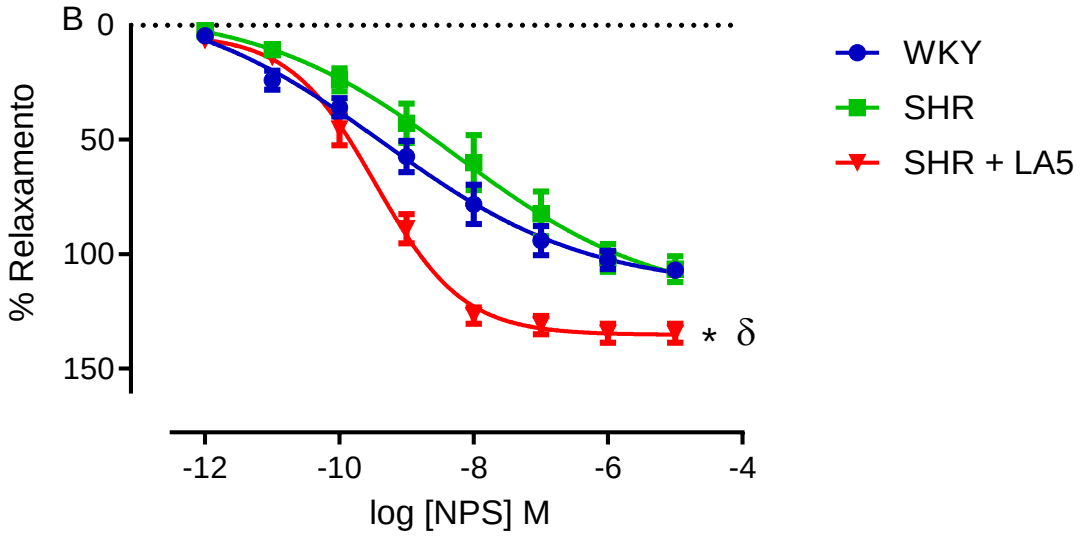


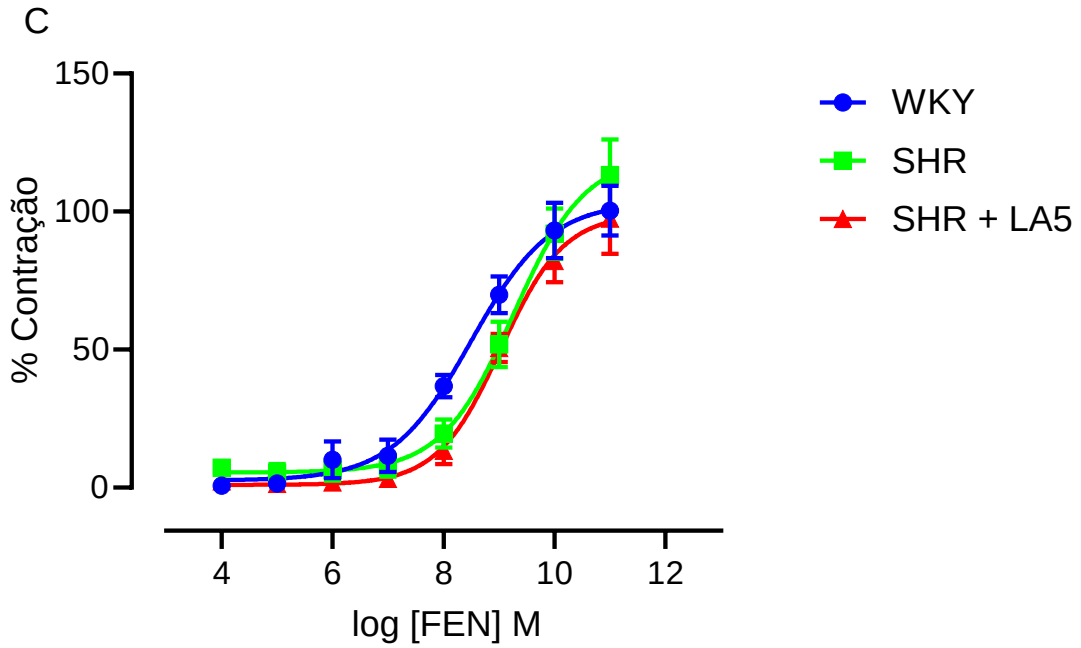
4.5 A suplementação com *L. acidophilus* LA-05 por 8 semanas melhora a reatividade vascular de animais com espontaneamente hipertensos

No Gráfico 5A é possível observar o vasorrelaxamento produzido pela ACh em anéis da artéria mesentérica superior isolados de animais normotensos e hipertensos. O grupo SHR apresentou prejuízo no perfil de relaxamento produzido pela ACh com efeito máximo ($E_{máx}$) reduzido quando comparado ao grupo WKY ($E_{máx}$: 71,35±9,37 *versus* 94,18±3,32%, $p<0,05$). Anéis isolados de animais SHR que receberam LA-5 apresentaram melhor relaxamento em resposta à ACh quando comparados ao grupo SHR não suplementado ($E_{máx}$: 111,23±6,45 versus 71,35±9,37%, $p<0,05$). No Gráfico 5B é possível observar o efeito vasorelaxante induzido pelo SNP nos anéis mesentéricos isolados de ratos normotensos e hipertensos que receberam ou não LA5 via oral por 8 semanas. Os grupos controle (WKY e SHR) não apresentaram diferenças significativas em relação ao $E_{máx}$ induzido pelo NPS ($E_{máx}$: 106,95±2,73 versus 106,41±5,52%, respectivamente). Curiosamente, a suplementação com LA5 foi capaz de melhorar o efeito relaxante máximo induzido pelo NPS quando comparado os grupos WKY e SHR não tratado ($E_{máx}$: 134,40±4,02 versus 106,95±2,74 e 106,41± 5,52%, $p<0,05$). No Gráfico 5C é possível observar o percentual de contração na presença de Phe adicionada cumulativamente em anéis sem endotélio funcional. Nota-se que não houve diferença no perfil de contração entre os grupos WKY e SHR (100,2±9%*versus*113,1±12,9%). LA5 não teve impacto na reatividade vascular a FEN em ratos SHR (97,4±12,7%).

Gráfico 5 – Efeito do relaxamento promovido pela concentração cumulativa de acetilcolina (ACh) (A) em anéis mesentéricos e relaxamento promovido pela concentração cumulativa de nitroprussiato de sódio (NPS). (B) em anéis mesentéricos de animais suplementados com LA5 por 8 semanas via oral. Efeito da contração promovida pela concentração cumulativa de fenilefrina (FEN) nos anéis mesentéricos (C). Grupos: Controle normotenso (WKY, n=6); controle hipertenso (SHR, n=6) e SHR hipertenso suplementado com LA5 por 8 semanas de suplementação oral com LA5 (SHR+LA5, n=5). Valores expressos em média ± erro padrão da média e p<0,05. * *versus* CTL; δ *versus* SHR.






C
150
100
50
0
% Contração
4
6
8
10
12
log [FEN] M
WKY
SHR
SHR + LA5

Discussão

5. Discussão

No presente estudo descobrimos que a suplementação oral com o LA5 em ratos espontaneamente hipertensos (SHR) por 8 semanas reduziu a PAD sem alterar os níveis de PAS, PAM ou FC; reduziu as ondas LF da análise da VFC; impediu o comprometimento da sensibilidade do barorreflexo; e melhorou o estresse oxidativo e a reatividade vascular à ACh e NPS.

Este foi o primeiro estudo avaliando o efeito da cepa *L. acidophilus* LA-5 em ratos SHR. O modelo SHR é o que mais se assemelha à hipertensão essencial humana, com natureza multifatorial e múltiplas vias envolvidas no desenvolvimento da hipertensão. A PA dos SHR começa a aumentar na 5ª semana de vida, atingindo níveis considerados como hipertensão espontânea entre a 7ª e a 15ª semana (Fazan *et al.*, 2001). Realizamos suplementação de LA5 a partir da 4ª semana de vida dos ratos como estratégia preventiva contra o processo hipertensivo. Apesar de impedir o aumento da PAD, o consumo oral de LA5 por 8 semanas não foi capaz de prevenir o aparecimento da hipertensão, como podemos observar pelo aumento dos níveis de pressão arterial média e pressão arterial sistólica no grupo SHR+LA5. Resultados opostos em PAM e PAS foram encontrados com o uso de leite de soja fermentado por cinco bactérias lácticas, incluindo cepas de *L. acidophilus* em SHR de 7 semanas de idade tratados pelo mesmo período (Tsai *et al.*, 2006). Nesse estudo, o efeito anti-hipertensivo não pôde ser atribuído apenas à espécie *L. acidophilus*, pois vários probióticos foram utilizados, promovendo possivelmente uma ação sinérgica, o que não foi possível em nosso estudo.

Além disso, os efeitos na PA induzidos por produtos probióticos demonstraram ser dependentes da cepa (Gadelha *et al.*, 2022). Reforçando essa ideia, cepas como *L. fermentum* CECT5716 mais *Bifidobacterium breve* CECT7263 (Robles-Vera *et al.*, 2020) ou *L. casei* C1 (Yap *et al*., 2016) administradas a ratos jovens SHR por 13 ou 08 semanas, respectivamente, foram capazes de reduzir PAM e PAS. Apesar de atenuar o aumento da PA, essas cepas também não preveniram o aparecimento da hipertensão. A incapacidade de evitar a hipertensão provavelmente se deve à complexidade dos mecanismos fisiopatológicos presentes no modelo escolhido.

Independentemente do efeito sobre os níveis pressóricos, as cepas analisadas em estudos anteriores (Robles-Vera *et al.*, 2020; Yap *et al.*, 2016) assim como o LA5 em nosso estudo têm promovido efeitos cardioprotetores em condições hipertensivas.

Existe uma estreita ligação entre hiperatividade simpática e hipertensão (Grassi *et al.*, 2015). No modelo SHR, a elevação do drive simpático precede o aumento da PA e contribui diretamente para a lesão de órgãos-alvo (Dai *et al*., 2018). A capacidade dos probióticos de regular a atividade autonômica já foi descrita em alguns estudos com o gênero *Lactobacillus* em ratos Wistar alimentados com uma dieta hiperlipídica (Cavalcante *et al.*, 2019; Ferreira *et al.*, 2022; Oliveira *et al.*, 2020) e também em filhotes pertencentes à ratas expostas a dieta hiperlipídica (do Nascimento *et al.*, 2022). Estudos avaliando o efeito de cepas de *Lactobacillus* na atividade simpática de SHR ainda não haviam sido realizados. No presente estudo, verificamos que a suplementação oral com LA5 foi capaz de reduzir as ondas do componente LF da VFC em animais SHR, sugerindo que esse probiótico reduz a hiperatividade simpática presente na hipertensão. Porém as ondas do componente HF foram mantidas sem alterações nos grupos estudados, assim como a razão LF/HF.

Os mecanismos pelos quais as bactérias probióticas reduzem a atividade simpática não são totalmente compreendidos, mas as evidências sugerem que a sinalização GABAérgica (Bao *et al.*, 2016; Hussin *et al.*, 2020; Zareian *et al.*, 2015), redução da inflamação e do estresse oxidativo (Moura *et al.*, 2016; Geeta *et al.*, 2017; Cheng *et al.*, 2016; Zubcevic *et al.*, 2019) podem desempenhar um papel essencial a esse respeito. Neste estudo, verificamos que o LA-5 reduziu a peroxidação lipídica no soro dos animais pertencentes ao grupo SHA+LA5, sugerindo que a redução do estresse oxidativo contribui, pelo menos em parte, para a diminuição da atividade simpática presente nos animais hipertensos. Não investigamos o efeito do LA5 na inflamação ou na sinalização GABAérgica. Estudos futuros são necessários para investigar o envolvimento desses últimos mecanismos no efeito simpatolítico observado após a suplementação com LA5.

Estudos mostram que o aumento do estresse oxidativo central e periférico está associado com a hiperatividade simpática e com hipertensão (Braga *et al.*, 2011). Muitas cepas do gênero *Lactobacillus* possuem atividade

antioxidante e anti-hipertensiva comprovadas, a exemplo do *L. fermentum* CECT5716 (Toral *et al.,* 2018); *L. paracasei subsp. paracasei* NTU 101, *L. plantarum* NTU 102 (Liu e Pan 2010; Liu *et al.,* 2011), *L. plantarum* MNZR (Zareian *et al.,* 2015) e *L. plantarum* TWK10 (Liu, Chiou e Tsai 2016). Os mecanismos pelos quais os probióticos exercem seus efeitos antioxidantes incluem aumento da expressão e/ou atividade de enzimas antioxidantes como superóxido dismutase, glutationa peroxidase e catalase (Chiu *et al.*, 2007; Liu, Chiou e Tsai 2016; Moura *et al.*, 2016; Zareian *et al.*, 2015), além da eliminação de radicais livres que, se acredita, ocorrer por peptídeos antioxidantes produzidos durante a fermentação (Geeta e Yadav 2017; Lee *et al.*, 2010; Liu *et al.,* 2011; Zareian *et al.,* 2015).

Vale ressaltar que a inibição da ECA, uma atividade demonstrada por diversas cepas do gênero *Lactobacillus*, em teoria pode reduzir o estresse oxidativo por reduzir a sinalização angiotensinérgica. Sabe-se que a Ang II, ao se ligar ao seu AT_1R, ativa anicotinamida adenina dinucleotídeo fosfato(NADPH) oxidase, elevando os níveis de espécies reativas de oxigênio nas células, esta via tem sido relacionada à vasoconstrição, hiperatividade simpática entre outros eventos relacionados ao aumento da pressão arterial (Mais *et al.,* 2019). Assim, o sinergismo entre a inibição da ECA e outros mecanismos antioxidantes pode explicar em grande parte o potencial anti-hipertensivo de algumas cepas pertencentes ao gênero. Todavia, os estudos que avaliaram esses mecanismos utilizaram matrizes alimentícias fermentadas ou metabolitos derivados do processo fermentativo com cepas do gênero *Lactobacillus*. Os mecanismos envolvidos na redução do estresse oxidativo promovido pela administração da cepa isolada ainda carece de investigação.

A hiperatividade simpática e o estresse oxidativo contribuem para a disfunção do barorreflexo. Na hipertensão, o barorreflexo é disfuncional, com redução de sua sensibilidade, ou seja, para que os mecanorreceptores possam ser ativados e culmine em uma resposta compensatória, uma maior variação na pressão sanguínea seria necessária. Curiosamente, os animais suplementados com LA-5 via oral por 08 semanas apresentaram melhora na sensibilidade do barorreflexo. A capacidade antioxidante do LA-5 pode contribuir para o efeito observado. Ao contrário, *L. fermentum* 296, isoladamente ou em mistura (*L. fermentum* 139, *L. fermentum* 263 e *L.*

fermentum 296) reduziu a atividade simpática em animais com hipertensão, mas não alterou a sensibilidade barorreflexa (Cavalcante *et al.*, 2019; Ferreira *et al.*, 2022; Oliveira *et al.*, 2020), confirmando que os efeitos cardiovasculares induzidos por bactérias probióticas são dependentes da cepa.

A hipertensão está intimamente relacionada à disfunção endotelial, comumente reconhecida pela perda da capacidade endotelial de desempenhar suas funções na modulação do tônus vascular (Dinh *et al.*, 2004). Um fator importante para a disfunção endotelial envolve o aumento da produção de espécies reativas de oxigênio (ROS) e diminuição dos níveis de óxido nítrico (NO), prejudicando o relaxamento das células musculares lisas vasculares (Tschudi *et al.*, 1996; Roberts *et al.*, 2005; Kobayasi *et al.*, 2010). Como esperado, o grupo SHR apresentou uma atenuação da resposta à ACh, indicando um prejuízo no funcionamento da camada endotelial na produção de vasorrelaxamento. O grupo suplementado com LA5 apresentou melhor resposta relaxante à ACh, indicando que o probiótico preveniu a disfunção endotelial relacionada à hipertensão. Da mesma forma, a cepa *L. fermentum* CECT5716 preveniu a disfunção endotelial em um modelo de lúpus genético (Toral *et al.*, 2019) ou induzido pela ativação de TLR-7 (de laVisitación *et al.*, 2021); na hipertensão induzida por tacrolimus (Toral *et al.*, 2018) e no modelo SHR (Robles-Vera *et al.*, 2020).

Além de melhorar o relaxamento em resposta à ACh, encontramos uma melhora na resposta relaxante ao doador de NO, SNP, nos vasos isolados do grupo SHR+LA5, indicando que o LA5 melhora a sensibilidade da camada muscular lisa ao NO. No geral, a suplementação oral com LA-5 por 8 semanas preveniu o comprometimento relacionado à hipertensão na função vascular. Embora não tenhamos avaliado os níveis séricos de NO, levantamos a hipótese de que a redução do estresse oxidativo contribuiu para o aumento da biodisponibilidade do NO. Esse evento está relacionado à melhora da função endotelial e diminuição da PAD observada no grupo SHR+LA5. Não houve diferença significativa na reatividade vascular à FEN entre os grupos.

De modo geral, a cepa *L.acidophilus* LA-05, apesar de não se mostrar um agente com potencial anti-hipertensivo, apresentou resultados positivos no sistema cardiovascular de animais com hipertensão espontânea, uma vez que melhorou a sensibilidade do barorreflexo, reduziu a hiperatividade simpática,

reduziu o estresse oxidativo, melhorou a reatividade vascular e ainda reduziu modestamente a pressão arterial diastólica. Outros mecanismos anti-hipertensivos atribuídos ao gênero, como inibição da ECA e aumento da sinalização GABAérgica, se deu com base em estudos relacionando as ações do *Lactobacillus* às matrizes alimentícias gerando produtos ativos. Porém, pelos dados obtidos, não se pode descartar a habilidade da administração da cepa em induzir esses mecanismos de ação anti-hipertensivos, mas para isso mais estudos seriam necessários inúmeros estudos foram realizados com diferentes espécies do gênero *Lactobacillus* na HA, pouquíssimos avaliaram o *L. acidophilus*. Portanto, o presente trabalho amplia nosso conhecimento sobre o impacto da *L. acidophilus*, especificamente da cepa LA-5, na hipertensão.

Conclusão

6. Conclusão

Nossos dados demonstram que o consumo de *L. acidophilus* LA-5, na dose de 10^9 UFC, por oito semanas, reduziu a hiperatividade simpática, restaurou a sensibilidade do barorreflexa e melhorou a reatividade vascular em ratos espontaneamente hipertensos. Esses efeitos foram acompanhados por uma redução modesta na pressão arterial diastólica nesses animais. A diminuição do estresse oxidativo periférico contribui, pelo menos em parte, para os efeitos cardiovasculares observados. Esses resultados indicam que a suplementação com LA5 pode ser um adjuvante eficaz na prevenção de parâmetros cardiovasculares importantes na prevenção da HA. Em conjunto, esses achados nos estimulam a realizar novos estudos a fim de aprofundar os conhecimentos sobre os mecanismos pelos quais a cepa LA5 exerce os efeitos demonstrados. Ensaios clínicos também podem ser realizados para avaliar o potencial preventivo ou terapêutico do LA5 em pacientes hipertensos.

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Anexos

Anexos

Anexo 1 – Certificado de aprovação do CEUA (nº 4821160718) para desenvolvimento do projeto com uso de animais

CERTIFICADO

Certificamos que a proposta intitulada "Avaliação dos efeitos da suplementação com Lactobacillus acidophilus (LA □ 05) nos parâmetros cardiovasculares de animais normotensos e hipertensos", protocolada sob o CEUA nº 4821160718 (ID 000423), sob a responsabilidade de **Maria do Socorro França Falcão** - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Universidade Federal da Paraíba (CEUA/UFPB) na reunião de 01/11/2018.

We certify that the proposal "Evaluation of the effects of Lactobacillus acidophilus (LA - 05) supplementation on the cardiovascular parameters of normotensive and hypertensive ", utilizing 64 Heterogenics rats (64 males), protocol number CEUA 4821160718 (ID 000423), under the responsibility of **Maria do Socorro França Falcão** - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the Federal University of Paraíba (CEUA/UFPB) in the meeting of 11/01/2018.

Finalidade da Proposta: Pesquisa (Acadêmica)

Vigência da Proposta: de 08/2018 a 02/2022 Área: Ciências Farmacêuticas

Origem:	Unidade de Produção Animal IPeFarM						
Espécie:	Ratos heterogênicos	sexo:	Machos	idade:	6 a 8 semanas	N:	64
Linhagem:	Rattus Norvegicus - Wistar			Peso:	180 a 200 g		

Local do experimento: Laboratório de Controle Neural da Circulação e Hipertensão Arterial

João Pessoa, 04 de setembro de 2020

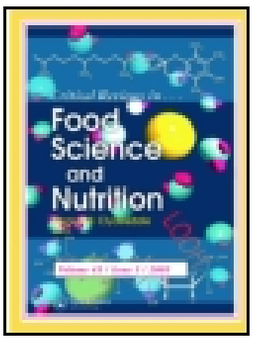
Profa. Dra. Jailane de Souza Aquino
Coordenadora da Comissão de Ética no Uso de Animais
Universidade Federal da Paraíba

Prof. Dr. Carlos Augusto Alanis Clemente
Vice-Coordenador da Comissão de Ética no Uso de Animais
Universidade Federal da Paraíba

Apêndice

Apêndice

Autor do trabalho intitulado **“***Lactobacillus* group and arterial hypertension: a broad review on effects and proposed mechanisms” publicado na revista Critical Reviews in Food Science and Nutrition com fator de impacto 11,208 e Qualis A1 para Farmácia pela Plataforma Sucupira da CAPES.



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REVIEW

Lactobacillus group and arterial hypertension: A broad review on effects and proposed mechanisms

Danilo Duarte de Assis Gadelha[a], José Luiz de Brito Alves[a], Paulo César Trindade da Costa[a], Mickael Sousa da Luz[b], Clênia de Oliveira Cavalcanti[b], Fabrícia França Bezerril[c], Juliana Franco Almeida[b], Josiane de Campos Cruz[b], Marciane Magnani[c], Camille Moura Balarini[a], Sandra Rodrigues Mascarenhas[b], Valdir de Andrade Braga[b] and Maria do Socorro de França-Falcão[b]

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ABSTRACT

Hypertension is the leading risk factor for cardiovascular diseases and is associated with intestinal dysbiosis with a decrease in beneficial microbiota. Probiotics can positively modulate the impaired microbiota and impart benefits to the cardiovascular system. Among them, the emended *Lactobacillus* has stood out as a microorganism capable of reducing blood pressure, being the target of several studies focused on managing hypertension. This review aimed to present the potential of *Lactobacillus* as an antihypertensive non-pharmacological strategy. We will address preclinical and clinical studies that support this proposal and the mechanisms of action by which these microorganisms reduce blood pressure or prevent its elevation.

KEYWORDS

ACE inhibition; antihypertensive; blood pressure; probiotic

Introduction

Systemic arterial hypertension (SAH) is a chronic, asymptomatic, silent disease that occurs because of a sustained rise in blood pressure (BP) levels. This disorder is associated with cardiovascular and metabolic disorders and increased mortality rates globally. The complexity of physiological BP control makes hypertension treatment difficult, and adequate control of BP often requires multiple medications (Noubiap et al. 2019). This can increase the number of side effects and decrease patient adherence to treatment, reinforcing the importance of non-pharmacological strategies in SAH management, including a healthy diet and physical activity (Mancia et al. 2014).

Recent studies have demonstrated that pathophysiological alterations in the gut microbiota may be associated with SAH (Cook and Chappell 2021; Muralitharan et al. 2020; T. Yang et al. 2018). Gut dysbiosis, a condition characterized by the loss of microbial homeostasis (i.e., microbial diversity and richness) and alteration of gut microbiota functionality, can promote sympathetic overactivity, low-grade systemic inflammation, endothelial dysfunction, and BP increase (Muralitharan et al. 2020; Santisteban et al. 2017; Toral et al. 2019a). On the contrary, SAH can also favor dysbiosis, which can contribute to the progression of the hypertensive state (Konopelski et al. 2021; Qin et al. 2018). From this perspective, microbiota-targeted therapies with probiotic have received specific attention as a safe approach to prevent and treat SAH (Aoyagi et al. 2017; Chi et al. 2020; Qi, Nie, and Zhang 2020).

Among the potential probiotic strains with antihypertensive properties, those belonging to the *Lactobacillus* genus have been highlighted to be involved in BP decrease. Although several studies have demonstrated the antihypertensive properties of *Lactobacillus*, an in-depth evidence based on preclinical and clinical findings focusing on hypertension therapy using *Lactobacillus* strains has not been found in the available literature.

Recently, the genus *Lactobacillus* has been reclassified owing to its enormous genomic diversity of more than 260 species. A total of 25 genera, including the emended *Lactobacillus*, were defined based on phenotypic, genotypic, and ecological features (Salvetti et al. 2018; Zheng et al. 2020). It is worth mentioning that the renaming of most *Lactobacilli* may confuse scientific reports about their biological effects since some names have mainly been used for decades. To reduce the shortcomings, the former name should always accompany the new name among the bracts (Pimentel et al. 2022), and we have adopted this format in the present manuscript.

This review provides a broad compilation of studies regarding *Lactobacillus* (and associated genera) strains with antihypertensive properties, as well as the dose and time interventions carried out using animal models and clinical trials. We have also discussed the potential underlying mechanisms and gaps that need to be addressed in future studies. This manuscript serves as a relevant, up-to-date, and well-cataloged reference for SAH therapy using strains of the *Lactobacillus* group.

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Gut dysbiosis and arterial hypertension

The human gastrointestinal tract is a complex ecosystem densely colonized by hundreds of bacterial species (Lloyd-Price et al. 2017). In addition, approximately 10-100 trillion of these microorganisms and their metabolites comprise the gut microbiota, which has 100 times more genes than the human genome (Ley, Peterson, and Gordon 2006). In healthy human beings, the gut microbiota mainly consists of the bacterial phyla *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, *Proteobacteria*, and *Tenericutes*. The phyla *Fusobacteria*, *Saccharibacteria*, *Spirochaetota*, *Synergistetes*, and *Verrucomicrobia* are present in less abundance (Almeida et al. 2019).

The specific composition of the gut microbiota varies between individuals and is influenced by many factors, including changes in diet, age, sex, and drug and antibiotic use (Dixit et al. 2021). The diversity and stability of the gut microbiota and the set of microbial interactions and symbiotic relationships substantially regulate diverse physiological functions in the host, such as immune system signaling, inflammatory responses, nutrient metabolism, and intestinal barrier regulation (Das and Nair 2019). Gut dysbiosis occurs when there is a decrease in the diversity and stability of the microbial population, with a reduction in the number of beneficial microorganisms and an increase in the number of pathobionts (Petersen and Round 2014).

The first strong evidence of the link between gut dysbiosis and SAH was shown in two independent studies published in 2015, wherein significant differences were demonstrated in the microbiota of hypertensive rats and a small cohort of hypertensive humans when compared with their respective controls (Mell et al. 2015; T. Yang et al. 2015). Recent clinical trials have confirmed differences in the microbiota between hypertensive and normotensive humans (Silveira-Nunes et al. 2020; Palmu, Lahti, and Niiranen 2021).

In this context, some mechanisms are essential for the development of SAH, including hyperactivation of the brain-gut-bone marrow and brain-gut-kidney axes, changes in short-chain fatty acids (SCFAs), and neurotransmitter production (Vallianou, Geladari, and Kounatidis 2020). The brain-gut-bone marrow axis contributes to local inflammation and several immune responses through hematopoietic stem cells that might migrate to the brain or gut, enhancing sympathetic activity (Vallianou, Geladari, and Kounatidis 2020). The brain-gut-kidney axis can activate renal sympathetic nerve activity and influence renal physiology by altering body fluid balance and plasma metabolite secretion and retention (Vallianou, Geladari, and Kounatidis 2020). Under hypertensive conditions, disruption of the integrity of the intestinal barrier and decreased SCFA-producing bacteria facilitate lipopolysaccharide translocation, low-grade inflammation, and increased oxidative stress (Lewis and Taylor 2020; Miranda et al. 2018; Santisteban et al. 2017; S. Kim et al. 2018), which can cause damage to critical organs for BP control and favor the development of hypertension (Cavalcanti Neto et al. 2018).

Some neurotransmitters, such as serotonin, dopamine, and norepinephrine, associated with gut dysbiosis, may promote autonomic nervous system dysfunction. The effects of these neurotransmitters could be exerted locally through the modulation of the intestinal vagal afferent feedback to the cardio-regulatory regions of the brain and centrally through the modulation of the efferent sympathovagal tone (Zubcevic et al. 2019). In addition, gut dysbiosis promotes an imbalance in nitric oxide (NO) and endothelin (ET) levels (Peluso et al. 2012; Vallianou, Geladari, and Kounatidis 2020), contributing to endothelial dysfunction and hypertension. Thus, these findings confirm the existence of a close link between dysbiosis and SAH, which is a complex relationship that involves several organs and signaling pathways and is influenced by both the microbiota and the host.

In the SAH has been found a reduction in the levels of *Lactobacillus* in the gut (Palmu et al. 2020; Razavi et al. 2019) and the administration of some *Lactobacillus* strains can improve the gut microbiota composition and exert antihypertensive effects in both animals and humans, as compiled in some recent reviews (Grylls, Seidler, and Neil 2021; J. Liu et al. 2020; Lugito et al. 2022; Qi, Nie, and Zhang 2020). The following sections present preclinical and clinical studies evaluating the effects of *Lactobacillus* strains on BP modulation.

Preclinical studies

The effect of *Lactobacillus* and its associated genera on BP has been shown in normotensive and hypertensive animals. Some experimental models of hypertension have been used so far, mainly in rats with spontaneous hypertension (SHR model). The age of the SHR animals, duration of treatment, evaluation methods, and strains varied; however, in all the studies, *Lactobacillus* strains were able to reduce BP in hypertensive animals. The central preclinical studies involving *Lactobacillus* strains in BP modulation are described below. Table 1 describes the methodological details and results, with some suggested mechanisms of action for the observed effect.

Lactobacillus helveticus

Lactobacillus helveticus has often been distinguished for its ability to hydrolyze proteins present in milk or other foods, generating bioactive peptides capable of lowering BP in animals and humans, mainly by inhibiting angiotensin II converting enzyme (ACE) (D. Pan, Luo, and Tanokura 2005; Yamamoto, Akino, and Takano 1993; Yamamoto, Maeno, and Takano 1999). The first study in this context was carried out by Yamamoto, Akino, and Takano in 1993, who purified a cell-wall-associated proteinase from *L. helveticus* CP790 (strain used to produce a fermented milk product in Japan) that could cleave casein isoforms. Some of the peptides generated showed the ability to inhibit ACE and reduce BP when hydrolysates of casein, fermented milk (Yamamoto, Akino, and Takano 1994), or isolated peptides (D. Pan, Luo, and Tanokura 2005) were administered orally to SHR rats.

Table 1. Preclinical studies assessing the effects of the *Lactobacillus* group on blood pressure of normotensive and hypertensive animals.

Strain tested	Models	Treatments	Main results	Suggested mechanism for the BP results	References
Lactobacillus helveticus CP790	SHR rats 20–23 weeks	Hydrolysate of casein or fermented milk (5 mg of peptides/kg) by gastric intubation were kept for 5 min.	Reduction of systolic blood pressure.	ACE inhibition.	Yamamoto, Akino, and Takano (1994)
Lactobacillus helveticus CPN4	SHR rats 18–21 weeks	Hydrolysate of casein or fermented milk (1 mg of peptides Tyr-Pro/kg) by gastric intubation were kept for 5 min.	Reduction of systolic blood pressure.	ACE inhibition.	Yamamoto, Maeno, and Takano (1999)
Lactobacillus helveticus and *Saccharomyces cerevisiae* (Calpis Milk, Calpis Food Industry Co., Ltd., Tokyo, Japan)	SHR rats 20 weeks	Single oral administration of the sour milk containing peptides VPP e IPP (5 ml/kg of BW); or peptides VPP and IPP in single (0.6 and 0.3 3 mg/kg, respectively) or serial doses.	Reduction of systolic blood pressure.	ACE inhibition.	Y. Nakamura et al. (1995)
Lactobacillus helveticus R211 and R389	SHR rats 18 weeks	Single dose in different days. Day 1: PBS; Day 2: 0.5 g/kg; Day 3; 1.0 g/kg; Day 5: 2.5 g/kgby gastric intubation.	Reduction blood pressure at 6 and 7 hours	ACE inhibition	Leclerc et al. (2002)
Lactobacillus helveticus LBK16H	SHR rats 6 weeks	Administration of IPP and VPPdissolved in drinking water or a sour milk containing these peptidesby 12 weeks.	Reduction blood pressure	ACE inhibition.	Sipola et al. (2001)
Lactobacillus helveticus LBK16H (milk A); *Lactobacillus helveticus* and *Saccharomyces cerevisiae* (milk B)	SHR rats 6 weeks	14 weeks by gavage with fermented milk containing VPP and IPP	Both milks reduced the BP of SHR rats. Milk A had a higher amount of VPP and IPP peptides and a better hypotensive effect, showing that peptides VPP and IPP are responsible by reduce blood pressure.	ACE inhibition.	Sipola et al. (2002)
Lactobacillus helveticus CHCC637 and *Lactobacillus helveticus* CHCC641	SHR rats 12 weeks	Fermented milk with each strain (10 ml/kg of body weight) by gavage - single dose.	After 4 hours it was found that the MAP of the rats had been reduced.	ACE inhibition.	Fuglsang, Nilsson, and Nyborg (2002)
Lactobacillus helveticus CHCC637 *and L. helveticus* CHCC641	Sprague Dawley rats	Fermented milk with each strain (10 ml/kg of body weight) by gastric intubation - single dose before Ang II infusion (0.3 mg/kg).	*L. helveticus* CHCC637 reduced blood pressure. Boths strains produced substances able to inhibition ACE.	ACE inhibition	Fuglsang et al. (2003)
Lactobacillus helveticus JCM1004	SHR rats 18 weeks	Single administration of doses of 1.80 and 3.60 mg of VPP or 1.20 and 1.80 mg of IPP per kg of body weight dissolved in PBS.	Reduction of systolic blood pressure.	ACE inhibition	D. Pan, Luo, and Tanokura (2005)
Lactobacillus helveticus IMAU 60208 or H9	SHR rats 8 or 15 weeks	Single oral dose or long-term administration of fermented milk over 7 weeks	Reduction of SAP, DAS and MAP after single dose administration and 2 weeks after long-term administration.	ACE inhibition.	Chen et al. (2014)
*Limosilactobacillus fermentum*296	Wistar rats fed with HFD	Oral administration of 10^9 CFU/mL/day for 4 weeks.	Reduction of systolic blood pressure. Reduction of total cholesterol, triglycerides and LDL.	Decreased sympathetic hyperactivity	Cavalcante et al. (2019)
*Limosilactobacillus fermentum*139, 263, and 296	Wistar rats fed with HFD	Administration of 1 mL of solution containing 10^9 CFU/mL of each strain for 4 weeks.	Reduction of blood pressure; mitigation of insulin resistance and hyperlipidemia.	Decreased sympathetic hyperactivity	Ferreira et al. (2022)
Limosilactobacillus fermentum 139, 263 and 296	Rat offspring exposed to a maternal HFD	Administration of 1 mL of solution (ratio 1:1:1, 1 x 10^9 CFU/mL) by oral gavage for 8 weeks.	Reduction of blood pressure. Reduction of total cholesterol and increase in HDL-c levels.	Decreased sympathetic hyperactivity	Oliveira et al. (2020)
Limosilactobacillus fermentum CECT5716	Female NZBWF1 mice Genetic SLE model	Administration of solution containing 5 x 10^8 CFU/day by oral gavage for 13 weeks.	Attenuated the BP increase, decreased endotoxemia.	Decresead renal oxidative stress, inflammation	de la Visitación et al. (2020)

(*Continued*)

Table 1. (Continued).

Straintested	Models	Treatments	Main results	Suggested mechanism for the BP results	References
Limosilactobacillus fermentum CECT5716	Female NZBWF1 mice Genetic SLE model	Administration of solution containing 5 x 10^8 CFU/day by oral gavage for 15 weeks.	Prevented hypertension in SLE model by improvement of gut microbiota and endothelial function.	Decreased plasma levels of pro inflammatory cytokines TNF-α, IFN-γ, IL-17a, and IL-21. Reduced vascular Th17 infiltration.	Toral et al. (2019b)
Limosilactobacillus fermentum 139, 263 and 296	Rat offspring exposed to a maternal HFD	Administration of *L. fermentum* formulation twice a day in a solution of approximately 3×10^9 CFU/mL by oral gavage for 4 weeks.	Reduction of blood pressure and oxidative stress; improvement of the renal function and alleviated renal dysfunction.	Increased SOD activity in renal córtex. Reduced of LPS-translocation, inflammation, sympathetic activity.	Do Nascimento et al. (2022)
Limosilactobacillus fermentum CECT5716 and *Bifidobacterium breve* CECT7263	SHR rats 05 weeks	Administration of 1 mL of solution containing 10^9 CFU/mL by oral gavage for 13 weeks	Prevent high blood pressure in genetic hypertension, development of endothelial dysfunction and dysbiosis.	Improved gut integrity with the subsequent reduced translocation of bacterial endotoxin into the circulation.	Robles-Vera et al. (2020)
Limosilactobacillus fermentum CECT5716 (LC40) and/or *Bifidobacterium breve* CECT7263 (BFM)	BALB/cByJRj mice Imiquimod-induced SLE	Administration LC40 (10^9 CFU/mL) and BFM (10^9 CFU/mL) by oral gavage once a day for 8 weeks.	Prevented hypertension and endothelial dysfunction in this lupus model induced by TLR-7 activation.	Decrease in O_2^- levels. Reduced Th17 and high Treg populations	de la Visitación et al. (2021)
Limosilactobacillus fermentum CECT5716	L-NAME rats	Administration LC40 (10^9 CFU/mL/day) by oral gavage for 4 weeks.	Did not prevent the hypertension, but prevented gut dysbiosis.	Decreased oxidative stress.	Robles-Vera et al. (2018)
Limosilactobacillus fermentum CECT5716	C57Bl/6J mice Tacrolimus-induced hypertension	Strain incorporated in drinking water and prepared daily, at a dose of 5 x 10^8 CFU/day/mouse, during 15 days. From the 8thdaytacrolimus was also administered.	Prevented the hypertension and endothelial dysfunction induced by tacrolimus by inhibiting gut dysbiosis.		Toral et al. (2018)
Limosilactobacillus fermentum CECT5716 LC40, or *Loigolactobacillus coryniformis* K8 + *Lactobacillu sgasseri* LC9	SHR rats 12 weeks	Oral administration of 3.3×10^{10} CFU/day by 5 weeks.	Reduction in SBP. Reduces cardiac and renal hypertrophy (K8/LC9)	Decreased oxidative stress and inflammation.	Gomez-Guzmán et al. (2015)
Lactiplantibacillus plantarum MNZ	SHR rats 10 weeks	Free GABA-enriched diet containing 4.36×10^8 CFU/mL for 10 weeks	Reduction of systolic blood pressure. Reduction in ET1 expression. Reduction of free radicals.	ACE inhibition	Zareian et al. (2015)
Lactiplantibacillus plantarum QS670	SHR rats	Single intragastric doses (5, 10, and 20 mL/kg) or chronic oral administration of 10 mg/mL/day (10^8 CFU/ml) for 4 weeks.	Reduction of Blood pressure in both assays.	ACE inhibition.	Xia et al. (2020)
Lactiplantibacillus plantarum HEAL19	L-NAME rats	2 g/rat/day of product fermented blueberries with *L. plantarum* HEAL19 10^9 CFU/animal/day for 4 weeks	Reduction blood pressure in 2 weeks.		Xu et al. (2013)
Recombinant *Lactiplantibacillus plantarum* NC8 (RLP)	SHR rats 12 weeks	One dose of 2×10^{11} CFU for 14 continuous days orally for 2 weeks.	Reduction of blood pressure.	ACE inhibition. Decrease TG, ET and Ang II. Increase NO level.	G. Yang, Jiang, et al. (2015)
Lactiplantibacillus plantarum DSM 15313	L-NAME rats	2 g/rat/day of blueberry powder with 10^9 CFU/day for 2 and 4 weeks	Reduction of blood pressure.		Ahrén et al. (2015)
Lactiplantibacillus plantarum TWK10	Wistar rat DOCA hypertension	TWK10-fermented soymilk water was dissolved in distilled water and administered orally by gastric intubation 450 mL/day/person (weight, 65 kg; height, 170 cm), which was converted to animal equivalent doses using the body surface area formula. Treatment of 11 weeks.	Improve learning and memory in DOCA-salt hypertension-induced VaD rats by acting as a blood pressure-lowering and neuroprotective agent.	ACE inhibition and NO production.	T. Liu, Chiou, and Tsai (2016)

(*Continued*)

Table 1. (Continued).

Straintested	Models	Treatments	Main results	Suggested mechanism for the BP results	References
Self-cloned *Lactiplantibacillus plantarum* Taj-Apis362	SHR rats 10 weeks	Acute oral administration of fermented yogurt plus GABA (30, 150, 300 mg/kg).	Reduction of systolic blood pressure from the 4th hour with a single dose of yogurt.	Decreased oxidative stress.	Hussin et al. (2020)
Lactiplantibacillus plantarum SR37-3 and *SR61-2*	L-NAME rats	Administration of 10 mL/kg/day milk fermented by the *Lactiplantibacillus plantarum* strain SR61-2 or SR7-3 by 4 weeks.	Reduction of systolic blood pressure, attenuate renal damage by inactivation of RAS.	ACE inhibition. Decreased Ang II, aldosterone, ET_1 and inflammatory markes (TNF-α, INF-β , IL-1β)	Yuan et al. (2022)
Lactiplantibacillus plantarum WJL	Wistar rats	Males offsprings of rats treated with 1 mL/day of suspension of L. plantarum WJL (10^9 log CFU/mL) during the pragnancy and lactation periods.	Reduction systolic and media blood pressure, improved vasomotor modulation.		K. Guimarães et al. (2020)
Lacticaseibacillus rhamnosus EBD1	SHR rats (250–300 g)	Oral administration of fermented whey (10 and 100 mg/Kg) for 6 weeks.	Reduction blood pressure from the 2nd week.	ACE inhibition	Daliri et al. (2019)
Lacticaseibacillus rhamnosus GG	Sprague–Dawley rats with obstructive sleep apnea	Oral administration of 10^9 CFU/2 mL of fermented milk for 4 weeks.	Reduction systolic blood pressure	Reduces inflammatory markers (TNF-Ɣ, TGF-ß1 and IL4)	J. Liu et al. (2019)
Lacticaseibacillus rhamnosus Lactobacillus brevis and *Enterococcus faecium*	SHRSP/Izm rats 11 weeks	Single-dose and chronic supplementation 2 g/kg (4 weeks) oral administration of prepared by dual fermentation using fungi and lactic acid bacteria with *Lacticaseibacillus rhamnosu, Lactobacillus brevis* and *Enterococcus faecium*.	Reduction of systolic blood pressure.	ACE inhibition	Alauddin et al. (2016)
Lactobacillus rhamnosus AC1	Sprague Dawley – DOCA Salt	Administration of ethanol extract (2.25 and 0.15 g/Kg) containing soymilk with *L. rhamnosus* AC1 $3x10^6$ CFU/mL by oral gavage daily for 5 weeks.	Reduction blood pressure and renal perivascular fibrosis.	Improved NO availability, reduces inflammatory markers (TNF-α and IL-6) and Ang II	Wu et al. (2022)
Lacticaseibacillus casei YIT9018	SHR rats 16 to 18 weeks for acute study 5 weeks for chronic study	Single oral administration of the extract from L. casei (LEx) – (1 to 10 mg/kg); Long term oral administration of LExfor 12 weeks(10 mg/kg once a week); Long term administrations of LEx for 3 weeks (10 mg/kg daily); Single oral administration of polysaccharide (SG-I); protein (PR-I); and nucleic acid (NA-1) fractions.	Polysaccharide portion was more efficient in reducing systolic blood pressure at 6 and 12 hours.		Furushiro et al. (1990)
Lacticaseibacillus casei YIT9019	SHR (16 – 18 weeks) and 2K1C rats (5 weeks)	Single oral administration of SG-1 (0.1 to 10 mg/kg) Single oral administration of moieties from SG-1 (5 mg/kg).	Reduction in systolic blood pressure at 6 and 12 hours at doses of 5 and 10 mg/kg.		Sawada et al. (1990)
Lacticaseibacillus casei YIT9018	SHR rats 18 weeks	Acute oral administration of 20 mg/kg – protein portions (20.6%), nucleic acids (10.3%–3.8% DNA + 6.5% RNA) and polysaccharides (18.5%).	Reduction of systolic blood pressure from 3 hours.	Increased concentration of PGI2	Furushiro et al. (1993)
Lacticaseibacillus casei CICC 20280 and 23184	SHR rats 6–8 weeks	1 mL of fermented milk + GABA for 12 days orally. The dose was 107 CFU	Reduction systolic blood pressure in SHR from the second day onwards, but did not sustain the drop throughout treatment.	ACE inhibition.	Bao and Chi (2016)

(*Continued*)

Table 1. (Continued).

Straintested	Models	Treatments	Main results	Suggested mechanism for the BP results	References
Lacticaseibacillus casei C1	SHR rats 8 weeks	A dose of 10^{11} CFU for 8 weeks orally.	Reduction systolic and diastolic blood pressure and reverts aorta remodeling.	ACE inhibition. Increase NO level.	Yap et al. (2016)
Lacticaseibacillus casei 01	Wistar rats	The suspension (1 mL) containing probiotics (9 log CFU/ mL or paraprobiotics was daily administrated by orogastric gavage for 28 days.	Improve hypertension (SAP, MAP and DAP) and cholesterol levels (total and LDL-cholesterol).		Brandão et al. (2021)
Lacticaseibacillus casei (Antibiophilus, Laboratoires Lyocentre, France)	Sprague Dawley pregnant	Administration oral of high fat plus $2x10^8$ CFU/day by 6 weeks.	Reduction systolic blood pressure.	Reduction of acetate in plasma level. Reduction renal mRNA Olfr78. Restored of reduction Ffar2	Hsu et al. (2018)
Lactobacillus casei Shirota and *Lactococcus lactis* YIT 2027	SHR rats 14 weeks	Administration of fermented low-fat milk product containing GABA (FMG). Expt1.: Single oral dose of 5 mL/Kg FMG. Expt2.: 3 weeks a diet with 100 mg/Kg (freeze-dried) FMG.	Expt1.: Reduction systolic blood pressure before 4 h administration FGM, but restored in 24 h. Expt2.: Reduction blood pressure sustaining for 10 weeks.	ACE inhibition.	Hayakawa et al. (2004)
Lactobacillus johnsonii La1	Wistar rats	Administration intraduodenal injection of LJLa1 ($1 \times 10^{8-9}$ CFU/2 ml saline).	Reduction blood pressure.	Improved parassympathetic outputs and reduction sympatthetic outputs.	Tanida et al. (2005)
Lactobacillus jensenii, *Lactobacillus acidophilus* and *Leuconostocmes enteroides* ssp. *cremoris* 358	SHR rats 14–18 weeks	Administration by gastric incubation at 4 hour intervals (0.1-100 mg/kg). After that, rats received single doses 200 mg/kg for 7 days.	Reduction blood pressure.	ACE inhibition	Pihlanto, Virtanen, and Korhonen (2010)
Lactobacillus bulgaricus and *Streptococcus thermophilus*	SHR rats 12 weeks	Gavage daily of fermented yogurt (*S. thermophilus* 6.1×10^8 CFU/ml and *L. bulgaricus* 4.8×10^8 CFU/ mL) for 7 weeks.	Reduction blood pressure, gut microbiota remodeling and increasing of short-chain fatty acids.		Kong et al. (2021)
Lacticaseibacillus paracasei subsp. *paracasei* NTU 101 and *Lactiplantibacillus plantarum* NTU 102	SHR	Experimental feeding doses of 101FM and 102FM were calculated on the basis of ACE activity and GABA concentrations.	Reduction blood pressure.	ACE inhibition.	C. Liu et al. (2011)

Similar effects were observed for the strains *L. helveticus* CPN4, a CP790 variant (Yamamoto, Maeno, and Takano 1999); *L. helveticus* CHCC637 or *L. helveticus* CHCC641 (Fuglsang, Nilsson, and Nyborg 2002); *L. helveticus* IMAU 60208 (H9) (Chen et al. 2014); *L. helveticus* R211; and R389 (Leclerc et al. 2002) and Calpis® milk containing *L. helveticus* and *Saccharomyces cerevisiae* (Y. Nakamura et al. 1995; Table 1). In all these studies, acute administration of fermented milk or isolated peptides was performed in rats (> 15 weeks of age) with established hypertension (Yamori 1984). It was possible to validate the hypotensive potential of the strains in the presence of hypertension and the possible mechanism of action for this effect; however, the assessment of an isolated impact after acute administration did not provide a solid basis to consider antihypertensive therapy with these strains.

In contrast, chronic assays were performed with six or eight-week-old SHR rats, a period in which hypertension began to develop in this model. In these animals, the use of ACE inhibitor (ACEi) peptides or milk fermented by *L. helveticus* LBK16H (Sipola et al. 2001) or Calpis® milk (Sipola et al. 2002) or *L. helveticus* IMAU 60208 (H9) (Chen et al. 2014) reduced BP levels despite not preventing the onset of hypertension. Therefore, the significant contribution of preclinical studies with *L. helveticus* was the discovery of ACEi peptides as an important hypotensive mechanism induced by the *Lactobacillus* group. They provided the basis for several clinical studies that focused on the effects of these peptides in individuals with hypertension.

Limosilactobacillus fermentum (former Lactobacillus fermentum)

The strain of *Limosilactobacillus (L.) fermentum* 296 recovered from Fragaria vesca L. (strawberry) decreased BP, sympathetic overactivity, and dyslipidemia in rats fed a high-fat

diet (HFD) (Cavalcante et al. 2019). Similar results were obtained when this strain was administered in association with *L. fermentum* 139, a strain recovered from *Mangifera indica* L. (mango), and *L. fermentum* 263 recovered from *Ananas comosus* (pineapple) (Ferreira et al. 2022). Male rat offspring exposed to a maternal HFD and treated with the same probiotic mixture showed the abovementioned effects and improved oxidative stress and kidney function (Do Nascimento et al. 2022; Oliveira et al. 2020). The cardiometabolic benefits reported for *L. fermentum* strains may be their antioxidant and anti-inflammatory properties (Freire et al. 2021a, 2021b). In all these trials, the supplementation lasted for six or eight weeks, and the results showed that besides the antihypertensive potential in the model used, these strains could improve different cardiometabolic parameters, thus expanding their potential beyond antihypertensive therapy. Thus, these data encourage the development of randomized controlled trials to assess the effects of the *L. fermentum* strain on cardiometabolic disorders.

Another well-studied strain is *L. fermentum* CECT5716 (LC40). An improvement in the gut microbiota and a decrease in BP was seen in 12-week-old SHR rats after long-term administration of LC40 for 5 weeks (Gomez-Guzmán et al. 2015). Like *L. helveticus* strains, administration of LC40 in 5-week-old SHR rats did not prevent the onset of hypertension but reduced the BP increase after 13 weeks of supplementation (Robles-Vera et al. 2020).

The preventive effect of LC40 was also shown in a model of hypertension induced by tacrolimus; in addition to a decrease in BP, there was an improvement in the microbiota and endothelial function and a reduction in intestinal inflammatory markers (Toral et al. 2018). Similar results have been reported in systemic lupus erythematosus (SLE) models, although the mean BP values of the SLE-treated group did not reach the values of the control group (de la Visitación et al. 2020, 2021; Toral et al. 2019b). The antihypertensive effect of LC40 has also been associated with its antioxidant and immunomodulatory properties (de la Visitación et al. 2020, 2021; Gomez-Guzmán et al. 2015; Robles-Vera et al. 2018; Toral et al. 2014, 2019b).

In L-NAME rats, treatment with LC40 for 4 weeks prevented gut dysbiosis, vascular oxidative stress, inflammation, and endothelial dysfunction but did not prevent hypertension induced by NOS inhibition (Robles-Vera et al. 2018). These findings show that the same strain can lead to different outcomes depending on the pathophysiological characteristics. The authors suggested that the differences in the microbiota between the hypertension models could explain the differences in the results obtained (Robles-Vera et al. 2018).

Lactiplantibacillus plantarum (former Lactobacillus plantarum)

C. Liu et al. (2011) showed the antihypertensive effects of *Lactiplantibacillus (L.) plantarum* NTU 102 (102FM), isolated from homemade Korean-style cabbage pickles (T. M. Pan, Chiu, and Guu 2002). This strain significantly decreased systolic and diastolic BP in SHR rats, and these effects were associated with ACE inhibition and gamma-aminobutyric acid (GABA) activity (C. Liu et al. 2011). Other studies have revealed this bacterium's immunomodulatory and antioxidant properties (C. Liu and Pan 2010; Chiu et al. 2007). Hypotensive and antioxidant effects were reported after the administration with *L. plantarum* MNZR in SHR rats (Zareian et al. 2015) or soymilk extract fermented with *L. plantarum* TWK10 in rats with vascular dysfunction induced by DOCA-salt hypertension (T. Liu, Chiou, and Tsai 2016). A recent study by Xia et al. (2020) showed that milk fermented with *L. plantarum* QS670 is a potential source of ACEi peptides and exerts antihypertensive effects in SHR rats after a single intragastric dose or long-term treatment for 4 weeks. Administration of *L. plantarum WJL* during pregnancy and lactation in dams fed a high-fat and high-cholesterol (HFHC) diet reduced blood pressure and recovered vascular function in male rat offspring (K. Guimarães et al. 2020).

Conflicting findings have been reported for *L. plantarum* in the L-NAME model. Xu et al. (2013) demonstrated that the intake of blueberries fermented by *L. plantarum* HEAL19 modulated the gut microbiota without lowering BP after 4 weeks of oral administration. In contrast, oral administration of blueberries fermented by *L. plantarum* DSM 15313 reduced BP in L-NAME rats 2 weeks after medication (Ahrén et al. 2015). Corroborating these data, milk fermented by *L. plantarum* strains SR37-3 and SR61-2 significantly lowered the BP of L-NAME rats and attenuated renal injury after 4 weeks of intervention (Yuan et al. 2022). These data reinforce the idea that the results are strongly strain-dependent.

It is because of the role of ACEi and GABA in the hypotensive effect of *L. plantarum* that some studies have been performed using recombinant strains focusing on these molecules, with exciting results in the cardiovascular system of animals. In a study by G. Yang et al. (2015), oral administration of *L. plantarum* expressing ACEi peptides (recombinant *L. plantarum* NC8 - RLP) in SHR rats decreased BP, endothelin (ET), Ang II production, and triglyceride levels with no observed side effects, indicating its potential application in hypertension and related diseases. Hussin et al. (2020) used self-cloned and expressed *L. plantarum* Taj-Apis362 recombinant cells with high glutamic acid decarboxylase (GAD) activity (UPMC90 and UPMC91) to improve GABA production in yogurt, which in turn had a potent antihypertensive effect in SHR rats.

Lacticaseibacillus casei (former Lactobacillus casei)

Furushiro et al. (1990) reported the antihypertensive effects of an extract of autologous *L. casei* YIT9018 cell lysate (LEx) in SHR rats after acute or chronic administration. The most effective antihypertensive compounds found in this extract were polysaccharide-glycopeptide complexes (SG-1) located in the cell wall of this bacterium, which were able to reduce systolic BP (SBP) when orally administered to SHR rats and renal hypertensive rats (Sawada

et al. 1990). Despite the reduction in BP after oral administration, there was no hypotensive effect in SHR rats after intraperitoneal and intravenous administration of SG-1 (Furushiro et al. 1993). These findings suggest the dependence of probiotic bacteria or their products on host gastrointestinal enzymes and physiological mechanisms to induce their effects.

The antihypertensive potential was also observed for *L. casei* C1, a strain isolated from probiotic drinks in Malaysia that was administered in SHR rats for 8 weeks (Yap et al. 2016); the low-fat milk product containing GABA (FMG) fermented by *L. casei* Shirota and *Lactococcus lactis* YIT 2027 that was given in SHR rats as a single dose for 3 weeks (Hayakawa et al. 2004); and fermented soybean milk (FSM) with *L. casei* strains obtained from the China Center of Industrial Culture Collection (CICC) that was administered in SHR rats for 12 days. *L. casei* CICC 20280 and 23184 lowered BP in SHR rats to an average level on the 4th day, but the BP returned to a high level until 12 days (Bao and Chi 2016). *L. casei* strains (10^7 CFU/ml) were added to soybean milk, and the mixture was fermented; however, the CFU present in the final solution was unclear. The authors also did not discuss the possible causes for the return of BP to baseline values, even with the administration of the product. Perhaps, there were a small number of strains with antihypertensive potential in the solution. Another study showed that early gut microbiota-targeted therapy with probiotic *L. casei* (Antibiophilus®) and prebiotic inulin prevented maternal HF-induced programmed hypertension in adult male rat offspring (Hsu et al. 2018).

Brandão et al. (2021) showed that the administration of live and ultrasound-inactivated *L. casei* in HFD rats induced different effects. Live and ultrasound-inactivated *L. casei* prevented the increase in total and LDL cholesterol levels, controlled insulin resistance, and modulated gut microbiota. However, only inactivated cells showed reduced BP. This study was the first to report a reduction in BP by inactivated probiotic cells. The hypotensive effect induced by this treatment is more associated with bacterial cell composition than metabolism of the bacteria when in the host. In front of the exposed, the species *L. casei* presents interesting results encouraging further studies on the described strains.

Lacticaseibacillus rhamnosus (former Lactobacillus rhamnosus)

Daliri et al. (2019) submitted soy protein for enzymatic hydrolysis and subsequent fermentation with *Lacticaseibacillus (L.) rhamnosus* EBD1, isolated from the Korean fermented soybean (doenjang) to obtain a product named P-SPI, with high levels of ACEi peptides. The authors demonstrated that P-SPI treatment for 6 weeks reduced SBP in SHR rats. Fermented rice bran (FRB) prepared by dual fermentation using *Aspergillus kawachii* and a mixture of lactic acid bacteria, including *L. rhamnosus*, *Levilactobacillus brevis*, and *Enterococcus faecium*, reduced BP and improved metabolic parameters in stroke-prone spontaneously hypertensive rats after single and chronic supplementation (Alauddin et al. 2016). In another study, *L. rhamnosus* GG mitigated the development of obstructive sleep apnea-induced hypertension in rats fed a high-salt diet (J. Liu et al. 2019). Fermentation of soymilk by *L. rhamnosus* AC1 reduced BP in DOCA-salt hypertensive rats by reducing inflammation and reverting dysbiotic microbiota (Wu et al. 2022)

Other species

Studies on *Lactobacillus johnsonii* and *Lactobacillus bulgaricus* have presented promising evidence for lowering BP. Intraduodenal injection of *L. johnsonii* (LJLa1) reduced BP and renal sympathetic nerve activity and enhanced gastric vagal nerve activity in urethane-anesthetized rats (Tanida et al. 2005). Likewise, probiotic yogurt containing *Streptococcus thermophiles* and *L. bulgaricus* exhibited antihypertensive effects in SHR rats by remodeling the gut microbiota (Kong et al. 2021). Milk fermented with 25 lactic acid bacteria including *L. casei*; *L. helveticus; L. rhamnosus*; *L. acidophilus; L. jensenii; and Limosilactobacillus reuteri* caused a transient reduction in BP in SHR rats (Pihlanto, Virtanen, and Korhonen 2010).

The Lacticaseibacillus paracasei subsp. paracasei NTU 101 (101FM) (*Lactobacillus paracasei subsp. paracasei*) strain, isolated from human feces (Lin, Chiu, and Pan 2004), decreased BP in SHR rats after single or chronic administration by ACE inhibition (C. Liu et al. 2011). This strain also reduced the disorganization of the aortic media layer (C. Liu et al. 2011) and exhibited antioxidant and immunomodulatory activities (C. Liu and Pan 2010; Tsai et al. 2008).

Several strains of the emended *Lactobacillus* genus are targets of the preclinical investigation. As discussed below, many of the described preclinical studies scientifically support the performance of clinical trials with strains with strong antihypertensive potential.

Clinical studies

Recent systematic reviews and meta-analyses have investigated the effects of *Lactobacillus* on SAH (J. Liu et al. 2020; Lugito et al. 2022). From a broader perspective, other meta-analyses have evaluated the impact of probiotics on BP, but most of the included studies involved the use of *Lactobacillus* strains (Chi et al. 2020; Qi, Nie, and Zhang 2020). All meta-analyses have concluded that *Lactobacillus* products have BP-lowering effects. The reduction reported in the meta-analysis was modest (maximum −3.05 mmHg for SBP and −1.51 mmHg for DBP); however, even a slight decrease in BP may have significant public health benefits with reduced risk for cardiovascular disease and mortality (Sleight et al. 2001).

J. Liu et al. (2020) found that the *lactobacillus*-induced BP-lowering effect is more significant when the daily dose is $>5 \times 10^9$ CFU/day, in capsule form, and a treatment duration of ≥8 weeks. In contrast, according to Lugito et al.

(2022), the decrease in BP was lower at > 8 weeks than at <8 weeks. According to Qi, Nie, and Zhang (2020), the probiotic`s BP-lowering effect could only last for a short-term period from 8 to 10 weeks. According to Chi et al. (2020), people treated for more than 4 weeks and dosage of more than 2×10^{10} CFU/day achieved a better effect on lowering BP. In the study by Lugito et al. (2022), a single supplementation had more significant effects than a multi-strain probiotic; the opposite was observed by Qi, Nie, and Zhang (2020). Chi et al. (2020) reported that the BP-lowering effect was unrelated to the number of strains.

The *Lactobacillus* group had a more significant impact on patients with borderline hypertension at baseline, suggesting that *Lactobacillus* can be used as a primary preventive strategy in the development of hypertension in these individuals (J. Liu et al. 2020). Furthermore, age (Chi et al. 2020) and ethnicity (J. Liu et al. 2020) appear to influence the effect of *Lactobacillus* on BP.

In view of the above, it is evident that *Lactobacillus* and its associated genera have the potential for use in the treatment or prevention of hypertension. The significant heterogeneity between the trials evaluated, in terms of sample size, the clinical status of the subjects, duration, dose of treatment, and the form of probiotic delivery, reinforce the need to carry out clinical trials with large samples only with hypertensive individuals and standardized treatment protocols. The analysis focused on ethnic and sex differences will also provide crucial information on the efficacy and safety of *Lactobacillus* in antihypertensive therapy.

Some studies have also shown no effect on BP after supplementation with *Lactobacillus* products (Ivey et al. 2015; Romão da Silva et al. 2020; Usinger et al. 2010; Xu et al. 2015). Since our focus is to address the effective strains in antihypertensive therapy, we have not discussed these studies. Here, we present some research using the most known strains of *Lactobacillus* and associated genera that have shown positive effects on SAH. The profiles of the patients, treatment protocols, primary findings, and suggested mechanisms are summarized in Table 2.

L. helveticus

Based on preclinical trials, clinical studies have been performed using strains of *L. helveticus* containing Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP) peptides. Early research examining normotensive and hypertensive subjects showed that the intake of powdered *L. helveticus* fermented milk tablets containing IPP and VPP reduced BP to the normal range in subjects with high–normal BP, preventing the incidence of SAH. The magnitude of the decrease in BP may be affected by hypertension status, with a more significant effect in hypertensive subjects than in non-hypertensive subjects (Aihara et al. 2005).

In the absence of antihypertensive medications, administration of *L. helveticus* LBK16H fermented milk containing IPP (7.5 mg/100 g) and VPP (10 mg/100 g) for 10 weeks reduced BP in hypertensive subjects in 24-h arterial BP measurements (ABPM), despite no differences in serum ACE activity (Jauhiainen et al. 2005). Aihara et al. (2005) suggested that despite no changes in plasma ACE activity, arterial tissue ACE activity may be reduced, as observed in SHR rats (Masuda, Nakamura, and Takano 1996). Jauhiainen et al. (2012) found similar results in BP using different concentrations of peptides in two intervention periods of 12 weeks each (IPP 1.2 and 5.8 mg/100 g; VPP 1.3 and 6.6 mg/100 g).

In addition to the antihypertensive effect, the same treatment regimens mentioned above could reduce arterial stiffness in hypertensive subjects (Jauhiainen et al. 2007a, 2010). According to the authors, the suggested mechanisms of action for the observed effects include ACE inhibition and high calcium, potassium, and magnesium concentration in the *L. helveticus* product (Jauhiainen et al. 2005, 2007a, 2010). In general, the subjects reported similar adverse events in both groups, and there were no changes in laboratory values, which is an important observation from a safety point of view. According to Jauhiainen et al. (2005, 2012), *L. helveticus* products and even a high amount of tripeptides can be considered safe alternatives for the dietary treatment of hypertension.

Using milk fermented by the same strain *L. helveticus* LBK16H but with different amounts of peptides (2.4 – 2.7 mg/1.5 dL of IPP and VPP) for 8 – 10 weeks, Tuomilehto et al. (2004) have demonstrated a modest reduction in BP in hypertensive patients, corroborating previous trials. Although these studies evaluated the antihypertensive effect of *L. helveticus* LBK16H in men and women, no sex-based differential analysis was performed. Extending the treatment period to 21 weeks also resulted in similar effects (Seppo et al. 2003).

Calpis® milk containing *L helveticus* and *Saccharomyces cerevisiae* reduced BP in elderly hypertensive patients without changes in other indices, such as heart rate, body weight, and blood serum variables (Hata et al. 1996). The antihypertensive effect of Calpis® milk was also observed in borderline hypertensive men who were not taking antihypertensive medication (Mizushima et al. 2004)

L. plantarum

In 2020, Lewis-Mikhael, Davoodvandi, and Jafarnejad published a meta-analyses of seven studies aiming to evaluate the effects of *L. plantarum* (alone or in combination with other probiotics) on BP. The duration of the trials varied from 2 to 12 weeks, and the doses varied from 1.0 x 10^9 CFU/day to 1.5 x 10^{12} CFU/day. *L. plantarum* caused a modest reduction in SBP (-1.58 mmHg) and DBP (-0.92 mmHg); this effect was more pronounced when *L. plantarum* was used alone and in individuals with established hypertension.

Notably, only two studies included in the meta-analyses had hypertensive individuals as samples (Sharafedtinov et al. 2013; Xu et al. 2015), and there were no changes in one of them (Xu et al. 2015) in the BP values after supplementation. Sharafedtinov et al. (2013) evaluated the effects of a

Table 2. Clinical studies assessing the effects of the *Lactobacillus* group on blood pressure of hypertensive patients.

Strain tested	Models	Treatments	Main results	Suggested mechanism for the BP results	References
Lactobacillus helveticus and *Saccharomyces cerevisiae*	30 hypertensive patients	100 mL sour milk containing *L. helveticus* (7×10^{11}/L) and *S. cerevisae* (2.5×10^{9}/L) containing 1.1 mg of Ile-Pro-Pro and 1.5 mg of Val-Pro-Pro (0.025 mg Ile-Pro-Pro and 0.033 mg Val-Pro-Pro/kg body wt); 8 weeks	Reduction in SBP from baseline at 4 and 8 weeks and in DBP at 8 weeks only in the treated group. These changes were maintained 4 weeks after the end of treatment.	ACE inhibition (found on cell lysates or supernatant fraction of the fermented milk)	Hata et al. (1996)
Lactobacillus helveticus LBK16H	60 mild hypertensive (36 men, 24 women)	Sour milk containing tripeptides ingestion 100 mL/day; 2.4–2.7 mg of Ile-Pro-Pro and 2.4–2.7 mg of Val-Pro-Pro for 13–17 weeks in two phases. 1th) phase: 8-10 weeks with washout period 3-4 weeks. 2th) phase: 5–7 weeks.	Reduction blood pressure with more pronounced effect on systolic blood pressure.	ACE inhibition	Tuomilehto et al. (2004)
Lactobacillus helveticus LBK16H	94 hypertensive (not receiving other treatment)	Before period of 4 weeks run-in, *L. helveticus* LBK-16H fermented milk with a high concentration of tripeptides (Ile-Pro-Pro 7.5 mg/100 g and Val-Pro-Pro 10 mg/100 g); 300 mL/day during 10 weeks.	Reduction in SBP and DBP in 24 hours ABPM, reduction in CRP (not significant).	ACE inhibition	Jauhiainen et al. (2005)
Lactobacillus helveticus	94 hypertensive (not receiving other treatment)	*L. helveticus* fermented milk with a high concentration of tripeptides (Ile-Pro-Pro 7.5 mg/100 g and Val-Pro-Pro 10 mg/100 g); 300 mL/day during 10 weeks.	Reduction in SBP and DBP in 24 hours ABPM; Improvement in ambulatory arterial stiffness index from baseline.	Improvement in endothelium-dependent relaxation (due to ACE inhibition and/or minerals), reduction in inflammation	Jauhiainen et al. (2007a)
Lactobacillus helveticus LBK16H	89 hypertensive (not receiving other treatment; 54 men and 35 women)	*L. helveticus* fermented milk with a different concentration of tripeptides for 24 weeks. First 12-week period: 200 mL/day; Ile-Pro-Pro 1.2 mg/100 g and Val-Pro-Pro 1.3 mg/100 g. Second 12-week period: 400 mL/day; Ile-Pro-Pro 5.8 mg/100 g and Val-Pro-Pro 6.6 mg/100 g.	Low-dose: no significant changes in the hemodynamic parameters. High-dose: decrease in aortic augmentation index (AIx), reduction in SBP and DBP. The treatment was more efficient in subjects with metabolic syndrome.	Improved endothelial release of nitric oxide (NO) is not involved; could positively influence arterial stiffness.	Jauhiainen et al. (2010)
Lactobacillus helveticus LBK16H	89 hypertensive (not receiving other treatment; 54 men and 35 women)	After 4 weeks run-in reciving 200 mL fermented milk, subjects taken 200 mL *L. helveticus* fermented milk with a low concentration of tripeptides (5 mg/day) for 3 months. After, the subjects taken 400 mL/day *L. helveticus* fermented milk with a high concentration of tripeptides (50 mg/day) for 3 months.	Reduction in SBP and DBP in 24 hours ABPM in high dose of peptides (50 mg/day), no different from placebo.		Jauhiainen et al. (2012)
Lactobacillus helveticus CM14	80 subjects without antihypertensive medication (40 with high-normal BP and 40 with mild hypertension)	Powdered *L. helveticus* fermented milk tablets (Ile-Pro-Pro 8.3 mg and Val-Pro-Pro 4.7 mg); 4 weeks.	Reduction in SBP and DBP both in high-normal and mild hypertension; no difference in HR.	ACE inhibition, no increase in plasma renin activity; mineral composition fermented milk (magnesium).	Aihara et al. (2005)
Lactiplantibacillus plantarum TENSIA	40 patients diagnosed with metabolic syndrome (obesity + hypertension)	50 g of cheese containing 10^{4} log CFU/day; 3 weeks	Reduction in systolic blood pressure both in control and treated groups	Antimicrobial activity, production of NO and polyamines, anti-oxidative ability, ACE inhibition	Sharafedtinov et al. (2013)

(*Continued*)

Table 2. (Continued).

Strain tested	Models	Treatments	Main results	Suggested mechanism for the BP results	References
Lacticaseibacillus casei YIT9018	28 hypertensive (14 men and 14 women)	*Lacticaseibacillus casei* cell lysate; 800 mg/day during 2 months (50% protein, 20% sugar, 15% nucleic acid, 10% ash)	Reduction in SBP, DBP and HR; Reduction in fasting plasma glucose and total cholesterol	Enhancement of PGI_2 biosynthesis by polysaccharide-glycopeptide fraction	Nakajima et al. (1995)
Lacticaseibacillus casei 01	30 hypertensive overweight women	50 g/gay of probiotic Minas Frescal cheese containing L. casei 01for 4 weeks	Reduction in SBP and DBP; improvement of lipid profile, hemoglobin, and hematocrit count.	ACE inhibition Changes in fatty acid profile Anti-oxidative ability	Sperry et al. (2018)
Lacticaseibacillus casei Shirota and *Lactococcus lactis YIT 2027*	39 mildly hypertensive patients	100 ml of fermented low-fat milk containing GABA (FMG) daily at breakfast time for 12 weeks followed by 2 weeks of no intake (weeks 13 and 14).	Reduction in BP	GABA signaling	Inoue et al. (2003)
Lacticaseibacillus casei Shirota	352 normotensive volunteers (125 men and 227 women), 65-93 years	0,9-40 × 10^9 cells per bottle; 5 years. Groups were divided in <3 times/week vs. ≥3 times/week.	Reduction blood pressure in normotensive individuals who took fermented milk ≥3 times/week.	Enhancement of PGI_2 biosynthesis by polysaccharide-glycopeptide fraction (SG-1);	Aoyagi et al. (2017)

hypocaloric diet containing cheese supplemented with *L. plantarum* TENSIA on patients with metabolic syndrome and hypertension. It is essential to highlight that approximately 80% of the individuals in each group took antihypertensive drugs, including Ca^{2+} antagonists, beta-blockers, ACEi, and diuretics. All patients treated with antihypertensive drugs showed a reduction in SBP and DBP, regardless of probiotic treatment. The authors suggested that the beneficial effects of this strain could be related to its ability to colonize the human intestinal tract, antimicrobial activity, NO production, and antioxidative capacity, which benefit vascular function. In addition, they described the ACE-inhibitory activity of *L. plantarum* TENSIA in vitro. Individual differences in responsiveness to probiotics may be explained by differences in the effectiveness of colonization by probiotic bacteria. In this regard, the consumed strain was detectable in only 64% of patients. These data reveal that scientific evidence supporting the use of *L. plantarum* in hypertension therapy is scarce.

L. casei

An early study evaluated the effects of ingestion of LEx for 8 weeks in hypertensive subjects (Nakajima et al. 1995). The authors observed mild reductions in SBP, DBP, and HR. These BP-lowering effects were attributed to the polysaccharide-glycopeptide fraction (SG-1) present in LEx. This compound was described to enhance PGI_2 biosynthesis, which would be responsible for lowering BP and promoting favorable side effects on glucose and lipid metabolism. The authors observed a reduction in fasting plasma glucose and total cholesterol levels.

The strain *L. casei* 01 added to Brazilian cheese reduced SBP and DBP and improved hypertensive overweight women's lipid profile, hemoglobin, and hematocrit count (Sperry et al. 2018). Reduction in SBP was seen in mildly hypertensive patients treated with FMG fermented by *L. casei* Shirota and *Lactococcus lactis* YIT 2027 for 12 weeks (Inoue et al. 2003). *L. casei* Shirota has also shown potential in preventing hypertension onset (Aoyagi et al. 2017). They accompanied 352 normotensive elderly over 5 years and observed that the development of arterial hypertension was 8% lower in subjects that ingested fermented products containing *L. casei* Shirota at least three times a week.

Mechanisms of action

Considering the previous results, it is evident that *Lactobacillus* strains and associated genera have hypotensive potential in animals and humans. The mechanisms of action responsible for reducing BP are diverse and involve interference with the renin-angiotensin system, oxidative stress, modulation of sympathetic activity, vascular tone, and inflammatory markers.

Inhibition of angiotensin I-converting enzyme (ACE)

Angiotensin I-converting enzyme (EC 3.4.15.1) (ACE) is a carboxypeptidase abundantly expressed in the plasma membrane of endothelial cells and a variety of tissues, such as the gastrointestinal tract (Bruneval et al. 1986). The main action of ACE is to convert angiotensin I (Ang I) into angiotensin II (Ang II), an octapeptide that induces a range of physiological effects culminating in BP increase after binding to its type 1 receptor (AT1R), as illustrated in Figure 1. The increase in BP by Ang II occurs through several mechanisms, including vasoconstriction, cell proliferation, increase in reactive oxygen species (ROS), inflammation, activation of immune responses, sympathetic activation, baroreflex dysfunction, and release of aldosterone and vasopressin, which in turn leads to the retention of salt and water (Ferrario and Mullick 2017). In addition, ACE is responsible for the breakdown of bradykinin, a vasodilatory peptide, thus contributing

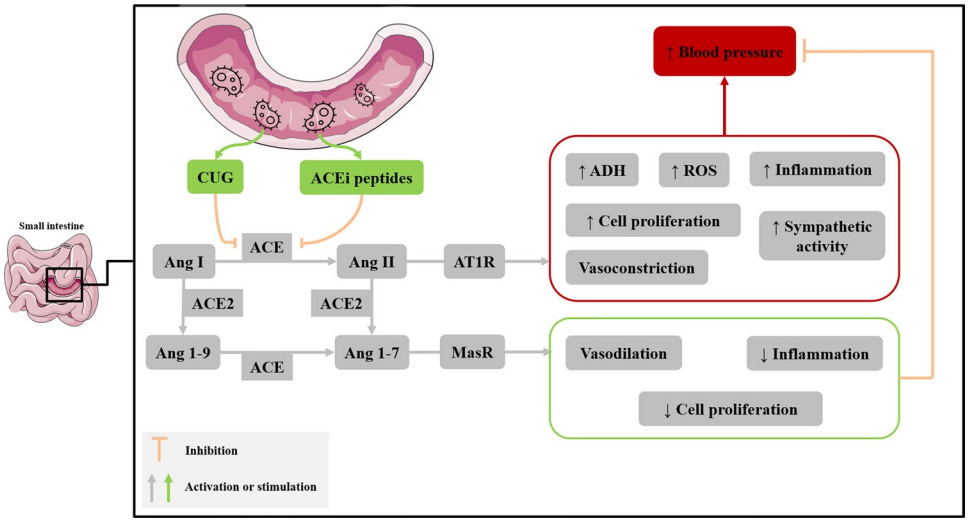

Figure 1. Potential mechanisms of action involved in the antihypertensive effect induced by ACEi peptides and CUG.

to vasoconstriction. Therefore, ACE overactivation contributes to the development of hypertension, making it an important target for treating this disorder.

Strains of *Lactobacillus* and associated genera have been highlighted for generating ACEi peptides during fermentation due to the presence of proteolytic enzymes in the plasma membrane, as previously described (Yamamoto, Akino, and Takano 1993). Peptides are formed after protein hydrolysis, mainly casein, and can be used as nitrogen sources necessary for bacterial growth. Some peptides can competitively inhibit ACE and reduce BP (Fuglsang, Nilsson, and Nyborg 2002), as illustrated in Figure 1.

Maeno, Yamamoto, and Takano (1996) showed the antihypertensive effect of a fraction of casein hydrolysate produced by *L. helveticus* CP790 in SHR rats, and the BP reduction was attributed to the heptapeptide Lys-Val-Leu-Pro-Val-Pro-Gln. This peptide showed weak ACE-inhibitory activity, which was enhanced after digestion by pancreatic enzymes, reinforcing the idea that host gastrointestinal enzymes modulate cardiovascular responses by interacting with peptides released by probiotic bacteria. The antihypertensive effect induced by the CP790 variant *L. helveticus* CPN4 occurred due to the Tyr-Pro dipeptide in a yogurt-like product fermented by this strain (Yamamoto, Maeno, and Takano 1999). ACE inhibition has also been attributed to the peptides Ile-Tyr (Yokoyama, Chiba, and Yoshikawa 1992), Leu-Val-Tyr-Pro-Phe-Pro-Gly-Pro-Ile-His-Asn-Ser-Leu-Pro-Gln-Asn, and Leu-Val-Tyr-Pro-Phe-Pro-Gly-Pro-Ile-His (Pihlanto, Virtanen, and Korhonen 2010).

Chen et al. (2014) analyzed the activity of ACEi peptides produced by 259 *L. helveticus* strains isolated from fermented Chinese and Mongolian foods. Among them, 37 strains exhibited ACE inhibitory activity of over 50%. Milk fermented by strain H9, a strain originating from kurut sampled from Tibet (China), and had the highest in vitro ACE inhibitory activities and detectable levels of VPP and IPP peptides. These tripeptides stood out because of their solid antihypertensive potential demonstrated in both animals and humans, as we can see in several studies stated in Tables 1 and 2.

The tripeptides IPP and VPP can be absorbed, at least partly, from the intestine without being decomposed by digestive enzymes, reaching the abdominal aorta, inhibiting ACE, and showing antihypertensive effects (Foltz et al. 2007; Jauhiainen et al. 2007b; Masuda, Nakamura, and Takano 1996; T. Nakamura et al. 2011).

The ACEi peptides generated during fermentation are diverse in their sequence and number of amino acids. As stated by Fuglsang, Nilsson, and Nyborg (2002), the proteolytic systems of lactic acid bacteria are nonspecific; that is, they cleave a protein in many places. An explanation for the ability of these peptides to inhibit ACE is the presence of free amino groups. In general, the higher the number of free amino groups formed during fermentation, the higher the chance that the same fermented substance will be able to inhibit ACE significantly (Fuglsang, Nilsson, and Nyborg 2002).

Some ACE inhibitor drugs, such as captopril and enalapril, are available as first-choice drugs for treating high BP in hypertensive patients, but they are accompanied by significant side effects (Whelton et al. 2017). Although ACEi peptides derived from probiotic bacteria are generally less potent than drugs with the exact mechanism of action (Tuomilehto et al. 2004), they can be used as adjuvants in hypertension therapy, allowing for dose adjustment of antihypertensive drugs and reduction of side effects.

In addition to peptides, uracil, which is also released during fermentation by *Lactobacillus*, has shown ACE inhibitory activity when combined with glycerol (CUG) (Figure 1) (Chung et al. 2020; Y. Liu et al. 2015). Future studies on ACE inhibition by peptide and non-peptide molecules should continue to broaden our understanding of the systemic effects of this inhibition and their contribution to the reduction of BP.

The inhibition of ACE not only reduces the levels of Ang II and its hypertensive effects but may also increase the levels of Ang 1–7, favoring the activation of the Mas receptor (MasR) and the events contrary to those triggered by AT1R, such as vasodilation, reduction of inflammation, and others with consequent BP decrease (Cook and Chappell

2021; Figure 1). Studies have been performed with *Lactobacillus* strains modified to deliver Ang 1–7 (Carter et al. 2020; Buford et al. 2020). However, no study has focused on intervention with ACEi peptides from *Lactobacillus* strains to evaluate the effect of ACE inhibition on Ang 1–7 and ACE2 levels in animals and humans.

GABA signaling

GABA is a non-proteinogenic amino acid that is the primary inhibitory neurotransmitter in the body. It plays a vital role in the central and peripheral nervous systems and influences many physiological processes, including BP modulation. Lactic acid bacteria naturally produce GABA from L-glutamate via an irreversible α-decarboxylation reaction catalyzed by the glutamic acid decarboxylase (GAD) enzyme expressed in these microorganisms (Hussin et al. 2020). However, the effective production of GABA requires a high concentration of glutamate, pyridoxal-5-phosphate cofactor, and a long fermentation time (Hasegawa et al. 2018; Santos-Espinosa et al. 2020). Some studies have been carried out to improve the production of GABA by *Lactobacillus*, aiming at a better yield with fewer substrates, cofactors, and simpler fermentation conditions (G. Yang et al. 2015; Hussin et al. 2020, 2021; J. Kim et al. 2022).

Some strains of *Lactobacillus* and associated genera have been highlighted for their production of GABA and antihypertensive action in hypertensive animals, such as *L. plantarum* MNZ (Zareian et al. 2015), *L. plantarum* NTU 102 (102FM) (C. Liu et al. 2011), *L. casei* CICC 20280 and CICC 23184 FSM (Bao and Chi 2016), and the self-cloned *L. plantarum* Taj-Apis362 recombinant with high GAD activity (Hussin et al. 2020). In the clinical trial, the antihypertensive effect of GABA has been examined in hypertensive people using fermented milk by *L. casei* Shirota (Inoue et al. 2003).

GABA is known to lower BP in animals and humans via several mechanisms. As it barely crosses the blood-brain barrier, GABA obtained from food seems to induce its antihypertensive effect, acting only peripherally (Boonstra et al. 2015). In general, the mechanisms underlying the hypotensive role of GABA include a decrease in sympathetic activity (Hayakawa et al. 2004; Hayakawa, Kimura, and Kamata 2002), a reduction of vasopressin-regulated water reabsorption (Donato et al. 2013), and vasodilation (Hayakawa et al. 2004; Kharazmi et al. 2015; Zareian et al. 2015).

The reduction in sympathetic activity occurs through the action of GABA on peripheral sympathetic nerve endings through activation of the $GABA_B$ receptor. This mechanism reduces noradrenaline release and promotes vasodilatation (Hayakawa, Kimura, and Kamata 2002; Hayakawa et al. 2004). The relaxing effect can also occur via the direct action of GABA on blood vessels through the activation of $GABA_A$ and $GABA_B$ receptors. NO increase and endothelin decrease are involved in GABA-induced vasodilatation; however, this effect requires further clarification (Kharazmi et al. 2015; Suzuki et al. 2012; Zareian et al. 2015; Figure 2).

The effects of GABA in the gut are still not fully understood; however, it has been shown that luminal GABA binds to $GABA_B$ receptors on enterochromaffin cells, stimulates 5-HT release, attenuates the sympathetic response, and has a hypotensive effect (Cookson 2021; Kimura, Hayakawa, and Sansawa 2002). GABA supplementation has been associated with reduced oxidative stress (Kawakami et al. 2018), but it is unclear whether GABA reduces oxidative stress or whether the antihypertensive effect induced by GABA contributes to the reduction of oxidative stress (Figure 2).

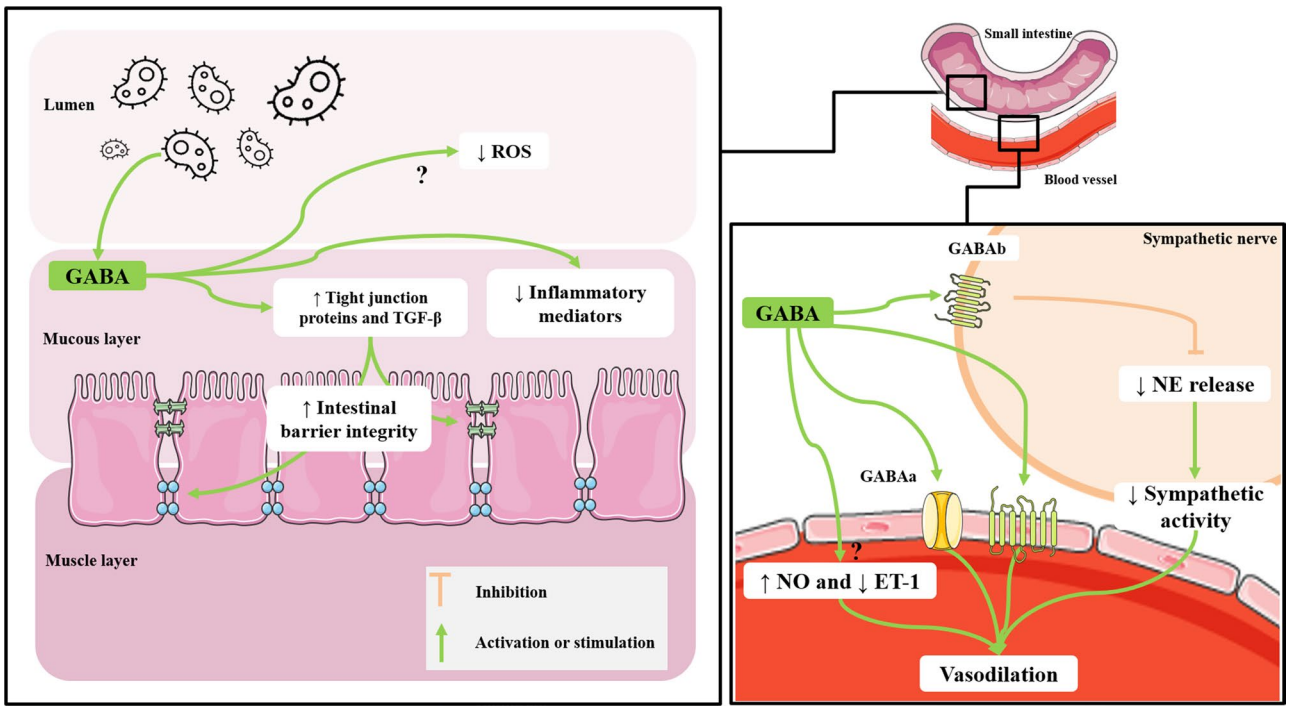

Figure 2. Potential mechanisms of action involved in the antihypertensive effect induced by GABA.

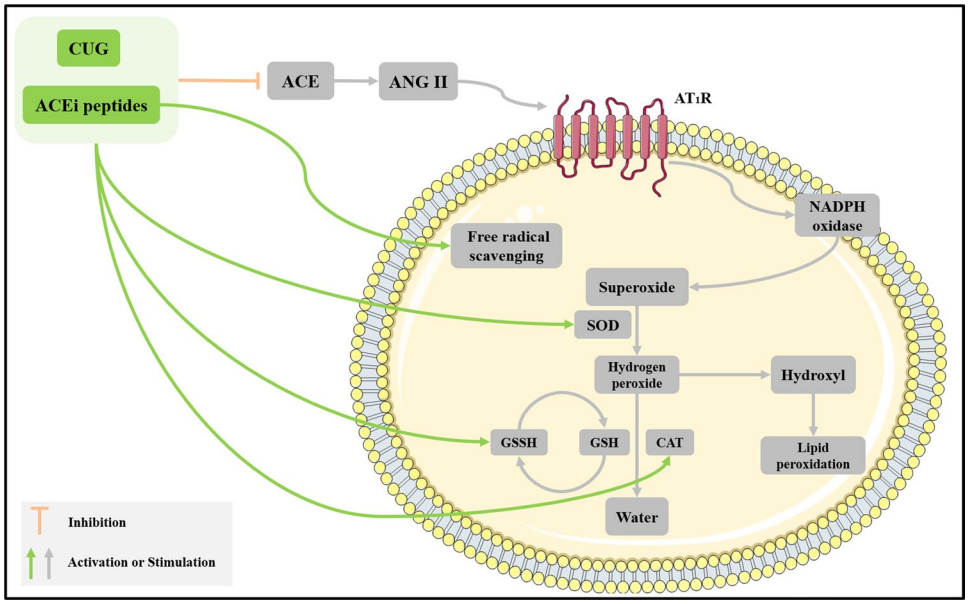

Figure 3. Mechanisms involved in the reduction of oxidative stress by ACEi peptides and CUG.

In addition, GABA alleviates inflammation caused by IL-1β and stimulates the expression of tight junction proteins and TGF-β cytokines, contributing positively to intestinal epithelial barrier integrity (Bajic et al. 2019). Cataldo et al. (2020) showed that GABA-enriched fermented strawberry juice could reduce the levels of peritoneal, intestinal, and serum TNF-α, IL-6, and CXCL1 increasing IL-10 and IFN-g-in rats exposed to an intraperitoneal challenge with lipopolysaccharide (LPS). These results suggest that the bioavailability of GABA in the gut can modulate BP through immunomodulatory mechanisms. However, the immunoregulatory effects of GABA-enriched products after fermentation with *Lactobacillus* and their influence on BP have not been studied in vivo models.

Reduction of oxidative stress

Several studies have shown that increased oxidative stress, both in the central and peripheral nervous systems is involved in sympathetic overactivity and hypertension (Braga et al. 2011), suggesting that probiotics with antioxidant action can be helpful in the treatment or prevention of hypertension. Various strains of *Lactobacillus* and associated genera have antioxidant and antihypertensive potential, such as *L. fermentum 296* (Freire et al. 2021b), *L. fermentum* CECT5716 (LC40) (Toral et al. 2018), *L. paracasei subsp. paracasei* NTU 101 (101FM), *L. plantarum* NTU 102 (102FM) (C. Liu and Pan 2010; C. Liu et al. 2011), *L. plantarum* MNZR (Zareian et al. 2015), and *L. plantarum* TWK10 (T. Liu, Chiou, and Tsai 2016).

The mechanisms involved in the antioxidant activity include increased expression and activity of antioxidant enzymes, such as superoxide dismutase, glutathione peroxidase, and catalase (Chiu et al. 2007; Moura et al. 2016; T. Liu, Chiou, and Tsai 2016; Zareian et al. 2015) and free radical scavenging, which is believed to occur by antioxidant peptides produced during fermentation (C. Liu et al. 2011; Lee et al. 2010; Geeta and Yadav 2017; Zareian et al. 2015; Figure 3).

It is worth noting that ACE inhibition, as demonstrated by several *Lactobacillus* strains, can theoretically reduce oxidative stress by decreasing angiotensinergic signaling (Figure 3). It is classically known that Ang II, when bound to its AT1R, activates NADPH oxidase, raising the ROS levels in the cells; this pathway has been related to vasoconstriction and sympathetic overactivity, among other events associated with BP increase (Masi, Uliana, and Virdis 2019). Thus, the synergism between ACE inhibition and other antioxidant mechanisms may largely explain the antihypertensive potential of some of the strains described so far.

Reduction of inflammatory markers

One of the characteristics of hypertension is a generalized inflammatory condition linked to changes in the microbiota (Vallianou, Geladari, and Kounatidis 2020). Bacterial wall components, such as lipopolysaccharide (LPS), and metabolic products, such as trimethylamine-N oxide (TMAO) and SCFAs, are involved in these processes (Caroff and Karibian 2003; Mehta et al. 2014; Pluznick et al. 2013; Poll, Cheema, and Pluznick 2020). SCFAs, commonly acetate, butyrate, and propionate, modulate BP, often favoring its reduction (Pluznick 2017), but the final effect stems from amount and the balance between these molecules besides the cell context.

SCFAs exert their effects in and outside the gut, modulating immune pathways, as shown in Figure 4. Through histone deacetylase (HDAC) inhibition and GPCR signaling, SCFAs stimulate the production of anti-inflammatory mediators, enhance the differentiation and activation of regulatory T-cells in both the gut and peripheral organs, and attenuate the migration and subsequent inflammatory response in macrophages and neutrophils (Corrêa-Oliveira et al. 2016). SCFAs activate peroxisome proliferator-activated receptor gamma (PPARγ) in the intestine, suppressing the production of proinflammatory cytokines and up-regulating tight junctions, anti-inflammatory Immunoglobulin A (IgA) and

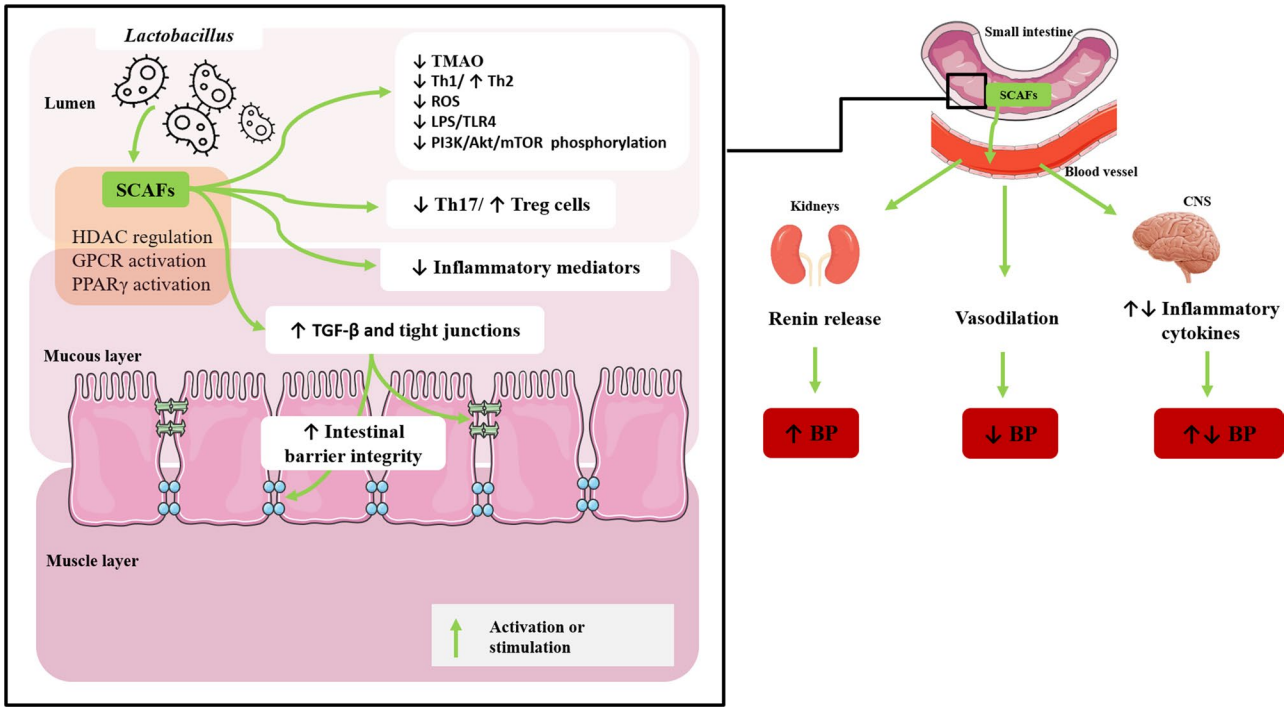

Figure 4. Mechanisms involved in the reduction of inflammatory markers and BP modulation by SCAFs.

β-defensin, thereby contributing to the maintenance of intestinal barrier integrity (Overby and Ferguson 2021). G-protein-coupled receptors for SCAFs (Gpr41, Gpr43, Gpr109a, Olfr78, and Olfr558) are present in several tissues such as kidney, brain, heart and vasculature. Activation of these receptors can induce renin release, vasodilatation and modulation of inflammatory cytokines, that affects BP (Poll, Cheema, and Pluznick 2020).

J. Liu et al. (2019) showed that *L. rhamnosus* GG mitigated the development of hypertension by reducing blood TMAO levels, modulating Th1/Th2 imbalance, and suppressing phosphorylation levels of ERK1/2, Akt and mTOR. This pathway is involved in T cell proliferation, activation and cytokine production (Figure 4). The strain *L. fermentum* CECT5716 (LC40) has been associated with Th17/Treg balance, improvement of endothelial function and reduced ROS production. These effects might be mediated by both the reduction in vascular LPS/toll-like receptor 4 (TLR4) pathway and the increase in Treg infiltration in the vasculature (Robles-Vera et al. 2020; Figure 4). Several studies have shown immunomodulatory and anti-inflammatory roles of the *Lactobacillus* group in hypertension models as presented in Table 1.

Reduction of sympathetic activity

Hypertension is closely related to sympathetic overactivity and baroreflex dysfunction (Braga et al. 2011; D. Guimarães and Braga 2012; Haspula and Clark 2018). Strains, such as *L. fermentum* 296, alone or in a mixture (*L. fermentum* 139, *L. fermentum* 263, and *L. fermentum* 296) reduced sympathetic activity in animals with hypertension but without changes in baroreflex sensitivity (Cavalcante et al. 2019; Ferreira et al. 2022; Oliveira et al. 2020). Another strain shown to reduce blood pressure and sympathetic activity is *L. johnsonii* (LJLa1) when administered to urethane-anesthetized rats (Tanida et al. 2005). Despite the absence of trials evaluating autonomic activity, the decrease in sympathetic activity may be part of the antihypertensive mechanism of many *Lactobacillus* strains.

The mechanisms by which probiotic bacteria reduce sympathetic activity are not fully understood, but evidence suggests that GABAergic signaling (Bao and Chi 2016; Hussin et al. 2020; Zareian et al. 2015) and reduction of oxidative stress and inflammation (Geeta and Yadav 2017; M.-C. Cheng and Pan 2017; Moura et al. 2016; Zubcevic et al. 2019) may play an essential role in this regard. It is worth mentioning that ACEi, by reducing Ang II levels, would result in reduced oxidative stress in neural regions of sympathetic modulation, with a consequent decrease in sympathetic outflow to the periphery (Braga et al. 2011; Nagase et al. 1996).

Modulation of vascular tone

Strains of *Lactobacillus* and its associated genera have demonstrated the ability to modulate vascular tone by increasing vasodilators and reducing vasoconstrictors, thereby reducing BP (Figure 5). Soy milk fermented with *L. plantarum* TWK10 increased the production of endothelial relaxing factors, such as NO and prostaglandin E2 (PGE2), and enhanced eNOS activity and superoxide anion scavenging in human umbilical vein endothelial cells (C. Cheng et al. 2013); the mechanisms responsible for these events are not yet fully understood. CUG generated after fermentation with *L. plantarum* TWK10 showed to inhibit ACE and induce NO production, reducing BP in SHR rats (Y. Liu et al. 2015; Figure 5). Similar results were found in DOCA-salt hypertension-induced

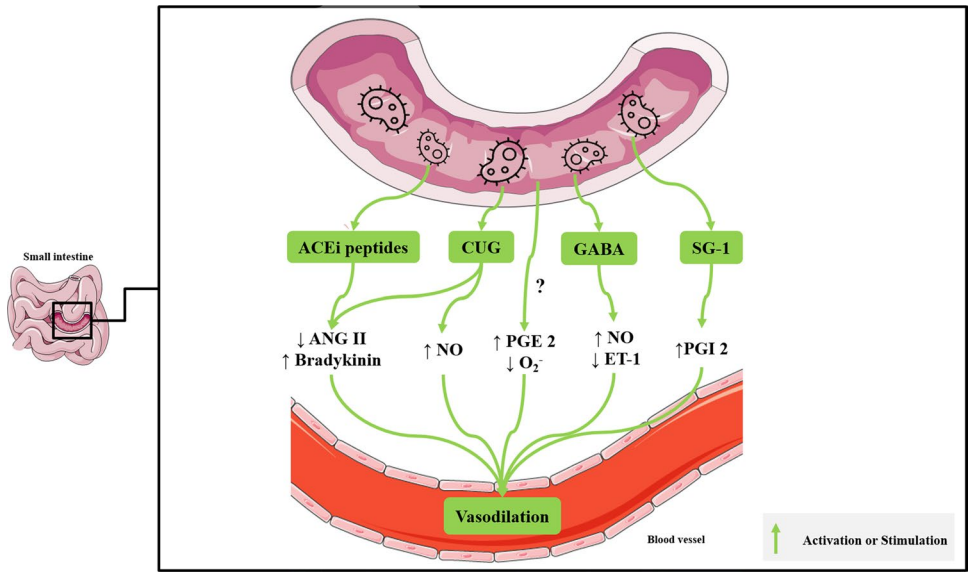

Figure 5. Mechanisms involved in the modulation of vascular tone by products derived from *Lactobacillus*.

VaD rats (T. Liu, Chiou, and Tsai 2016). It is unknown whether the effect on NO bioavailability results from ACE inhibition or a direct mechanism induced by CUG in blood vessels.

The antihypertensive effect of SG-l isolated from *L. casei* has been attributed to the synthesis of the vasodilator prostacyclin (PGI_2) (Furushiro et al. 1993; Nakajima et al. 1995). The reduction of vasoconstriction factor ET-1 protein in the aorta of SHR rats also appears to be responsible, at least in part, for the decrease in peripheral resistance and BP in SHR rats after supplementation with wheat-based fermented rice using GABA-producing *L. plantarum* MNZ (Zareian et al. 2015). ACE inhibition, demonstrated by several strains of *Lactobacillus*, also modulated vascular tone by reducing the effect of Ang II on blood vessels and sympathetic activity (Figure 5).

The modulation of vascular tone may be accompanied by other effects, such as a reduction in arterial wall thickness (Jauhiainen et al. 2007a) and an improvement in endothelial function (Gomez-Guzmán et al. 2015; M.-C. Cheng and Pan 2017; Robles-Vera et al. 2020) after supplementation with *Lactobacillus* strains in animals or humans. These events contribute to the improvement in the hypertensive state.

In summary, the main *Lactobacillus*-derived products with antihypertensive effect are ACEi peptides, CUG, GABA, polysaccharide-glycoprotein complexes (SG-1) and

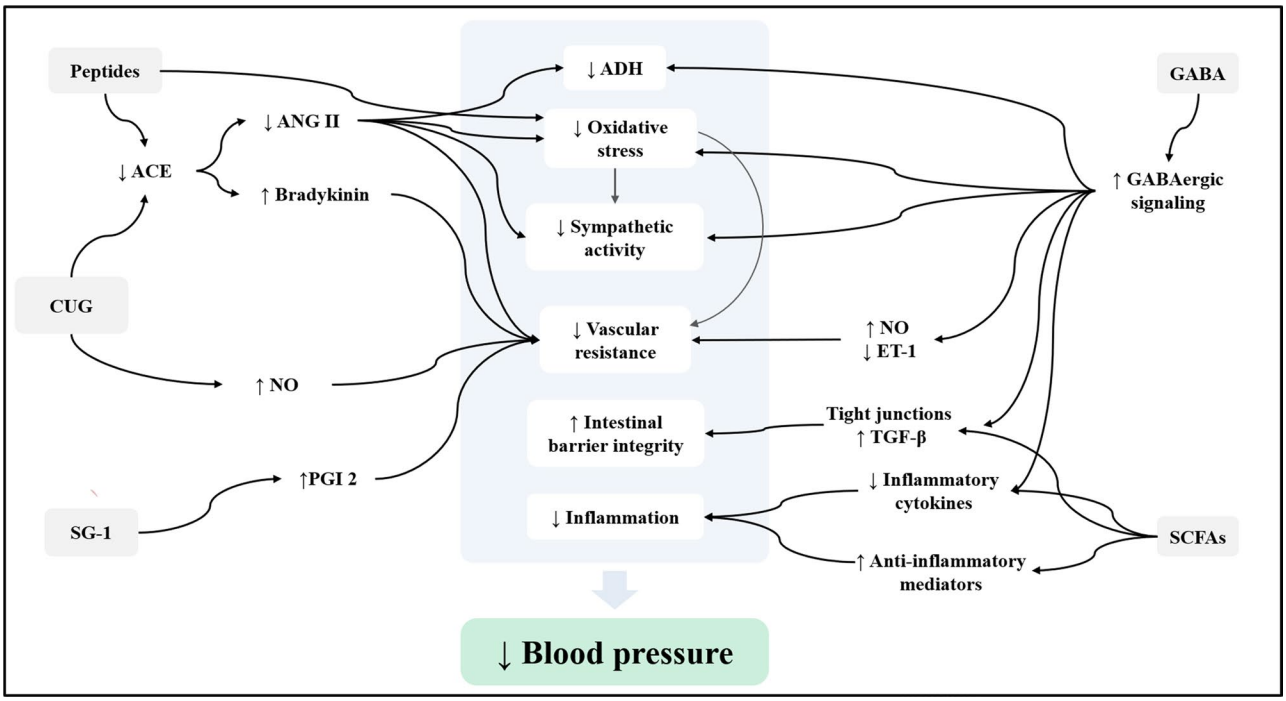

Figure 6. Potential mechanisms of action involved in the antihypertensive effect induced by products derived from *Lactobacillus*.

SCAFs. Figure 6 illustrates these products and the main mechanisms of action they activate to reduce BP. We can observe some points in common between them, ACEi peptides and CUG inhibit ACE, reducing ADH release, oxidative stress, sympathetic activity, vascular resistance and other effects. Inhibition of ADH and oxidative stress may also result from GABA, which in turn reduces inflammatory markers and improves intestinal barrier integrity as well as SCAFs. The reduction of vascular resistance can occur through GABA, peptides, CUG and SG-1, which increase the bioavailability of vasodilators such as NO, bradykinin, PGI2 and reduce vasoconstrictors such as Ang II and ET-1. All these events contribute to the reduction of BP. We did not rule out the participation of other metabolites in the antihypertensive effect observed for *Lactobacillus*. This encourages further studies focused on other molecules present in the composition of bacteria or generated from their metabolism.

Conclusion

This review addresses strategies focused on the prevention and treatment of arterial hypertension by restoring gut microbiota homeostasis in the *Lactobacillus* group. The administration of these bacteria or products fermented with specific strains provides the body with molecules capable of inducing significant cardiovascular changes and reducing BP. Therefore, products containing *Lactobacillus* can be used alone as a preventive agent or with antihypertensive drugs, allowing adjustments in the therapeutic regimen of hypertensive patients.

The antihypertensive effect varies according to strain, dose, treatment duration, and administration route. Among the strains with antihypertensive potential, *L. helveticus* LBK16H, *L. casei* YIT9018, and *L. casei* Shirota showed significant results in preclinical and clinical trials. The strains *L. fermentum* 139, 263, and 296 seem promising based on preclinical findings in hypertensive rats and are strong candidates for future clinical studies.

Many clinical studies have been performed on *Lactobacillus* strains in nonhypertensive individuals. Studies carried out on patients with hypertension had relatively small sample sizes. Therefore, it is essential to conduct studies with larger samples comprising only patients with hypertension and with a standardized treatment protocol. The investigation of adverse effects and the duration of the antihypertensive effect after supplementation are essential issues that should be investigated in clinical trials of probiotic products.

The mechanisms of action involved in the BP-lowering effect are diverse, including inhibition of ACE, increase in GABAergic signaling, reduction of oxidative stress, and inflammatory markers. The decline in sympathetic activity and endothelial function improvement results from many of these mechanisms, culminating in a decrease in BP. Although much has already been discovered, some questions regarding the signaling pathways induced by *Lactobacillus* strains need to be clarified. Therefore, future mechanistic studies associated with more extensive clinical trials will justify using *Lactobacillus* and its associated genera in managing SAH. Certainly, advances in probiogenomics studies and bioengineering will reveal the molecular basis for how *Lactobacillus* can down- or upregulate gene expression, as also develop novel engineered probiotic bacteria destinated to treating SAH.

Disclosure statement

The authors report no conflict of interest.

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Lactobacillus acidophilus LA-5 improves baroreflex sensitivity and vascular reactivity in hypertensive rats

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Abstract

Background and aims: The objective of this study was to determine the cardiovascular effects of the probiotic *Lactobacillus acidophilus* LA-5 (LA5) in spontaneously hypertensive rats (SHR). Methods: Four-week old Wistar Kyoto rats (WKY) and aged matched SHR were randomly distributed into three groups: control WKY; control SHR and supplemented SHR+LA5. The strain (10^9 CFU/mL) was daily administrated by orogastric gavage for 8 weeks. After supplementation we assessed the blood pressure, heart rate, autonomic modulation, baroreflex sensitivity, oxidative stress and vascular reactivity. Results: Oral supplementation with LA5 reduced the diastolic arterial pressure levels in SHR+LA5 when compared to control SHR group (122.0±4.3 *versus* 153.0±6.7, $p<0.05$) without changes in the systolic and mean arterial pressure. LA5 reduced the low frequency (LF) oscillation in the frequency domain of heart rate variability (7.38±0,22 *versus* 9.5±0.75%, $p<0.05$) and restored baroreflex sensitivity, observed in both induced (-2.48±0.32 *versus* -0.94±0.09 bpm/mmHg, $p<0.05$) and spontaneous baroreflex (0.44± 0.04 *versus* 0.77±0.1 ms/mmHg, $p<0.05$). Additionally, probiotic supplementation lowered oxidative stress and improved the vascular reactivity to ACh and SNP. Conclusion: Together, these data suggest that probiotic therapy for 8 weeks with LA5 prevents deleterious effects related to hypertension, such as sympathetic overactivity, baroreflex and endothelial dysfunctions, and peripheral oxidative stress. These effects may be related to a modest reduction in diastolic blood pressure, but they were not accompanied by significant changes in the systolic and mean arterial pressure of the hypertensive rats.

Keywords: hypertension, endothelial dysfunction, probiotics.

1. Introduction

Systemic arterial hypertension (SAH) is a serious and chronic clinical condition that is considered the main risk factor related to cardiovascular, cerebrovascular and chronic

kidney diseases, increasing both early morbidity and mortality worldwide. Therefore, controlling blood pressure (BP) levels provides a great public health benefit (Chobanian, 2011; Forouzanfar *et al.*, 2017; Wilkins *et al.*, 2010). Studies have indicated dietary strategies as important adjuvants in the treatment of hypertension or its prevention (Valenzuela *et al.*, 2020).

The human gastrointestinal tract is a complex ecosystem colonized by trillions of microorganisms, which together with their metabolites constitute the gut microbiota (Ley *et al.*, 2006). The diversity and stability of the gutmicrobiota and microbial and symbioticinteractions regulate diverse physiologicalfunctions in the host, including the blood pressure (Das and Nair 2019; Palmu *et al.*, 2020; Palmu *et al.*, 2021). When there is an imbalance in gut microbiota, with a decrease in the diversity and stability ofthe microbial population and an increase in the intestinal permeability, what we call dysbiosis occurs (Petersen and Round, 2014). Several studies have demonstrated that gut dysbiosis is associated with SAH (Muralitharan *et al.*, 2020; Silveira-Nunes *et al.*, 2020; Yang *et al.*, 2018). In summary, dysbiosis can promote sympathetic overactivity, low-grade systemic inflammation, endothelial dysfunction, thus increasing BP (Muralitharan *et al.*, 2020; Santisteban *et al.*, 2017; Toral *et al.*, 2019). On the contrary, SAH can also favor dysbiosis, which can contribute to the progression of the hypertensive state (Konopelski *et al.*, 2021; Qin *et al.*, 2018).

In this context, microbiota-targeted therapies with probiotic have been proposed for treatment or prevention of SAH (Aoyagi *et al.*, 2017; Chi *et al.*, 2020; Qi *et al.*, 2020). Among the potentially probiotic strains with antihypertensive properties, those belonging to the *Lactobacillus* stand out in the BP modulation. In the SAH has been found a reduction in the levels of *Lactobacillus* in the gut (Palmu *et al.*, 2020; Razavi *et al.*, 2019) and the administration of some *Lactobacillus* strains improved the gut microbiota composition and exerted antihypertensive effects in both animals and humans, as compiled in a recent review by Gadelha *et al.* (2020).

Lactobacillus acidophilus is widely recognized to have probiotic effects and is one of the most commonly suggested organisms for dietary use, being added in various food matrices (Sanders, 2003; Shah, 2007). Studies using a mix containing a *L. acidophilus* demonstrated an ACE inhibitory role (Ankolekar *et al.*, 2012; Pihlanto *et al.*, 2010); BP reduction in SHR rats (Tsai *et al.*, 2006) and elderly patients with metabolic syndrome, with an improvement in other cardiometabolic parameters (Cicero *et al.*, 2021). The strain *L. acidophilus* LA-5 is among the primary commercial strains of *L. acidophullus* species (Bull *et al.*, 2013), nevertheless, studies with this strain in the cardiovascular system are still scarce. Rezazadeh *et al.* (2021) demonstrated that a probiotic yogurt containing *L. acidophilus* LA-5 and *Bifidobacterium lactis* Bb12 improved the insulin sensitivity, oxidative stress and uric acid levels in patients with metabolic syndrome. Moura *et al.* (2016) also showed an antioxidant effect and an improvement in the lipid profile of Wistar rats fed with dairy dessert containing *L. acidophilus* LA-5. The data presented indicate a cardioprotective potential for this strain. In spite of that, the *L. acidophilus* LA-5-induced effects on hypertension has not yet been investigated. The objective of this study was to investigate the effects induced by supplementation with *L. acidophilus* LA-5 (LA5) on the BP modulation, oxidative stress and vascular reactivity in spontaneously hypertensive rats, in order to evaluate its potential as an adjuvant in the management of SAH.

2. Material and methods

2.1 Experimental design

All experimental procedures were approved by the Animal Care and Use Committee of the Federal University of Paraíba (CEUA, UFPB, Paraiba, Brazil, protocol Nº. 4821160718). Four-week-old male SH and WKY rats were housed under controlled conditions of temperature (22±1 ºC) and humidity with a 12 h light/dark cycle and had free access to standard chow (Labina®, Purine) and water. Water and feed consumption were measured daily throughout the treatment period.

Animals were randomly divided into three groups: normotensive control (WKY); spontaneously hypertensive rats (SHR); and SHR supplemented with LA5 (SHR+LA5). The suspension (1 mL) containing probiotics (10^9 CFU/mL) was daily administrated by orogastric gavage for 28 days. After supplementation cardiovascular parameters were evaluated by *in vivo* experiments. After that, superior mesenteric artery and serum were removed for *in vitro* and biochemical analyzes, respectively.

2.2 Probiotic strain preparation

The probiotic strain *Lactobacillus acidophilus* LA-5 (LA5) was obtained from Christian Hansen (Valinhos, SP, Brazil). The commercial lyophilized culture was kept frozen at -18°C and for use it was rehydrated in MRS broth and inoculated 24 hours at 37°C. Strain inoculum was obtained by preparing suspensions in sterile saline from overnight cultures in MRS broth (HiMedia, Mumbai, India). Cells were harvested by centrifugation (4500 × g, 15 min, 4°C), washed twice with phosphate-buffered saline (PBS; pH 7.2), resuspended and homogenized using a vortex (30 s) in the same diluent to obtain standard cell suspensions that corresponded to viable counts of 9 log CFU/mL (optical density at 660 nm of 1.0). Cell suspensions were daily prepared for administration to animals.

2.3Cardiovascular analysis

Eight weeks after LA5 supplementation, vascular catheters were implanted in the femoral artery and vein for blood pressure and heart rate measurements and administer medication, respectively. After a 24 hours recovery period, pulsatile arterial pressure (PAP) of conscious rats was recorded using a pressure transducer (MLT0380/D, ADInstruments, Sydney, Australia) coupled to an amplifier and an acquisition system (PowerLab, ADInstruments, Bella Vista, NSW, Australia) using specific software (LabChart 5.0, ADInstruments, Bella Vista, NSW, Australia). Systolic arterial pressure (SAP), mean arterial pressure (MAP), diastolic arterial pressure (DAP), and heartrate (HR) were derived from PAP online.

As described by Romão da Silva *et al*. (2020), the heart rate (HR) and the heart rate variability (HRV) were determined. The HRV in the frequency domain was evaluated by spectral analysis. Sequences of consecutive systolic interval values were extracted from time series of 4,000 to 4,500 consecutive heartbeats (approximately 10 minutes of continuous recording of pulsatile blood pressure), using the “heart rate variability - HRV” module of LabChart 8.0.1 to Windows software (ADInstruments Pty Ltd, Australia). The power of the oscillatory components obtained from rats belonging to the experimental groups was integrated into low frequency (LF; 0.2–0.75 Hz) and high frequency (HF; 0.75–2.5 Hz) bands and the results were expressed as percentage (%). LF bandsare representative of the modulatory effects of sympathetic activity, whereasthe HF bands are associated with parasympathetic or respiratory modulation. The ratio of LF to HF power (LF/HF ratio) was used to estimate sympathovagal balance (Shaffer; Ginsberg, 2017).

Baroreflex sensitivity (BRS) was assessed by inducing reflex responses to vasoactive agents. Reflex changes in HR induced by transient changes in BP were analyzed. These changes were induced by intravenous injection of phenylephrine (Phe, 8 µg/Kg), α_1 adrenergic agonist; and sodium nitroprusside (SNP, 25 µg/Kg), nitric oxide donor; in order to obtain pressor and depressor responses, respectively. To determine the baroreflex sensitivity, we adopted the technique of peak response to the vasoactive agent (ΔHR/ΔBP)and the response was expressed in bpm/mmHg. Additionally, the spontaneous baroreflex (SBRS) was evaluated from the baseline BP and HR of the animals, using the Hemolab software (Analyser version 9.3). In this last analysis, the reflex response is observed in pulse interval (ms/mmHg).

2.4 Oxidative stress measurement

Blood samples were collected for the dosage of thiobarbituric acid reactive substances (TBARS) (Cavalcanti *et al.*, 2016). In this assay, malondialdehyde (MDA), a final product of lipid peroxidation, reacts with thiobarbituric acid to produce a red complex, which works as an indicator of serum oxidative stress. Blood samples from animals was collected and centrifuged at 2000 g, 4º C, for 15 min to obtain serum. 400 µL of perchloric acid (7%) was added to the sample, mixed and centrifuged for 20 minutes. The supernatant was collected, and added to thiobarbituric acid (0.6%). The mixture was heated at 40°C for 1 hour and the absorbance was determined on a Chem Well T spectrophotometer at a wavelength of 532 nm. The analyses were performed in duplicat and a standard MDA curve with concentrations from 0 to 100 nmol/ml was constructed before reading the samples. The results were expressed as nmol MDA/ml.

2.5 Vascular reactivity assays

After euthanasia, the superior mesenteric artery of the rats was removed and cleaned from connective and adipose tissues. Rings (2-5 mm) were placed in physiological Tyrode's solution, maintained to 37ºC, gassed with a carbogenic mixture (95% O_2 and 5% CO_2) at pH 7.4. All preparations were stabilized under a resting tension of 0.75 g for 1 h. The solution was replaced every 15 min to prevent the accumulation of metabolites. The tension was recorded by a force transducer (PowerLabTM, ADInstruments, MA, U.S.A.). In order to confirm the presence of functional endothelium, the rings were pre-contracted with Phe (10 µM) and exposed to ACh (10 µM). Relaxations exceeding 80% indicated a functional endothelium and were included in the study. A maximum contraction was induced using a high potassium concentration (K^+: 60 mM). After wash out, we performed concentration–response curves to Phe (10^{-10} to 10^{-4} M), ACh (10^{-10} to 10^{-4} M), or SNP (10^{-12} to 10^{-5} M), the last two in rings pre-contracted with Phe (10 µM). Contractile responses to Phe were compared with the maximum contraction obtained following tissue stimulation with high [K^+] as described by Carvalho-Galvão *et al*. (2018). Relaxation responses to cumulative concentrations of ACh and SNP were calculated as percentage of inhibition of Phe-induced maximal contraction. Results were expressed as maximum effect (ME) and pD_2 (negative logarithm to base 10 of the EC_{50}).

2.6 Statistical analysis

Data were expressed as mean ± S.E.M. One-way ANOVA followed by Tuckey's post-test and Two-way ANOVA followed by Bonferroni's post-test were performed when

appropriate. Differences were considered when $p<0.05$. The program used was GraphPad Prisma version 5.00®.

3. Results

3.1 LA5 does not prevent blood pressure increase in SHR group

As expected, the SHR group exhibited higher MAP levels than WKY group(162.8±4.3 mmHg *versus* 118.6±4.7 mmHg, respectively, $p<0.05$) (Figure 1A). Oral supplementation of LA5 for eight weeks did not attenuate the MAP increase in SHR+LA5 (171.4±13 mmHg) (Figure 1A). Similar results were observed in systolic arterial pressure, SHR group presented higher SAP levels than WKY group (198.33±9.3 *versus* 120.1±3.0mmHg, $p<0.05$), which has not been modified by LA5 consumption (192.6±12.2 mmHg) (Figure 1B). On the contrary, DAP levels were higher in the SHR compared to WKY group (153.0±6.7 *versus* 77.3±5.0mmHg, $p<0.05$) and LA5 supplementation significantly reduced DAP in the SHR+LA5 group (122.0±4.3 mmHg, $p<0.05$) (Figure 1C). HR was not significantly different amongst the groups (WKY: 331.6±24 bpm; SHR: 341.8±20.3 bpm; and SHR+LA5: 330.4±11.8 bpm) (Figure 1D).

3.2 LA5 decreases the sympathetic overactivity

The effect of administration of LA5 for 8 weeks in young SHR on autonomic modulation was analyzed by spectral analysis and is shown in Figure 2. Representative spectral (Figure 2A) and the group graph (Figure 2B) highlight the expected increase in oscillation in the LF range in SHR compared with WKY rats (9.5±0.75 *versus* 6.34±0,40%, respectively, $p<0.05$, Figure 2B). Supplementation with LA5 decreased the magnitude of oscillation in the LF in SHR+LA5 group (7.38±0,22%, $p<0.05$). The HF component and the LF/HF ratio were not different between the groups (Figure 2C, D). These data suggest that LA5 reduces the sympathetic overactivity present in SHR animals, without promoting significant changes in the sympathovagal balance.

3.3 LA5 improves the baroreflex sensitivity

As expected, the SHR group presented a reduction in BRS when compared with WKY rats (-0.94±0.09 *versus* -2.91±0.35 bpm/mmHg, respectively, $p<0.05$). Supplementation with LA5 was able to prevent the impairment of BRS in hypertensive animals, since a significant difference can be seen when comparing SHR+LA5 and SHR groups (-2.48±0.32 *versus* -0.94±0.09 bpm/mmHg, $p<0.05$, Figure 3A). Unsurprisingly, SBRS was significantly impaired in SHR compared with the WKY group (0.44±0.04 *versus* 0.85±0.2 ms/mmHg, respectively, $p<0.05$) and supplementation with LA5 prevented the reduction in SBRS (0.77± 0.1 ms/mmHg, $p<0.05$, Figure 3B).

3.4 LA5 reduces the oxidative stress

Using an indirect quantification method of oxidative stress with malonylaldehyde (MDA), it is possible to observe that hypertensive rats had a higher serum MDA levels when compared to WKY group (60.45 ± 2.89 *versus* 33.26 ± 1,04 nmol/ml, $p<0.05$) (Figure 4). Oral supplementation with LA5 for 8 weeks was able to reduce MDA levels as observed in the SHR+LA5 compared with control SHR (33.78 ± 1,61 *versus* 60.45 ± 2.89 nmol/ml, $p<0.05$).

These results suggest that oral supplementation with LA-5 for 8 weeks is able to reduce oxidative stress in hypertensive animals.

3.5 LA5 improves the vascular reactivity

In Figure 5A it is possible to observe the vasorelaxation produced by ACh in superior mesenteric artery rings isolated from normotensive and hypertensive animals. The SHR group showed impairment in the relaxation profile produced by ACh with reduced maximum effect when compared to the WKY group (ME: 71.35±9.37 *versus* 94.18±3.32%, $p<0.05$). Rings isolated from SHR animals that received LA5 showed better relaxation to ACh when compared to the unsupplemented SHR group (ME: 111.23±6.45 *versus* 71.35±9.37%, $p<0.05$). In the Figure 5B it is possible to observe the vasorelaxant effect induced by SNP in the isolated mesenteric rings of normotensive and hypertensive rats that received or not, LA5 orally for 8 weeks. The control groups (WKY and SHR) did not show significant differences regarding the ME induced by the SNP (ME: 106.95±2.73 *versus* 106.41±5.52%, respectively). Interestingly, supplementation with LA5 was able to improve the maximal relaxing effect induced by SNP when compared to the WKY and untreated SHR groups (ME: 134.40±4.02 *versus* 106.95±2.74 and 106.41± 5.52%, $p<0.05$). In Figure 5C, it is possible to observe the percentage of contraction in the presence of Phe cumulatively added in rings without functional endothelium. It is noticed that there was no difference in the contraction profile between WKY and SHR groups (WKY: 100.2±9%, SHR: 113.1±12.9% and SHR+LA5: 97.4±12.7%). LA5 had no impact on vascular reactivity to Phe in SHR rats.

4. Discussion

In the present study, we found that oral supplementation of LA5 in spontaneously hypertensive (SH) rats for 8 weeks reduced DAP without changes in SAP, MAP levels or HR; reduced the LF waves of the HRV analysis; prevented the impairment of the baroreflex sensitivity; and improved the oxidative stress and vascular reactivity to ACh and SNP.

This was the first study evaluating the effect of the strain *L. acidophilus* LA-5 in SHR rats. SHR model is the one that most closely resembles human essential hypertension, with a multifactorial nature and multiple pathways involved in the hypertension development. The BP of SHR start to increase at 5th week of life, reaching levels considered as spontaneous hypertension between the 7th and 15th week (Fazan Jr, 2001). We performed LA5 supplementation from the 4th week of life of the rats as a preventive strategy against the hypertensive process. Despite preventing the increase in the DAP, oral consumption of LA5 for 8 weeks was not able to prevent the onset of hypertension, as we can see by the increased MAP and SAP levels in the SHR+LA5 group. Opposite results in MAP and SAP were found with the use of fermented soymilk by five lactic acid bacteria, including *L. acidophilus* strains in 7-week-old SHR treated for the same period (Tsai *et al.*, 2006). In this study, antihypertensive effect cannot be attributed solely to the *L. acidophilus* species, since several probiotics were used, thus promoting a synergistic action, which did not occur in our study. In addition, the effects on BP induced by probiotic products have been shown to be strain dependent (Gadelha *et al*., 2022). Reinforcing this idea, strains such as *Limosilactobacillus fermentum* (former *Lactobacillus fermentum*) CECT5716 plus *Bifidobacterium breve* CECT7263 (Robles-Veras*et al.*, 2020) or *Lacticaseibacillus casei* (former *Lactobacillus casei*) C1 (Yap *et al.*, 2016) administered to young SHR rats for 13 or 08 weeks, respectively, were able to reduce MAP and SAP. Despite attenuating the BP increase, these strains also did not prevent the onset of hypertension. The inability to avoid the hypertension is probably due to the complexity of the pathophysiological mechanisms present in the chosen model.

Regardless of the effect on BP levels, the strains analyzed in previous studies (Robles-Veras *et al.*, 2020; Yap *et al.*, 2016) as well as LA5 in our study have promoted cardioprotective effects that may reflect beneficial in hypertensive conditions.

There is a close link between sympathetic overactivity and hypertension (Grassi *et al.*, 2015). In the SHR model, elevation of sympathetic drive precedes the BP increase, and directly contributes to target organ damage (Dai *et al.*, 2018). The ability of probiotics to regulate autonomic activity has already been described in some studies with the *Lactobacillus* genus in Wistar rats (Tanida *et al.*, 2005) and Wistar rats fed a high-fat diet (HFD) (Cavalcante *et al.*, 2019; Ferreita *et al.*, 2022; Oliveira *et al.,* 2020) or rat offspring exposed to a maternal HFD (do Nascimento *et al*., 2022). Studies evaluating the effect of *Lactobacillus* strains on the sympathetic activity of SHR have not yet been performed. In the present study, we found that oral supplementation with LA5 was able to reduce the waves of the LF component of the HRV in SHR animals, suggesting that this probiotic reduces the sympathetic veractivity present in the hypertension.

The mechanisms by which probiotic bacteria reduce sympathetic activity are not fully understood, but evidence suggests that GABAergic signaling (Bao and Chi, 2016; Hussin *et al.*, 2020; Zareian *et al.*, 2015); reduction of inflammation and oxidativestress (Geeta and Yadav, 2017; Moura *et al.*, 2016; Pan, 2017; Zubcevic *et al.*, 2019) may play an essential role in this regard. In this study, we found that LA-5 reduced lipid peroxidation in the serum of animals belonging to the SHA+LA5 group, suggesting that oxidative stress reduction contributes, at least in part, to the decrease of sympathetic activity present in the hypertensive animals. We did not investigate the effect of LA5 on inflammation or GABAergic signaling. Future studies are needed to investigate the involvement of these mechanisms in the sympatholytic effect seen after supplementation with LA5.

Sympathetic hyperactivity and oxidative stress contribute to baroreflex dysfunction. Baroreflex monitors changes in BP in each heartbeat and promotes changes in BP and HR in order to compensate for these variations (Irigoyen *et al.*, 2001; Krieger, 1964; Tu *et al.*, 2019). In hypertension, the baroreflex is dysfunctional, with a reduction in its sensitivity. Interestingly, animals supplemented with LA-5 orally for 08 weeks showed an improvement in their baroreflex sensitivity. The antioxidant capacity of LA-5 may contribute to the observed effect. Contrary, *L. fermentum* 296, alone or in a mixture (*L. fermentum* 139, *L. fermentum* 263, and *L. fermentum* 296) reduced sympathetic activity in animals with hypertension but did not change the baroreflex sensitivity (Cavalcante *et al.*, 2019; Ferreira *et al.*, 2022; Oliveira *et al.*, 2020), confirming that the cardiovascular effects induced by probiotic bacteria are strain dependent.

Hypertension is closely related to endothelial dysfunction, commonly recognized by the loss of the endothelial ability to perform its functions in modulating vascular tone (Dinh *et al.*, 2014). An important factor for endothelial dysfunction involves the increased production of reactive oxygen species (ROS), and a decrease on the nitric oxide (NO) levels, impairing the relaxation of smooth muscle vascular cells (Kobayasi *et al.*, 2010; Roberts *et al.*, 2005; Tschudi *et al.*, 1996). As expected, the SHR group presented an attenuation of the response to ACh, indicating an impairment in the functioning of the endothelial layer in producing vasorelaxation. The group supplemented with LA5 showed a better relaxing response to ACh, indicating that the probiotic prevented hypertension-related endothelial dysfunction. Similarly, the strain *L. fermentum* CECT5716 (LC40) prevented endothleial dysfunction in a lupus model induced by TLR-7 activation (de la Visitación *et al.*, 2021); genetic SLE model (Toral *et al.*, 2019); in the tacrolimus-induced hypertension (Toral *et al.*, 2018) and in SHR model (Robles-Veras *et al.*, 2020).

In addition to improving ACh relaxation, we found an improvement in the relaxing response to the NO donor, SNP, in the isolated vessels of the SHR+LA5 group, indicating that

LA5 improves the sensitivity of the smooth muscle layer to NO. Overall, oral supplementation with LA-5 for 8 weeks prevented hypertension-related impairment in the vascular function. Although we did not assess serum NO levels, we hypothesized that reduced oxidative stress contributed to an increase in the NO bioavailability. This event is related to improvement of the endothelial function and DAP decrease observed in the SHR+LA5 group. There was no significant difference in the vascular reactivity to Phe between the groups.

Numerous studies have been carried out with different species of the *Lactobacillus* genus in the SAH, very few evaluated the *L. acidphilus*. Therefore, the present work expands our knowledge about the impact of *L. acidophulis,* specifically the LA-5 strain, in the hypertension.

5. Conclusion

Our data demonstrate that the consumption of *L. acidophilus* LA-5, at a dose of 10^9 CFU, for eight weeks reduced the sympathetic overactivity, restored baroreflex sensitivity, and improved the vascular reactivity in spontaneously hypertensive rats. These effects were accompanied by a modest reduction in DAP in these animals. The decrease of the peripheral oxidative stress contributes, at least in part, to the seen cardiovascular effects. These results indicate that LA5 supplementation may be an effective adjuvant for prevention or treatment of the SAH. Altogether, these findings encourage us to carry out new studies in order to deepen the knowledge about the mechanisms by which the LA5 strain exerts the demonstrated effects. Clinical trials may also be carried out to evaluate the preventive or therapeutic potential of LA5 in hypertensive patients.

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Conflict of interest

The authors declare no competing interests.

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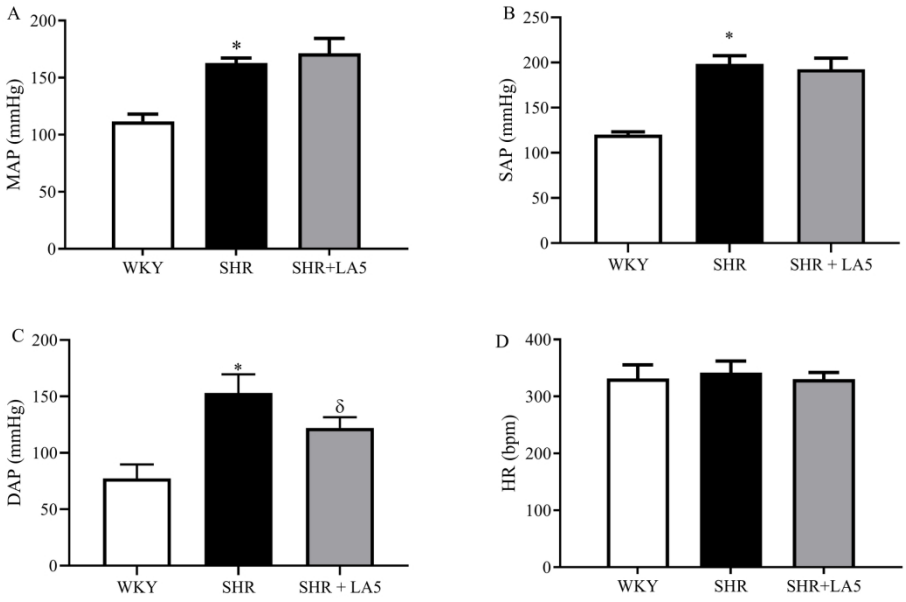

Figure 1 – Effects of L. acidophilus LA-5 on MAP(A), SAP(B), DAP(C) and Heart Rate (D) of hypertensive rats. Groups: Normotensive control (WKY, n=6); hypertensive control (SHR, n=6) and hypertensive SHR supplemented with LA5 for 8 weeks of oral treatment with LA5 (SHR+LA5, n=5). Values expressed as mean ± standard error of mean and $p<0.05$. * vs. CTL; δ vs. SHR.

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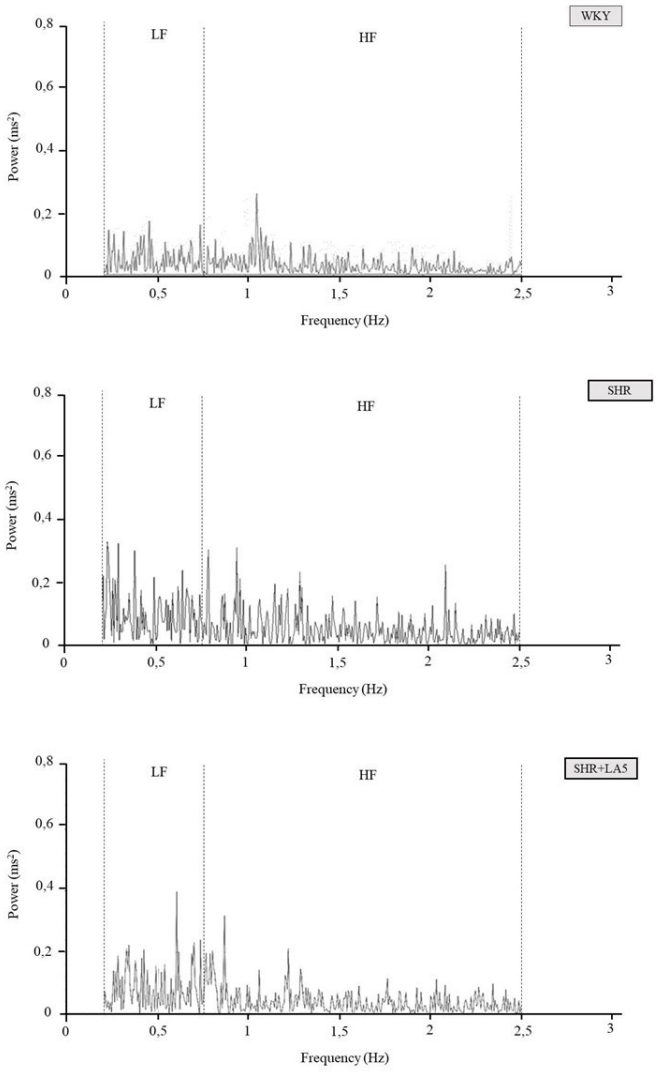

Figure 2 – Evaluation of the effect of LA5 supplementation for 8 weeks on spectral analysis of heart rate variability in animals with hypertension. A - Representative traces of the spectral analysis of one animal from each group.

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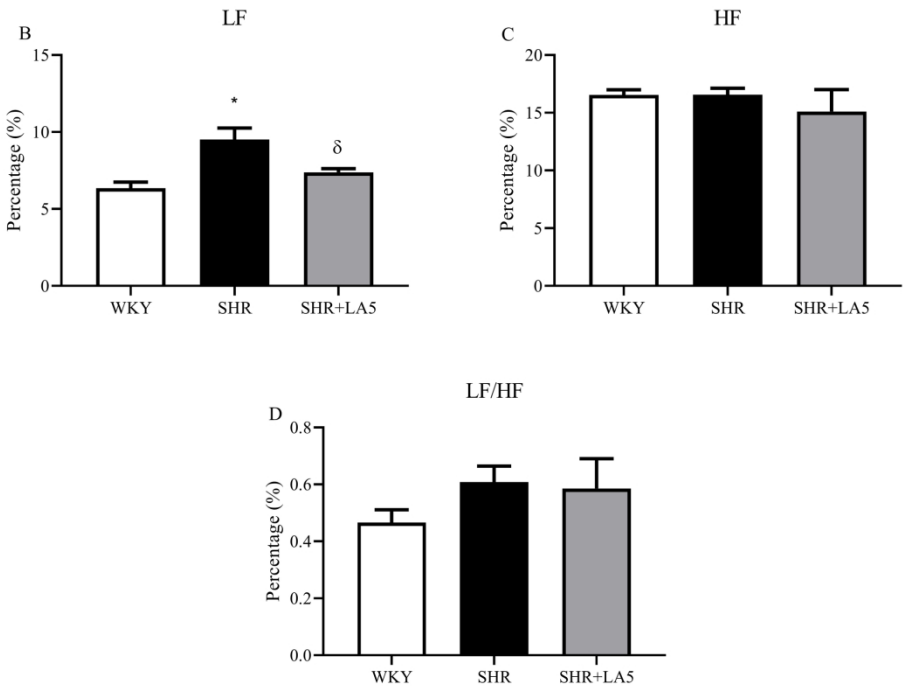

Figure 2 – Evaluation of the effect of LA5 supplementation for 8 weeks on spectral analysis of heart rate variability in animals with hypertension. B –Low frequency (LF), C – High frequency (HF) and D – Balance of LF/HF. Groups: Normotensive control (WKY, n=6); hypertensive control (SHR, n=6) and hypertensive SHR supplemented with LA5 for 8 weeks of oral treatment with LA5 (SHR+LA5, n=5). Values expressed as mean ± standard error of mean and $p<0.05$. * vs. CTL; δ vs. SHR.

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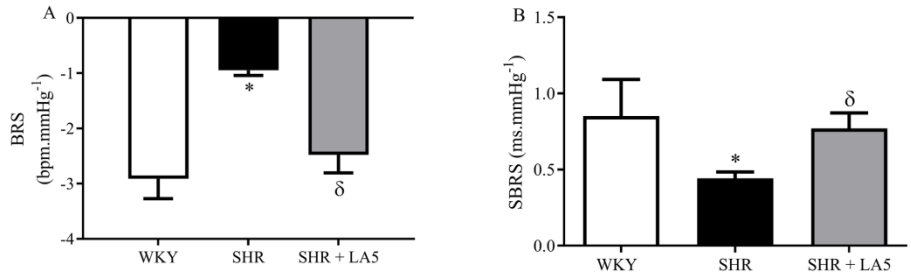

Figure 3 – Effects of LA5 probiotic supplementation on baroreflex sensitivity induced by vasoactive drugs (A) and spontaneous baroreflex(B) of hypertensive rats. Groups: Normotensive control (WKY, n=6); hypertensive control (SHR, n=6) and hypertensive SHR supplemented with LA5 for 8 weeks of oral treatment with LA5 (SHR+LA5, n=5). Values expressed as mean ± standard error of mean and $p<0.05$. * vs. CTL; δ vs. SHR. The vertical bars represent the mean ± SEM of the slope of each group, *$p < 0.05$ vs. WKY and δ$p <0.05$ vs. SHR. The difference between the groups was analyzed by one-way ANOVA, followed by Tukey's post hoc test.

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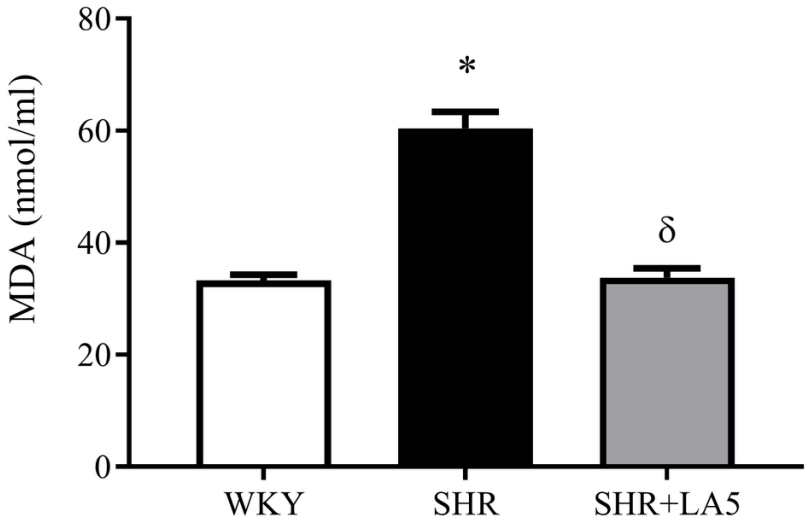

Figure 4 - Effect of oral LA5 supplementation for 8 weeks in plasma of animals that received oral treatment with LA5. Groups: Normotensive control (WKY, n=6); hypertensive control (SHR, n=6) and hypertensive SHR supplemented with LA5 for 8 weeks of oral treatment with LA5 (SHR+LA5, n=5). Values expressed as mean ± standard error of mean and $p<0.05$. * vs. CTL; δ vs. SHR.

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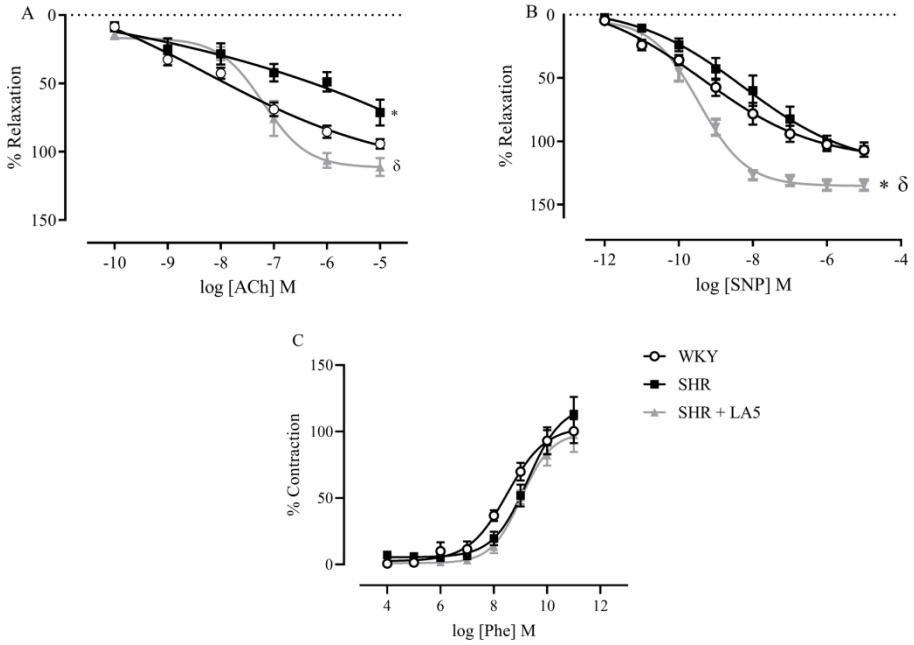

Figure 5 – Effect of relaxation promoted by the cumulative concentration of acetylcholine (ACh) (A) in mesenteric rings and relaxation promoted by the cumulative concentration of sodium nitroprusside (SNP) (B) in mesenteric rings of animals treated with LA5 for 8 weeks oral route. Effect of contraction promoted by cumulative concentration of phenylephrine (Phe) in mesenteric rings (C). Groups: Normotensive control (WKY, n=6); hypertensive control (SHR, n=6) and hypertensive SHR supplemented with LA5 for 8 weeks of oral treatment with LA5 (SHR+LA5, n=5). Values expressed as mean ± standard error of mean and $p<0.05$. * vs. CTL; δ vs. SHR.

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Colaboração no trabalho intitulado “Borneol reduces sympathetic vasomotor hyperactivity and restores depressed barorreflex sensitivity in rats with renovascular hypertension” na revista Hypertension Research com fato de impacto 5,528 e Qualis A2 para Farmácia pela Plataforma Sucupira da CPAES.

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ARTICLE

Borneol reduces sympathetic vasomotor hyperactivity and restores depressed baroreflex sensitivity in rats with renovascular hypertension

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Abstract

Borneol is a bicyclic monoterpene that has long been used in traditional Chinese medicine to increase blood–brain barrier permeability and has shown promising cardiovascular effects. The present study aimed to evaluate the effect of borneol on vascular tone, blood pressure, autonomic function, and baroreflex sensitivity in normotensive and hypertensive rats. A combination of in vitro and in vivo assays was performed in 2-kidneys-1-clip hypertensive rats (2K1C) and their controls (sham). We assessed the in vivo effect of oral treatment with borneol on blood pressure, heart rate, autonomic function, and baroreflex sensitivity in sham and 2K1C rats. Additionally, the vasorelaxant effect of borneol in the superior mesenteric artery isolated from rats and its mechanism of action were evaluated. Oral administration of borneol (125 mg/kg/day) reduced blood pressure, sympathetic vasomotor hyperactivity, and serum oxidative stress and improved baroreflex sensitivity in 2K1C rats. In vessel preparations, borneol induced endothelium-independent vasodilatation after precontraction with phenylephrine or KCl (60 mM). There was no difference in the vascular effect induced by borneol in either the 2K1C or the sham group. In addition, borneol antagonized the contractions induced by $CaCl_2$ and reversed (S)-(−)-Bay K 8644-induced contraction. These data suggest that borneol presents antihypertensive effects in 2K1C rats, which is associated with its ability to improve autonomic impairment and baroreflex dysfunction. The borneol-induced relaxation in the superior mesenteric artery involves L-type Ca^{2+} channel blockade. This vascular action associated with the antioxidant effect induced by borneol may be responsible, at least in part, for the in vivo effects induced by this monoterpene.

Keywords 2-kidneys-1-clip hypertension · Ca^{2+} channel blockade · Mesenteric superior artery · Vasorelaxation

Introduction

Hypertension is a multifactorial clinical condition that is difficult to treat due to the complexity of the underlying pathophysiological mechanisms. Two or more drugs are usually used to treat hypertension in routine clinical practice. However, a specific group of patients is resistant to the treatment, meaning that their blood pressure does not decrease even with a combination of three or more drugs [1]. At a time when cardiovascular diseases are the leading cause of death from noncommunicable diseases worldwide, the search for new therapeutic alternatives to improve the effectiveness of treatment and the quality of life of these patients is timely and of extreme clinical interest.

Medicinal plants represent an important source for new or adjuvant therapies together with existing therapies [2]. In this regard, borneol is a monoterpene present in several medicinal plants and has been demonstrated to have many biological activities, such as antibacterial [3], antifungal [4], and analgesic [5] activities. In the cardiovascular system, borneol relaxes rings of the thoracic aorta isolated from normotensive rats [6, 7] and has antihypertensive properties in rats with L-NAME-induced hypertension [8].

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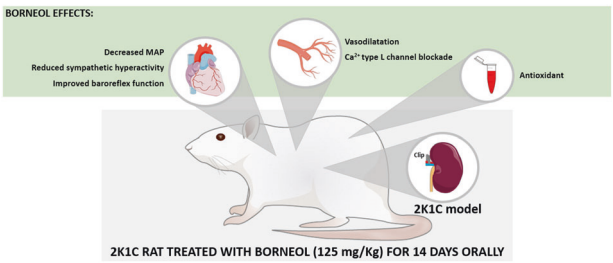
Graphical Abstract

Schematic illustration of the effects induced by borneol on various cardiovascular parameters in rats with 2-kydneys-1-clip hypertension



The hypertensive process is closely related to baroreflex dysfunction, sympathetic hyperactivity, increased peripheral vascular resistance, and systemic oxidative stress [9–11]. These characteristics are also present in several experimental models of hypertension, such as renovascular hypertension, and are important aspects for antihypertensive drugs [12, 13]. In this context, our hypothesis is that borneol reduces blood pressure in rats with renovascular hypertension and reduces sympathetic hyperactivity, baroreflex dysfunction, and oxidative stress present in these animals. The vasorelaxant and antioxidant properties are involved in the effects induced by this monoterpene.

Therefore, we aimed to evaluate the borneol-induced vasorelaxant activity on superior mesenteric arteries from normotensive and hypertensive rats and the effect of oral treatment with borneol on cardiovascular parameters and serum oxidative stress in these animals. Renovascular hypertension was induced by the 2-kidneys-1-clip (2K1C) model, which is characterized by high oxidative stress with reduced baroreflex sensitivity and sympathetic hyperactivity [14, 15].

Methods

Animals

All experimental procedures were approved by the Animal Care and Use Committee of the Federal University of Paraiba and complied with the ARRIVE guidelines. Male Wistar rats (*Rattus norvegicus*) weighing between 200 and 300 g were housed under controlled temperature (22 ± 1 °C) and humidity conditions with a 12 h light/dark cycle and had free access to standard chow (Labina®, Purina) and water. The specific number of animals per group is presented in each protocol.

For the in vivo experiments, animals were subdivided into the (1) normotensive group treated with vehicle (sham); (2) normotensive group treated with borneol (sham + borneol); (3) hypertensive group treated with vehicle (2K1C); and (4) hypertensive group treated with borneol (2K1C + borneol). Borneol was administered by gavage for 14 days at a 125 mg/kg/day dose based on a previous study [8, 16]. For the in vitro experiments, rats were divided into the sham (normotensive) and 2K1C (hypertensive) groups. The vehicles used were cremophor and mineral oil for in vitro and in vivo experiments, respectively.

Induction of renovascular hypertension

For the induction of renovascular hypertension, the 2K1C model was performed as previously described [17], adapted from the Goldblatt model [15]. Rats weighing between 160 and 170 g were anesthetized with ketamine and xylazine (75 mg/kg and 10 mg/kg, respectively, i.p.), the right renal artery was carefully exposed through a retroperitoneal incision, and a silver clip (0.2 mm opening) was implanted around the artery to decrease renal blood flow. After 6 weeks, the hypertension was established. Sham animals underwent the same surgical process, except for the implantation of the silver clip. The weight of the animals was monitored during the entire experimental period (time of induction of 2K1C hypertension and treatment); furthermore, at the end of the protocol, the kidneys were removed for evaluation.

Drugs and solutions

For the in vivo assays, we used acetylcholine hydrochloride (ACh); atropine; hexamethonium chloride; mineral oil; (−) phenylephrine hydrochloride (Phe); sodium nitroprusside (NPS) (Sigma–Aldrich, Brazil); heparin (Parinex® 5000 IU/ml - Hypolabor Brazil Laboratory); ketamine hydrochloride (Quetamin® Laboratory - Vetnil, Brazil); and xylazine (Dopaser® - Calier Laboratory, Spain). All drugs were diluted in saline solution.

The following substances were used in in vitro experiments: acetylcholine hydrochloride (ACh), cremophor, (−)

phenylephrine hydrochloride (Phe), (S)-(−)-Bay K 8644, and tetraethylammonium (TEA) (Sigma–Aldrich, Brazil). All drugs were dissolved in distilled water. We also used a nutritive solution (Tyrode solution) with the following composition (in mM): NaCl, 158.3; KCl, 4.0; $CaCl_2$, 2.0; $MgCl_2$, 1.05; NaH_2PO_4, 0.42; $NaHCO_3$, 10.5; and glucose, 5.6 [18]. A modified Tyrode solution was used in certain protocols, as a nominally Ca^{2+}-free solution, as well as a K^+-depolarizing solution (KCl 60 mM), with or without $CaCl_2$.

In vivo experiments

Blood pressure and heart rate recordings

Six weeks after hypertension induction or sham procedure, the sham and 2K1C rats were treated with vehicle or borneol for 14 days. On the 14th day, vascular catheters were implanted, as reported [19], into the femoral artery and vein for blood pressure and heart rate measurements and drug administration, respectively. After a 24 h recovery from catheter implantation surgery, pulsatile arterial pressure (PAP) of conscious rats was recorded using a pressure transducer (MLT0380/D, ADInstruments, Sydney, Australia) coupled to an amplifier and an acquisition system (PowerLab, ADInstruments, Bella Vista, NSW, Australia) using specific software (LabChart 5.0, ADInstruments, Bella Vista, NSW, Australia). Systolic arterial pressure (SAP), mean arterial pressure (MAP), diastolic arterial pressure (DAP), and heart rate (HR) were derived from PAP online.

Autonomic function and baroreflex sensitivity studies

As previously described [20], the autonomic function was assessed in conscious freely moving rats after treatment with borneol using a pharmacological method with intravenous injections of hexamethonium (30 mg/kg), a ganglionic cholinergic blocker, and atropine (2 mg/kg), a muscarinic cholinergic blocker, to evaluate autonomic vascular sympathetic and parasympathetic function, respectively [20]. Each injection was separated by a 3 h recovery period. Changes in MAP (ΔMAP) or HR (ΔHR) were calculated after administration of these blockers and compared between different groups.

Additionally, an indirect evaluation of the sympathetic autonomic modulation of vascular resistance was performed by analyzing blood pressure (BP) variability in the frequency domain, as previously described [20]. A total of 10 min of PAP recordings obtained at baseline were processed by computer software (LabChart, ADInstruments) that employs an algorithm to detect beat-to-beat inflection points in the pulse pressure signal. Beat-by-beat series with systolic BP (SBP) values were generated and loaded into customized software (CardioSeries V2.4, http://www.danielpenteado.com). The power of the oscillatory components obtained from rats belonging to all groups was integrated into low-frequency bands (LF; 0.2–0.75 Hz) and high-frequency bands (HF; 0.75–3.0 Hz), and the results were expressed as absolute values ($mmHg^2$). AP oscillations in the LF range are representative of the modulatory effects of the sympathetic activity that controls vascular tone, and AP oscillations in the HF range refer to respiratory or parasympathetic modulation. In addition, the sympathovagal index was evaluated by the LF/HF ratio [21].

To evaluate the baroreflex sensitivity (BRS), after 40 min of AP and HR baseline recordings, reflex responses were obtained using the vasoactive drugs Phe (8 μg/kg) and SNP (25 μg/kg) according to the modified Oxford method, as previously described [19]. Additionally, spontaneous baroreflex sensitivity (SBRS) was evaluated using baseline recording and the sequence method [19] with Hemolab software (HaraldStaussScientific, Analyzer version 9.3).

Oxidative stress assay

Serum samples from these animals were used for the measurement of thiobarbituric acid reactive substances (TBARS) as previously described [19]. In this assay, malondialdehyde (MDA), a final product of lipid peroxidation, reacts with thiobarbituric acid to produce a red complex, which works as an indicator of serum oxidative stress. The analyses were performed in duplicate, and the absorbance was determined on a ChemWell T spectrophotometer at a wavelength of 532 nm. A standard MDA curve with concentrations from 0 to 100 nmol/ml was constructed before reading the samples, and the results were expressed as nmol MDA/ml.

In vitro experiments

Evaluation of the vasorelaxant effect induced by borneol in the superior mesenteric artery

Sham and 2K1C rats were sacrificed, and the superior mesenteric artery was removed and cleaned of perivascular tissue in Tyrode solution. Vessels were cut into segments (2–5 mm in length), mounted vertically on two Δ-shaped stainless-steel wires in an organ bath chamber (10 mL) with Tyrode solution at 37 °C and pH 7.4, and continuously bubbled with 95% O_2 and 5% CO_2. All preparations were stabilized under a resting tension of 0.75 g for 1 h. At this time, the Tyrode solution was changed every 15 min to prevent the accumulation of metabolites. The force of contraction was isometrically recorded by a force transducer (Panlab Organ Bath Systems, ML0146/C-V, MA, USA) connected to an acquisition system (PowerLabTM, ADInstruments, Australia).

The endothelial integrity was verified by the ability of acetylcholine (10 μM) to induce more than 80% relaxation of vessels precontracted with phenylephrine (10 μM). Less than 10% relaxation to acetylcholine was taken as evidence that the vessel segments were functionally denuded of the endothelium. Segments with or without functional endothelium were contracted with Phe (10 μM), and a concentration–response curve to borneol (10^{-12} to 10^{-3} M) was constructed. In another set of experiments, borneol was added to precontracted vessels with a depolarizing solution of 60 mM KCl.

To evaluate the involvement of K^+ channels in the effect induced by borneol on the mesenteric superior artery, some preparations were exposed to TEA (3 mM), which is a nonselective K^+ channel blocker [22]. This inhibitor was added 30 min before the application of Phe. In addition, to assess the participation of Ca^{2+} channels in borneol-induced relaxation, we evaluated the effect of borneol on $CaCl_2$-induced contractions. The vascular segments were exposed to a depolarizing solution with KCl (60 mM) and then washed in Tyrode solution (nominally Ca^{2+} free). After that, the rings were exposed to a depolarizing KCl (60 mM) solution (nominally Ca^{2+} free) to obtain a concentration–response curve to $CaCl_2$ (10^{-6} to 3×10^{-2} M), cumulatively added to the medium. The process was repeated, and new concentration–response curves to $CaCl_2$ were reached in the presence of different concentrations of borneol (3×10^{-5}, 10^{-4} or 10^{-3} M). The results were expressed as percentages of the maximal response for the $CaCl_2$ only-induced response, and the curves were statistically compared. Additional experiments were performed in rings precontracted with (S)-(−)-Bay K 8644 (200 nM). In the tonic phase of contraction induced by this Ca_V-L activator, borneol was cumulatively added (10^{-12} to 10^{-3} M). The relaxation induced by borneol has always been expressed as a reverse percentage of contraction induced by contracting agents.

Statistical analysis

Data are expressed as the mean ± S.E.M. Data were analyzed by one-way or two-way ANOVA for multiple comparisons between means when appropriate, followed by Tukey's test. Statistical comparisons were performed using Prism 6 (GraphPad Software, San Diego, CA). The difference was considered significant when $p < 0.05$. The specific test is presented with the results for each protocol.

Results

Effect of oral treatment with borneol on body and kidney weight

Table 1 shows that treatment with borneol or vehicle did not affect body weight. A reduction in the right kidney weight of the 2K1C rats was observed and compared with the left kidney from the same group and the kidney from the sham group. These values confirm the blood perfusion reduction of the right kidney in hypertensive animals due to obstruction of the renal artery from clip insertion.

Borneol decreases blood pressure in rats with renovascular hypertension

Figure 1A shows a representative tracing of the baseline pulsatile arterial pressure (PAP), mean arterial pressure (MAP), and heart rate (HR) of one animal from each group treated or not with borneol.

As expected, the 2K1C group presented higher MAP levels than the sham group (169 ± 9, $n = 8$ vs. 119 ± 2 mmHg, $n = 7$, $p < 0.05$). The MAP values presented by 2K1C rats were associated with a reduction in the right kidney and confirmation of renovascular hypertension. Treatment with borneol was able to reduce MAP in the 2K1C + borneol

Table 1 Body, right kidney, and left kidney weights of the animals

Groups	Body weight variation (g)	Right kidney (g)	Right kidney/ Body weight (mg/g)	Left kidney (g)	Left kidney/ Body weight (mg/g)
SHAM $n = 8$	35.5 ± 6	1.19 ± 0.06	3.9 ± 0.2	1.2 ± 0.02	4 ± 0.1
SHAM + borneol $n = 6$	28.25 ± 3	1.17 ± 0.02	4.1 ± 0.1	1.1 ± 0.02	4 ± 3
2K1C $n = 7$	27.25 ± 5	0.92 ± 0.02*, #	3.3 ± 1.7	1.4 ± 0.02	4.8 ± 6
2K1C + borneol $n = 8$	16.92 ± 3	0.98 ± 0.11*, #	3.3 ± 0.3	1.4 ± 0.04	4 ± 0.3

Values represent the mean ± SEM, *$p < 0.05$ vs. sham; #$p < 0.05$ vs. 2K1C left kidney. The difference between groups was analyzed by one-way ANOVA, followed by Tukey's post hoc test

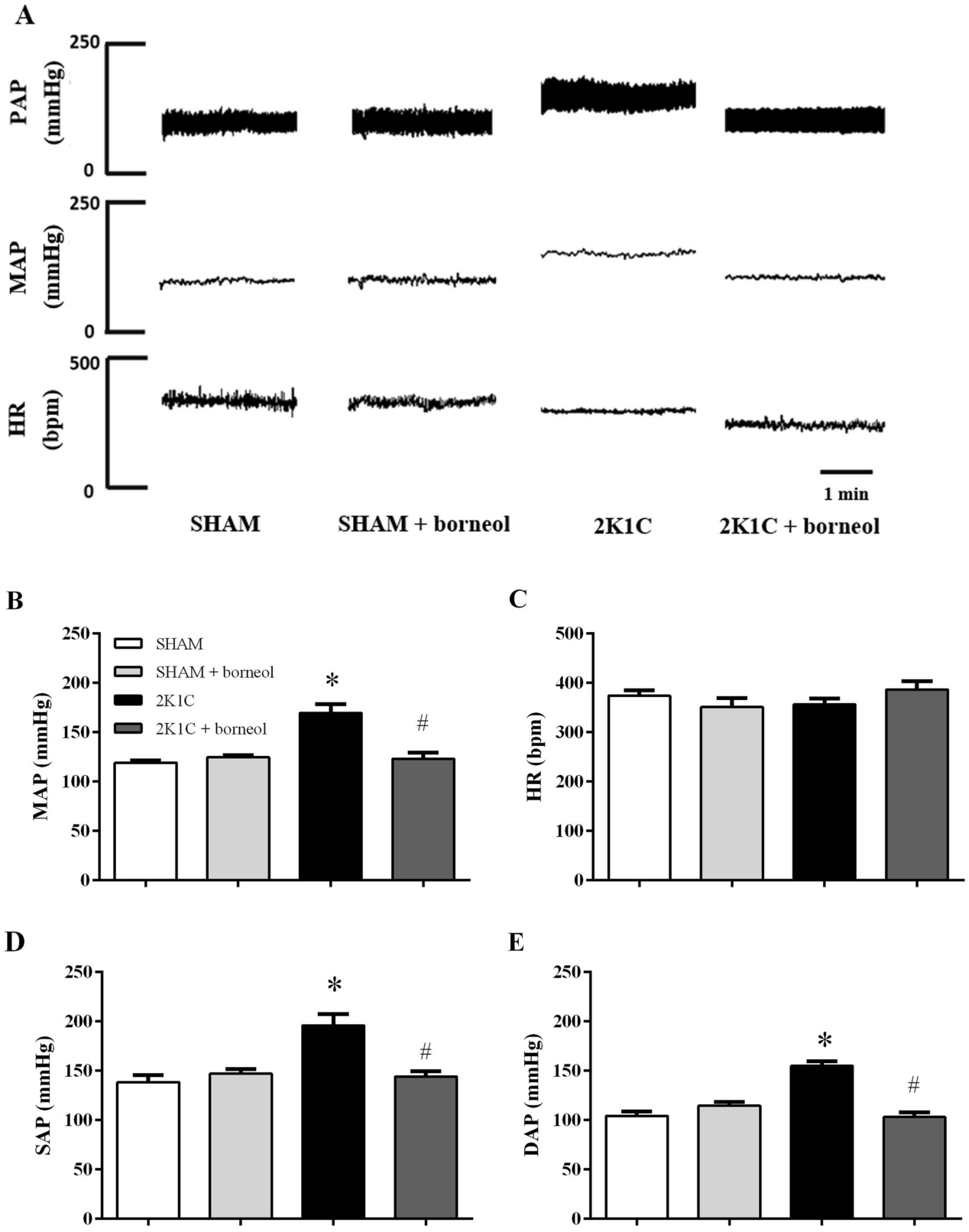


Fig. 1 **A** Representative tracings of one animal from each group demonstrating the pulsatile arterial pressure (PAP), mean arterial pressure (MAP), and heart rate (HR). Effect of oral treatment with borneol (125 mg/kg/day) for 14 days on the MAP (**B**), HR (**C**), SAP (**D**), and DAP (**E**). The difference between groups was analyzed by one-way ANOVA, followed by Tukey's post hoc test. The vertical bars represent the mean ± SEM, $^{*}p < 0.05$ vs. sham, and $^{\#}p < 0.05$ vs. 2K1C

group (123 ± 6 mmHg, $n = 7$, $p < 0.05$). No changes were detected in the sham + borneol group when compared to the normotensive control (124 ± 2 mmHg, $n = 6$), demonstrating that borneol acts only in the presence of hypertension (Fig. 1B).

We observed similar effects when we analyzed systolic and diastolic blood pressure separately. SAP and DAP were higher in the 2K1C group than in the sham group [SAP: 196 ± 11 vs. 138 ± 7 mmHg; DAP: 155 ± 4 vs. 104 ± 5 bpm, respectively, $p < 0.05$), and the 2K1C group treated with borneol showed SAP and DAP levels (144 ± 5 mmHg and 103 ± 4 bpm, respectively) that were similar to those of the sham group (Fig. 1D, E).

There was no difference in the HR between all groups (sham: 374 ± 11 bpm; sham + borneol: 350 ± 19 bpm; 2K1C: 356 ± 12 bpm; 2K1C + borneol: 386 ± 17 bpm) (Fig. 1C).

Borneol reduces sympathetic autonomic activity in rats with renovascular hypertension

Sympathetic and vagal tone was measured as ΔMAP or ΔHR after pharmacological blockade with hexamethonium and atropine, respectively (Fig. 2). Sympathetic vasomotor activity was increased in the 2K1C group compared to the sham group ($\Delta MAP = -73.43 \pm 4.9$, $n = 6$ vs. -40.3 ± 6.1 mmHg, $n = 6$, respectively, $p < 0.05$), while vagal tone was blunted ($\Delta HR = 54.73 \pm 8.54$, $n = 6$ vs. 116.9 ± 11.44 bpm, $n = 10$ $p < 0.05$) (Fig. 2A, B). Borneol treatment reduced sympathetic vasomotor hyperactivity in 2K1C rats (-48.5 ± 7.3 mmHg, $n = 8$, $p < 0.05$) compared to the untreated 2K1C group (Fig. 2A). However, borneol did not change the vagal tone in these animals.

To confirm the changes in sympathetic vasomotor activity, we assessed the SBP variability of all groups. Representative spectra (Fig. 2C) highlight the expected increase in oscillation in the LF range in 2K1C compared with sham rats (9.16 ± 0.52, $n = 7$ vs. 4.24 ± 0.95 $mmHg^2$, $n = 8$, $p < 0.05$, respectively, Fig. 2D). Treatment with borneol decreased the magnitude of oscillation in the LF range in 2K1C (3.59 ± 0.68 $mmHg^2$, $n = 7$, $p < 0.05$, Fig. 2D) and did not produce significant changes in sham + borneol rats ($n = 5$). No significant changes related to HF or the sympathovagal index (LF/HF) were observed in any of the groups (Fig. 2E, F).

Borneol improves baroreflex sensitivity in rats with renovascular hypertension

To determine the role of borneol on BRS, baroreceptor reflex responses were initially evaluated after bolus administration of Phe and SNP. 2K1C rats presented a reduced BRS when compared to the sham group (-1.3 ± 0.1, $n = 7$ vs. -2.64 ± 0.3 $bpm.mmHg^{-1}$, $n = 10$, $p < 0.05$). Of note, oral treatment with borneol (125 mg/kg/day) was able to restore the baroreflex sensitivity in 2K1C rats (-3.66 ± 0.34 $bpm.mmHg^{-1}$, $n = 7$, $p < 0.05$) (Fig. 3A). The SBRS was significantly impaired in the 2K1C group compared to the sham group (-1.38 ± 0.24, $n = 6$ vs. -2.2 ± 0.23 $bpm.mmHg^{-1}$, $n = 8$, $p < 0$, 05). Consistent with the previous data, borneol was also able to improve the SBRS (-3.56 ± 0.64 $bpm.mmHg^{-1}$, $n = 9$) (Fig. 3B).

Borneol reduces the level of serum MDA

Quantification of MDA, a final product of lipid peroxidation, was performed in all groups as an indirect measure of oxidative stress. As expected, 2K1C animals showed increased levels of MDA compared to the sham group (2K1C: 22.8 ± 1.35, $n = 5$ vs. 14.7 ± 0.58 nmol/mL, $n = 4$, Fig. 4), suggesting a higher degree of oxidative stress. Oral treatment with borneol for 14 days was able to reduce this indicator in the 2K1C + borneol group (15.5 ± 1.51 nmol/mL, $n = 3$, $p < 0.05$, Fig. 4). Treatment of sham animals with borneol did not modify this parameter (8.9 ± 1.84 nmol/mL, $n = 6$).

Borneol induces vasorelaxation in Phe- or KCl (60 mM)-precontracted mesenteric artery rings isolated from sham and 2K1C rats

Cumulative addition of borneol (10^{-12} to 10^{-3}) induced vasorelaxation in Phe-precontracted mesenteric rings from sham rats in the presence or absence of functional endothelium (ME = $76.4 \pm 6.5\%$, $n = 6$; ME = $84.7 \pm 3.9\%$, $n = 9$, respectively). A similar effect was observed in rings from the 2K1C group (ME = $89.2 \pm 9.3\%$, $n = 5$; ME = $98.3 \pm 5.0\%$, $n = 5$, respectively) (Fig. 5A), suggesting that borneol-induced relaxation is not dependent on the vascular endothelium. Additionally, no differences were observed for pD_2 values in PHE-precontracted rings from the 2K1C and sham groups with functional endothelium (5.8 ± 0.3 vs. 5.7 ± 0.4, respectively) or without endothelium (5.2 ± 0.3 vs. 5.1 ± 0.2, respectively).

The relaxation induced by borneol in depolarizing agent KCl 60 mM-precontracted rings without endothelium was not different when compared to Phe in sham (ME = $80.3 \pm 14\%$; $pD_2 = 4.67 \pm 0.26$, $n = 7$) and 2K1C rats (ME = $108.5 \pm 8.5\%$; $pD_2 = 4.66 \pm 0.17$, $n = 4$) (Fig. 5B, C), suggesting a common point in the relaxation pathway induced by this monoterpene, the Ca^{2+} channels.

Vasorelaxation elicited by borneol involves modulation of Ca^{2+} channels

Cumulative administration of $CaCl_2$ (10^{-5}– 3×10^{-3} M) promoted concentration-dependent contraction in

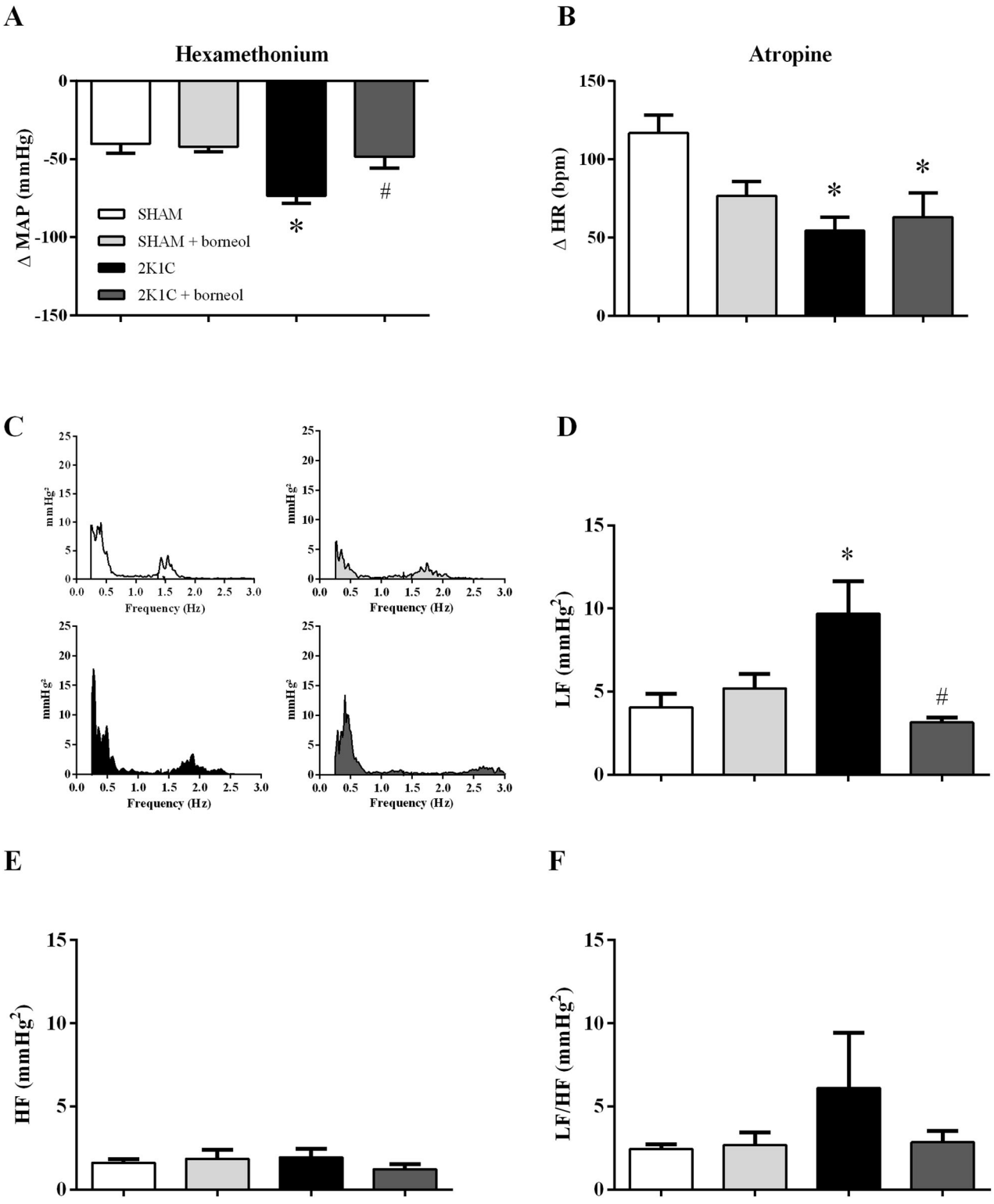


Fig. 2 Borneol reduces sympathetic hyperactivity in 2K1C rats. Effect of oral treatment with borneol (125 mg/kg/day) for 14 days on sympathetic (**A**) and parasympathetic activity (**B**) from the sham and 2K1C groups. Representative spectra of SBP (**C**), average magnitudes of LF (**D**) and HF (**E**) components of SBP and the sympathovagal index (**F**) from sham and 2K1C rats treated or not with borneol. The vertical bars represent the mean ± SEM, $^{*}p < 0.05$ vs. sham; $^{\#}p < 0.05$ vs. 2K1C. The difference between the groups was analyzed by one-way ANOVA, followed by Tukey's post hoc test

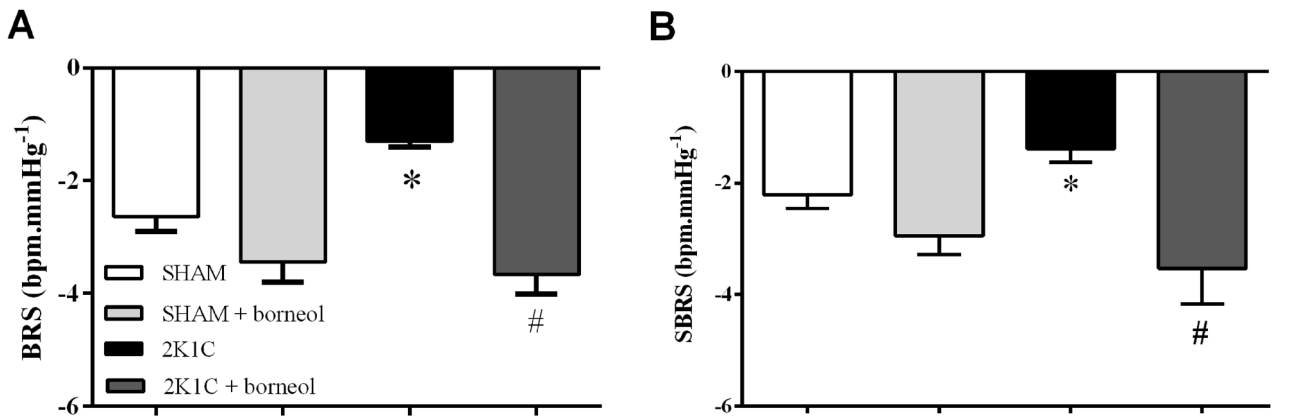


Fig. 3 Borneol restores baroreflex sensitivity in 2K1C rats. Effect of oral treatment with borneol (125 mg/kg/day) for 14 days on baroreflex sensitivity induced by vasoactive drugs (**A**) and spontaneous baroreflex sensitivity (**B**). The vertical bars represent the mean ± SEM of the slope of each group, $^{*}p < 0.05$ vs. sham and $^{\#}p < 0.05$ vs. 2K1C. The difference between the groups was analyzed by one-way ANOVA, followed by Tukey's post hoc test

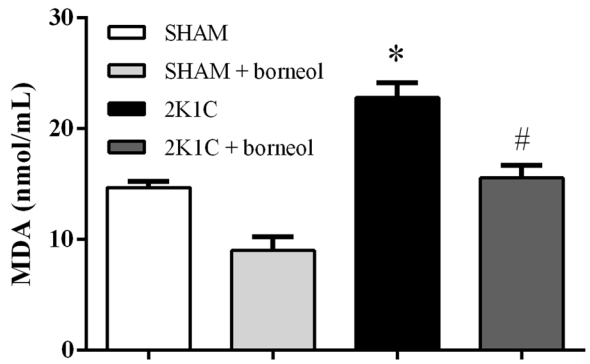


Fig. 4 Borneol reduces the level of serum MDA in 2K1C rats. The vertical bars represent the mean ± SEM, $^{*}p < 0.05$ vs. sham; $^{\#}p < 0.05$ vs. 2K1C. The difference between the groups was analyzed by one-way ANOVA, followed by Tukey's post hoc test

mesenteric artery rings isolated from sham (ME = 86.7 ± 6.3) (Fig. 6A) and 2K1C rats (ME = 82.7 ± 11.6) (Fig. 6B). Borneol at concentrations of 10^{-4} (in the sham group) and 10^{-3} M (in the sham and 2K1C groups) significantly reduced the maximal effect of $CaCl_2$ when compared to the curve control (sham: 38.9 ± 1.6% and 13.2 ± 6.5% vs. 86.7 ± 6.3; 2K1C: 56.2 ± 9.9% and 13 ± 6 % vs. 82.7 ± 11.6, respectively, $p < 0.05$, $n = 6$/group). This result suggests that blocking Ca^{2+} channels is part of the relaxant effect induced by borneol. The cumulative administration of borneol in (S)-(−)-Bay K 8644-precontracted rings confirmed this idea (ME = 90.9 ± 13.0; $n = 8$), demonstrating that L-type Ca^{2+} channels are involved in the effect induced by borneol (Fig. 6C). Nonselective blockade of K^{+} channels by TEA (3 mM) did not change the standard relaxing curve in response to borneol (ME = 111.5 ± 3.2%; $n = 10$ vs. 84.7 ± 3.9%, $n = 9$) (Fig. 6D), ruling out the involvement of these channels in the relaxation caused by borneol in this vascular bed.

Discussion

Here, we documented that oral treatment with borneol for 14 days decreased blood pressure in 2K1C rats without changes in the sham group or heart rate. Furthermore, the treatment was able to improve baroreflex sensitivity and to decrease sympathetic vasomotor hyperactivity and serum oxidative stress in hypertensive animals. Additionally, borneol induces relaxation in the superior mesenteric artery isolated from sham and 2K1C rats, and this effect involves L-type Ca^{2+} channel blockade.

The antihypertensive effect of borneol has already been demonstrated in L-NAME hypertension; [8] here, we demonstrate that borneol also reduces blood pressure in rats with renovascular hypertension. It is important to emphasize that we did not observe alterations in the HR of the sham and 2K1C animals in the 8th week post-clipping, when the cardiovascular parameters were evaluated. This is attributed to the model used, which no longer shows differences in HR from 4 or 6 weeks after induction of hypertension [17, 19, 20, 23, 24]. Treatment with borneol also did not change the HR of either group.

2K1C hypertension is based on renal blood flow and glomerular filtration rate reduction, which over 6 weeks promotes the loss of mass in the clipped kidney associated with hypertrophy of the nonclipped kidney [14, 15]. This kidney hypertrophy was observed in our animals in association with the blood pressure increase, confirming the effectiveness of the experimental model adopted. Renal blood flow reduction stimulates the renin-angiotensin-aldosterone system (RAAS), which in turn provides an increase in sympathetic tone, blood volume, and vasoconstriction, among other effects [9, 10, 24, 25]. In this model of 2K1C hypertension, plasmatic renin and angiotensin II levels are elevated until the 4th week after clip insertion;

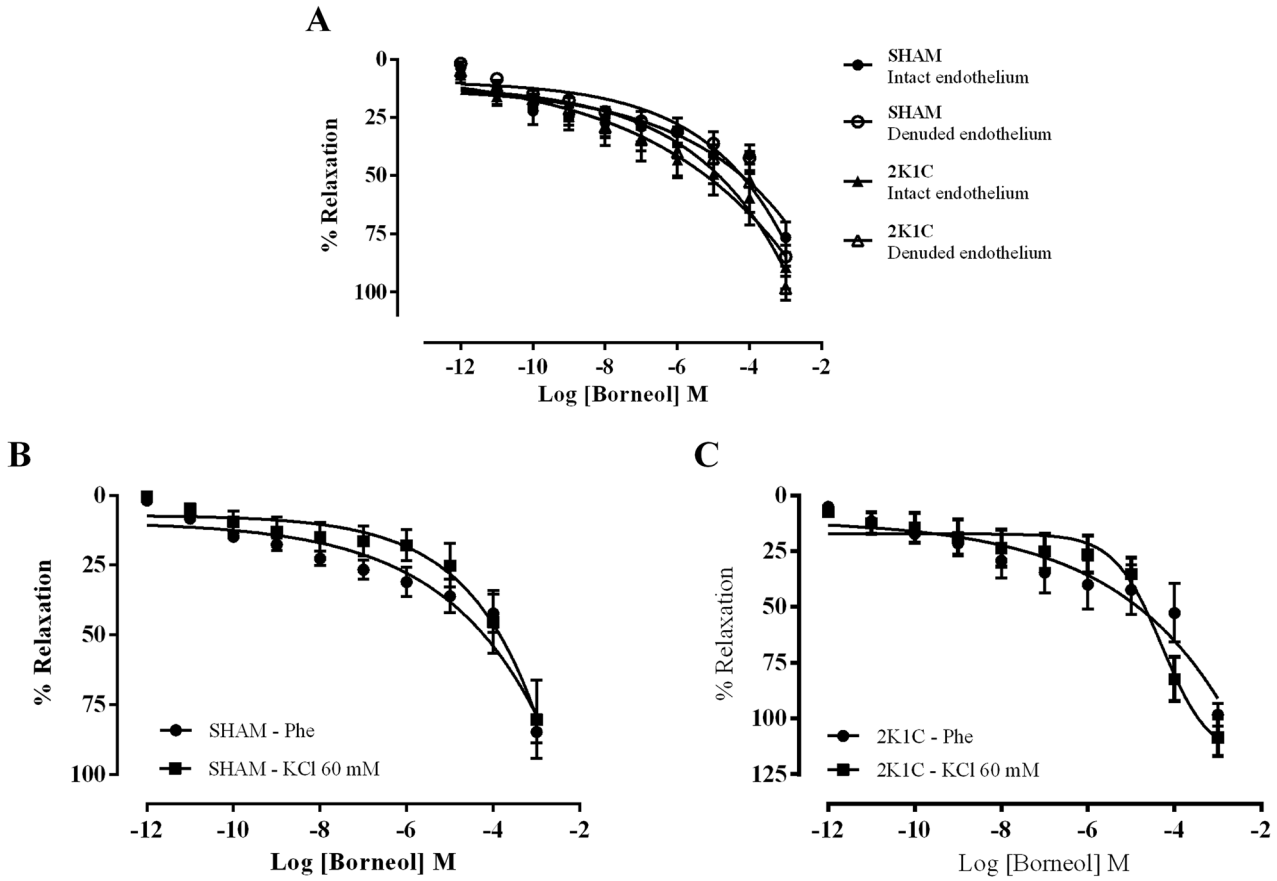


Fig. 5 Borneol-mediated vasorelaxation. Concentration–response curves to borneol (10^{-12} to 10^{-3}) in mesenteric artery rings isolated from sham and 2K1C rats precontracted with Phe (1 μM) in the presence or absence of functional endothelium (**A**). Concentration–response curves to borneol in rings without functional endothelium precontraction with Phe (1 μM) or KCl 60 mM in sham (**B**) and 2K1C rats (**C**). The results are expressed as the means ± SEM. The difference between groups was analyzed by two-way ANOVA

from that point on, hypertension is a consequence of this initial change in the RAAS and its effects [14, 26, 27]. It is noteworthy here that our treatment started in the 6th week after clipping, when renin and angiotensin II levels were already in the normal range. Nevertheless, changes in the autonomic control of blood pressure remain in 2K1C rats.

Cabral and Vasquez [28] had already demonstrated that in 2K1C rats, the HR and cardiac sympathetic outflow peaked at 15 days and returned to control levels by 30 days after the renal artery was clipped. On the other hand, sympathetic vasomotor activity seems to be more persistent and induces important vascular changes that contribute to the development and maintenance of 2K1C hypertension [23, 29, 30]. Due to this scientific evidence and the lack of effects induced by borneol on HR, we focused our analysis on sympathetic vasomotor activity using hexamethonium and spectral analysis of systolic blood pressure in the LF range. Borneol reduced sympathetic vasomotor hyperactivity in the 2K1C group.

Scientific evidence reveals that ROS accumulation in neural areas of blood pressure modulation, such as the subfornical organ, paraventricular nucleus, and rostral ventrolateral medulla, is involved in increased sympathetic outflow. This effect is associated with peripheral and central RAAS stimulation and baroreflex dysfunction [9, 31]. The close relationship between 2K1C hypertension, sympathetic hyperactivity, and baroreflex dysfunction has been demonstrated in several studies [24, 32–34]. One of the hypotheses by which oxidative stress increases sympathetic discharge is the modulation of ion channels by reactive species [35]. In addition, the increase in circulating ROS also reduces NO bioavailability and promotes vasoconstriction, favoring hypertension [36].

We found that oral treatment of hypertensive rats for 2 weeks with borneol was able to restore baroreflex sensitivity and reduce lipid peroxidation in animal serum, as shown in the TBARS assay. Corroborating our data, other authors [8] have also pointed out the antioxidant role of borneol in NO-deficient hypertension. In this context, we suggest that the antioxidant role of borneol may contribute, at least in part, to the in vivo cardiovascular effects observed in 2K1C rats.

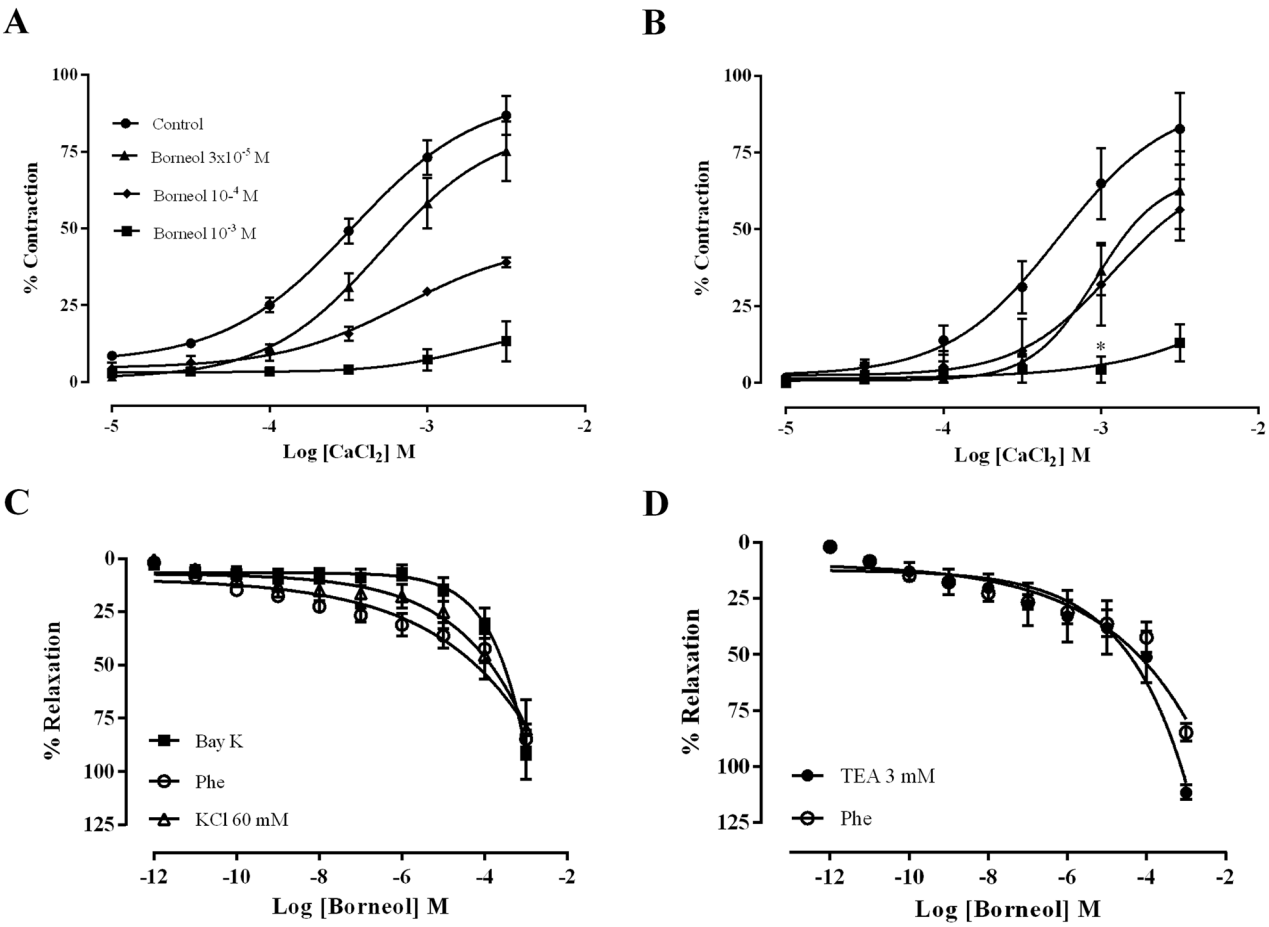


Fig. 6 Borneol-mediated vasorelaxation involves Ca^{2+} channels. Concentration–response curves to $CaCl_2$ (10^{-5} to 3×10^{-3}) in nominally Ca^{2+}-free depolarizing solution (KCl 60 mM) in the mesenteric arteries from sham (**A**) and 2K1C rats (**B**) in the absence or presence of borneol (10^{-3}, 10^{-4} or 3×10^{-5} M). Concentration–response curves to borneol (10^{-12} to 10^{-3} M) in (S)-(−)-Bay K 8644-precontracted rings from sham rats (**C**) or Phe-precontracted rings from sham rats with or without TEA (3 mM) (**D**). The results are expressed as the mean ± SEM. The difference between groups was analyzed by two-way ANOVA: $*p < 0.05$ vs. the control curve

In addition to its antioxidant effect, the vasorelaxant capacity of borneol may also contribute to its antihypertensive effect. Borneol promoted endothelium-independent relaxation in rat superior mesenteric arteries. We also consider this characteristic important since endothelial dysfunction is a common condition in hypertension. Thus, vasodilator drugs capable of promoting relaxation independent of the endothelial layer have advantages over those that use the endothelium to promote its effects. The relaxing effect of borneol has already been demonstrated in aortas from normotensive rats [6, 7], in which borneol was effective in blocking Ca^{2+} channels and activating K^+ channels present in the membrane of vascular smooth muscle cells (VSMCs). In our study, we found that the relaxing effect induced by borneol also occurred in the superior mesenteric artery from sham and 2K1C rats. This effect involves L-type Ca^{2+} channel blockade, as we could see in the (S)-(-)-Bay K 8644 assay. On the other hand, K^+ channels have no involvement in borneol-induced vasodilatation since the previous incubation with TEA (3 mM), a nonselective K^+ channel blocker, did not alter the effect induced by this monoterpene. These data suggest that borneol acts differently depending on the vascular bed. The difference in responses between conductance and resistance vessels for vasoactive agents is already classically known in the literature [37, 38]. It is important to highlight that resistance vessels have more pronounced participation in determining mean blood pressure than conductance vessels such as the aorta [39]. Regardless, the vasorelaxant action of borneol in vessels of different sizes may represent an advantage of this compound in the reduction of blood pressure.

In summary, this study demonstrated that borneol relaxes superior mesenteric arteries isolated from normotensive rats and that this effect is maintained in the presence of hypertension. We also demonstrated that oral treatment with borneol reduces serum oxidative stress and sympathetic vasomotor hyperactivity and restores depressed baroreflex sensitivity in rats with renovascular hypertension. All of these effects are related to the antihypertensive role of borneol.

Notably, borneol can easily penetrate the blood–brain barrier (BBB) and facilitate the access of other molecules to the central nervous system [40–43]. These properties strengthen the importance of our study, in which we showed for the first time that borneol can alter autonomic activity. However, further studies are needed to clarify whether this effect occurs by central actions, peripheral actions, or both of borneol. Altogether, the data obtained point to the promising role of borneol and encourage us to further study the properties of this molecule on the cardiovascular system and its autonomic modulation.

In view of the benefits of borneol on the cardiovascular system demonstrated by this study, our data contribute to the potential of its application in humans. For this, using the formula described by Reagan-Shaw [44], the adequate dose of borneol for humans would be 20.27 mg/kg. Controlled release formulations could be synthesized, but other physicochemical, pharmacokinetic and toxicity studies would need to be performed first.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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Role of Renin-Angiotensin System Components in Atherosclerosis: Focus on Ang-II, ACE2, and Ang-1–7

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Atherosclerosis is the leading cause of vascular disease worldwide and contributes significantly to deaths from cardiovascular complications. There is a remarkably close relationship between atherosclerotic plaque formation and the activation of renin-angiotensin system (RAS). However, depending on which RAS pathway is activated, pro- or anti-atherogenic outcomes may be observed. This brief review focuses on the role of three of the most important pieces of RAS axis, angiotensin II (Ang-II), angiotensin converting enzyme type 2 (ACE2), and angiotensin 1–7 (Ang-1–7) and their involvement in atherosclerosis. We focused on the effects of these molecules on vascular function and inflammation, which are important determinants of atherogenesis. Furthermore, we highlighted potential pharmacological approaches to treat this disorder.

Keywords: angiotensin converting enzyme type 2, angiotensin II, angiotensin 1–7, atherosclerosis, endothelial dysfunction, inflammation

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INTRODUCTION

Cardiovascular diseases remain the leading cause of adult death worldwide (Herrington et al., 2016). Nowadays, it is already established that hypertension is a modifiable risk factor for cardiovascular diseases and the reduction in blood pressure is accompanied by a reduction in cardiovascular risk (Herrington et al., 2016). On the other hand, the persistent burden of cardiovascular events despite a highly effective control of conventional risk factors, suggests that other mechanisms might underlie a proportion of these events (Libby et al., 2019).

Atherosclerosis can be considered the primary origin of most cardiovascular diseases (Husain et al., 2015). As previously reviewed by us and by others, atherosclerosis consists of an inflammatory response of arterial wall to injuries. This inflammation is often initiated by endothelial dysfunction and progresses to cellular adhesion molecules (CAM) expression, adhesion of circulating leukocytes to the endothelial cells (Koleva et al., 2016), leucocyte migration and the formation of a fibrous cap around a lipidic core, which compromises vascular lumen (Freitas-Lima et al., 2015). In addition to its traditional role in hypertension, the long-term blood pressure control system (the renin-angiotensin system – RAS) is directly involved in the development of atherosclerotic lesions due to its mainly effects on endothelial function, inflammation,

fibrosis, coagulation balance, plaque stability, and structural remodeling (Montezano et al., 2014; Husain et al., 2015).

Along with the classic cascade in RAS, which involves the conversion of angiotensinogen to angiotensin I (Ang-I) by renin, followed by its cleavage to angiotensin II (Ang-II) by angiotensin converting enzyme (ACE), other peptides and enzymes related to RAS are important in atherogenesis (**Figure 1**; Montezano et al., 2014). In this context, we highlight the role of the angiotensin converting enzyme type 2 (ACE2), which is typically responsible to form angiotensin 1–7 (Ang-1–7) from Ang-II. The heptapeptide is described to oppose Ang-II effects by mediating vasodilation, growth-inhibition, anti-inflammatory responses, and anti-thrombotic effects (Montezano et al., 2014).

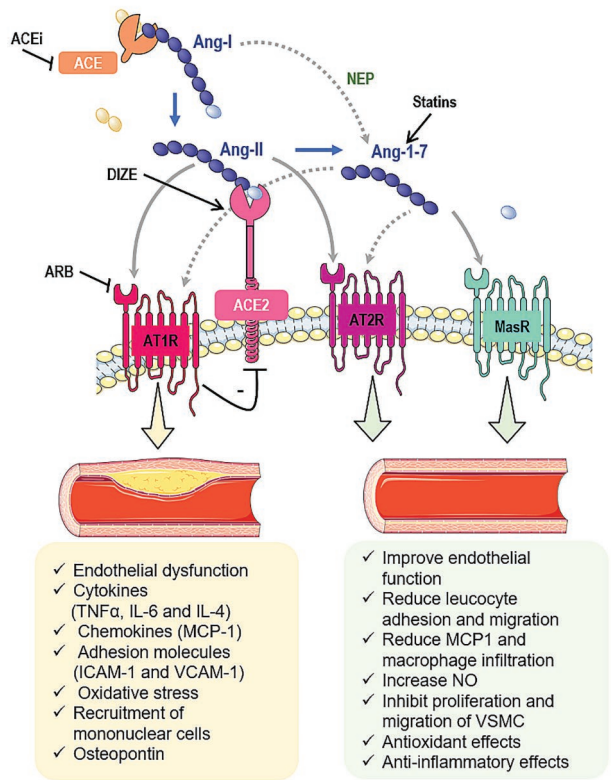

FIGURE 1 | Interactions between the renin-angiotensin system (RAS) and atherosclerosis. Angiotensin II (Ang-II) is formed from the angiotensin I (Ang-I) cleavage by angiotensin converting enzyme (ACE). Ang-II can bind to Ang-II type 1 (AT1R) or type 2 (AT2R) receptors. Ang-II undergoes the action of angiotensin converting enzyme type 2 (ACE2) to be converted into angiotensin 1–7 (Ang-1-7), which classically interacts with Mas receptor (MasR). Furthermore, Ang-1–7 can bind to AT2R or it can induce the β-arrestin pathway through its interaction with AT1R. Ang-1–7 also can be produced by neprylisin (NEP) from Ang-I. In summary, the ACE/Ang-II/AT1R pathway induces atherosclerosis while the ACE2/Ang-1–7/MasR and Ang-II and Ang-1–7/AT2R pathways inhibit the atherosclerotic events. In addition, some pharmacological tools have been shown to interfere in some components of RAS cascade and prevent the atherosclerosis, such as statins, ACE inhibitors (ACEi), angiotensin receptor blockers (ARB) and diminazene aceturate (DIZE). Gray arrows indicate classic pathways while gray dotted arrows indicate alternative pathways. Black lines indicate potential pharmacological approaches to stimulate/increase (arrows) or block/decrease (lines) components of RAS.

Considering that, this review is devoted to summarize the effects of Ang-II, ACE2, and Ang-1–7 in atherosclerosis, highlighting the promising interventions that could lead to RAS modulation and atherosclerosis treatment.

ANGIOTENSIN II AND ATHEROSCLEROSIS

Ang-II is the main effector of RAS (Colafella et al., 2019). The effects of Ang-II are mediated by its binding into the angiotensin type 1 and type 2 receptors (AT1R and AT2R, respectively). These receptors are G protein-coupled receptors that tend to present opposing activities (Kellici et al., 2015). AT1R is primarily responsible for the classic pro-hypertensive activity of Ang-II, whereas the AT2R is reported to present antagonistic effects to the AT1R (**Figure 1**; Ding et al., 2016).

It has been shown that Ang-II directly induces endothelial dysfunction and increases endothelial oxidative stress through the production of reactive oxygen species (ROS) such as superoxide anions (O_2^-) derived from the complex enzyme nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase). This occurs predominantly through interaction with endothelial AT1R (Ziegler et al., 2020), which mediates increase in Ca^{2+} concentration in endothelial cells, promoting activation of calmodulin and interaction with the Nox5/Ca^{2+} calmodulin binding domain (Montezano et al., 2010; Piqueras and Sanz, 2020). Nox5 is a member of the NADPH oxidase family which is not found in rodents but is highly expressed in coronary arteries obtained from individuals with coronary artery disease (Guzik et al., 2008; Gray and Jandeleit-Dahm, 2015). In atherosclerosis, oxidative and inflammatory processes involve increased expression and activation of Nox5 in both vascular cells and resident macrophages (Touyz et al., 2019).

Activation of Nox5 mediated by Ang-II produces O_2^-, activates RhoA and leads to the subsequent stimulation of Rho-associate kinase in human umbilical arterial endothelial cells culture (Escudero et al., 2015). The RhoA/ROCK pathway is an upstream regulator of mitogen-activated protein kinases (MAPKs – p38MAPK and ERK1/2), which promotes transactivation of several transcription factors, including NF-κB (Piqueras and Sanz, 2020). NF-κB regulates the expression of numerous genes, such as cytokines, tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6), chemokines (monocyte chemoattractant protein – MCP-1), adhesion molecules (P-selectin, ICAM-1, and VCAM-1), the inflammatory enzyme cyclooxygenase type 2 (COX-2), and angiotensinogen (Durante et al., 2012; Liang et al., 2015). Moreover, activation of NF-κB seems to be an important signal transducer involved in the upregulation of oxidized low-density lipoprotein (ox-LDL)–mediated AT1R expression (**Figure 2**; Li et al., 2000).

It is likely that TNF-α, released upon Ang-II stimulation of the AT1R, in combination with IL-4 acts as a paracrine molecule, inducing selective adhesion of mononuclear cells to the arterial endothelium through increased expression of CAM, and the release of varied chemokines involved in the recruitment of mononuclear cells (Piqueras and Sanz, 2020).

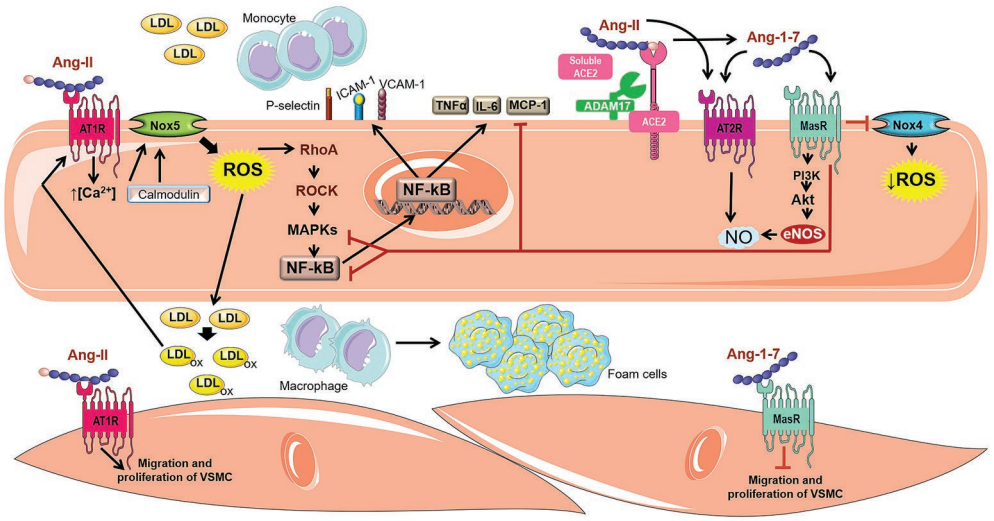

FIGURE 2 | Involvement of Ang-II, ACE2, and Ang-1–7 in atherogenic pathways. The Ang-II binding into AT1R can activate Nox5 through a calcium/calmodulin-dependent pathway. The activated Nox5 induces the formation of ROS and stimulates the RhoA/ROCK pathway, which in turn, activates MAPKs and induces the transactivation of several transcription factors such as NF-κB. The expression of several genes is regulated by NF-κB, for instance cytokines (TNF-α and IL-6), chemokines (MCP-1), adhesion molecules (P-selectin, ICAM-1 and VCAM-1), which are involved in Ang-II-induced migration of mononuclear leukocytes. In addition, Ang-II is cleaved by ACE2 and produces Ang-1–7, an important RAS counter-regulator. Ang-1–7 shows the potential to negatively regulate atherogenic pathways, inducing anti-inflammatory effect, weakening monocyte migration and decrease of vascular lipids accumulation. These actions attributed to Ang-1–7 are related to the reduction of oxidative stress and the synthesis of inflammatory cytokines due to inhibition of the Nox4 and NF-κB-mediated pathways. Furthermore, Ang-1–7 stimulates the PI3K/Akt pathway, leading to phosphorylation of eNOS and NO formation, which improves the endothelial function. Ang-1–7 is also capable of promoting endothelial activation of AT2R, which also stimulates the NO cascade. In VSMC, Ang-1–7 inhibits muscle cell migration and proliferation, in contrast to Ang-II which possess proliferative and hypertrophic effects.

Shu et al. (2019) demonstrated that Ang-II induces monocyte chemotactic protein-induced protein expression (MCPIP1) through an AMPK/p38 MAPK dependent pathway. The increase in MCPIP1 expression triggered apoptosis in macrophages, contributing to atherosclerotic plaque vulnerability.

In addition, Ang-II induces the expression of osteopontin, a multifunctional protein found in many cell types, including macrophages, endothelial cells, smooth muscle cells (SMCs), and epithelial cells. Osteopontin is found in atherosclerotic lesions, especially in association with macrophages and foam cells, suggesting that this protein plays an important role in the development and progression of atherosclerosis (Ding et al., 2016). The molecular mechanisms related to osteopontin involve recruitment of inflammatory cells and migration of foam cells through the binding to integrins (Giachelli and Steitz, 2000).

Ang-II also up-regulates the LOX-1 gene. LOX is a transmembrane glycoprotein that serves as a receptor for oxidized LDL (Lubrano and Balzan, 2016). In the endothelium, binding of oxLDL to LOX-1 causes increase in leukocyte adhesion molecules, activates apoptosis pathways, increases ROS and induces endothelial dysfunction. In a pro-inflammatory environment, LOX-1 is positively regulated in macrophages and is associated with more than 40% of oxLDL uptake, contributing to the formation of foam cells (Kattoor et al., 2019). In addition, oxLDL increases the generation of ACE, which in turn induces the Ang-II formation. This octapeptide increases the expression of LOX-1, which positively regulates the expression of AT1R, contributing to a self-perpetuating pro-atherogenic cycle. It has also been reported that ACE inhibitors and AT1R blockers (ARBs) decrease the expression of LOX-1 (Lubrano and Balzan, 2016).

According to experimental and clinical data, ACE inhibitors and ARBs appear to have beneficial anti-atherosclerotic effects (Tousoulis et al., 2015). Studies have shown that enalapril ameliorated oxidative vascular injury, suppressed NADPH oxidase activity, decreased inflammatory mediators and regulated the antioxidant defense system in apolipoprotein E-deficient mice (ApoE-KO; Suarez-Martinez et al., 2014; Husain et al., 2015), an animal model commonly used to study atherosclerosis.

It has been shown that the ARB olmesartan significantly reduced vascular inflammation in hypertensive patients, with a significant reduction in serum levels of many inflammatory markers, such as C-reactive protein, TNF-α, IL-6, and MCP-1 (Fliser et al., 2004; Durante et al., 2012). Moreover, long-term therapy with valsartan has been associated with atherosclerosis regression in individuals with thickening of the carotid wall. These effects were accompanied by concomitant improvements in oxidative stress markers, inflammation, and peripheral smooth muscle function (Ramadan et al., 2016).

ACE2 AND ATHEROSCLEROSIS

The first evidence of a relationship between ACE2 and atherosclerosis was demonstrated by Zulli et al. (2006). They have shown the immunolocalization of ACE2 protein in macrophages and SMC

actin-positive cells from rabbit atherosclerotic plaques. After this study, several experimental and clinical evidence have confirmed the involvement of ACE2 in atherosclerosis, suggesting its anti-atherogenic role (Dong et al., 2008; Lovren et al., 2008).

Dong et al. (2008) have found that ACE2 overexpression on aortic plaques attenuate the progression of early lesions in rabbits that underwent to endothelial injury and received atherogenic diet, probably by conversion of Ang-II to Ang-1–7. In this scenario, there was a reduction in local inflammation, lipid deposition, macrophage infiltration, and MCP-1 expression, in addition to an increase in collagen content, resulting in stabilized plaques. Similar results were found in rabbits fed with a high-cholesterol diet. The anti-atherosclerotic effects of ACE2 were associated with inhibition of proliferation and migration of vascular SMC and improvement of endothelial function. Additionally, ACE2 produced down-regulation of ERK1/2, p38 MAPK, JAK-STAT, and Ang-ll/ROS/NF-κB signaling pathways and upregulation of the PI3K-Akt pathway (Zhang et al., 2010).

Likewise, overexpression of ACE2 in ApoE-KO mice attenuated atherosclerotic lesion size and improved endothelial homeostasis, at least in part, through a mechanism that involves reduction of Ang-II-induced ROS generation (Lovren et al., 2008). In accordance to these data, Zhang et al. (2015a) also have shown that inhibition of inflammatory response, such as reduction of Ang-II-induced expression of adhesion molecules and cytokines prevent atherosclerotic plaque evolution in ApoE-KO animals overexpressing ACE2.

The protective role of ACE2 on atherosclerosis was also supported by the use of ACE2-deficient mice model (ACE2-KO). ACE2-deficiency in both LDL receptor-deficient mice (LDLR-KO) and ApoE-KO backgrounds resulted in larger atherosclerotic lesions when compared to their respective controls. Furthermore, the increased atherosclerotic vulnerability was associated to intraplaque inflammatory profile (Thomas et al., 2010; Thatcher et al., 2011; Sahara et al., 2014). On the other hand, the protective role of ACE2 on atherosclerosis in humans is not well-established yet.

In 2008, Sluimer and colleagues demonstrated the presence of ACE2 in humans. They detected ACE2 protein in human veins, healthy and atherosclerotic arteries, expressed in endothelial cells, SMCs, and macrophages. In addition, they found ACE2 messenger RNA (mRNA) and protein in early and advanced atherosclerotic lesion from humans. Despite total protein expression of ACE2 was similar during all stages of atherosclerosis, ACE2 activity was lower in advanced lesions, suggesting differential regulation of ACE2 in progression of atherosclerosis (Sluimer et al., 2008).

Anguiano et al. (2016) have found that baseline circulating ACE2 activity was enhanced in chronic kidney disease patients with atherosclerotic plaques when compared to patients with no plaque, suggesting that higher circulating ACE2 activity is associated with higher risk for silent atherosclerosis. Accordingly, Zhou et al. (2020) have shown an increase in circulating ACE2 protein levels in women with coronary heart disease (CHD) when compared to healthy group. This increase was associated with multi-vessel lesions, corroborating with the reports by Anguiano et al. (2016) and indicating the ACE2 as a compensatory mechanism in coronary atherosclerosis.

ACE2 is an integral cell membrane protein that can undergo cleavage or shedding and release its catalytically active ectodomain into surrounding milieu. The main promoter of ACE2 shedding is A Disintegrin and Metalloprotease 17 (ADAM17), which has been involved in atherosclerosis (Canault et al., 2006, 2007). This evidence and the results found by Zhou et al. (2020) allowed these authors to conclude that the increase in circulating ACE2 level is due to increasing tissue ACE2 synthesis from mRNA and augmented ACE2 protein shedding followed by its increase in circulation. All together these data show the increased circulating ACE2 protein levels or activity as biomarkers of atherosclerosis and encourage further studies in this direction.

Some therapeutic strategies for atherosclerosis targeting ACE2 have been thought, either with new drugs or drugs already used in the clinic. A recent study has demonstrated that overexpression of ACE by plasmid-mediated transfection in both primary monocytes and THP-1 cells leads to a marked decrease of ACE2 mRNA expression and induces a pro-atherogenic phenotype with elevated gene expression of the cellular adhesion molecules ICAM-1, VCAM-1, and macrophage colony-stimulating factor (MCSF). All these effects were partly reversed by captopril and losartan (Trojanowicz et al., 2017).

In that context, Zhang et al. (2015b) have shown that losartan inhibited the evolution of atherosclerotic plaques in high-cholesterol fed rabbits as well as increased the ACE2 protein expression in the plaques. In addition, Ang-II downregulated ACE2 protein expression and activity in SMC cell culture and losartan significantly blocked Ang-II-induced reduction of both ACE2 protein and activity. These data indicate that Ang-II generation by ACE can affect the expression and activity of ACE2 and ACE inhibitors or AT1R antagonists can upregulate ACE2 and favor its anti-atherogenic effects.

ACE2-activating drugs also seem promising, with emphasis on diminazene aceturate (DIZE) (Qaradakhi et al., 2020), which has several protective effects, such as improvement of metabolic profile and reduction of lipogenesis in mice (Macedo et al., 2015), anti-hypertensive effects in renovascular hypertensive rats (De Maria et al., 2016), and improvement of pulmonary endothelial function in Sprague Dawley rats (Shenoy et al., 2013). Thatcher et al. (2014) have found that DIZE decreases formation and severity of Ang-II-induced abdominal aortic aneurysms (AAA). Ang-II-induced AAA is characterized by progressive leukocyte accumulation, extracellular matrix degradation, luminal expansion, and thrombus (Saraff et al., 2003), being closely related to atherosclerosis. In addition, Fraga-Silva et al. (2015) have demonstrated that DIZE enhances the stability of atherosclerotic plaques in ApoE-KO mice and reduces the expression of ICAM-1 and VCAM-1. Although the mentioned studies have been performed on animal models, they suggest DIZE as a potential drug for the treatment of atherosclerosis and related cardiovascular diseases.

ANGIOTENSIN 1–7 AND ATHEROSCLEROSIS

Ang-1–7 was investigated three decades ago as an important counter-regulator component of RAS, promoting hypotension

and bradycardia after microinjection in dorsal motor nucleus of the vagus (Santos et al., 1988; Campagnole-Santos et al., 1989). The classical formation of Ang-1–7 occurs through ACE2 action on Ang-II. Alternatively, Ang-1–7 is formed by the cleavage of Ang-1–9 facilitated by ACE. Moreover, Ang-I can be directly converted into Ang-1–7 by action of neutral endopeptidase (neprylisin – NEP; Santos et al., 2003; Santos, 2014).

The formation of Ang-1–7 in vascular endothelium was first identified by Santos et al. (1992) using human aortic and human umbilical vein endothelial cells (HUVEC; Santos et al., 1992). Robust studies have shown that Ang-1–7 induces MasR activation, a G protein-coupled receptor which stimulates the PI3K/Akt pathway leading to phosphorylation of endothelial nitric oxide (NO) synthase and consequent NO production and releasing (Sampaio et al., 2007). Of note, Ang-1–7 is able to promote AT2R endothelial activation, which stimulates the bradykinin–NO cascade (Walters et al., 2005; Villela et al., 2015). NO is one of the most important factors released by endothelium. This gas is involved in vascular homeostasis and its decrease induces endothelial dysfunction (Cheng et al., 2009; Forstermann and Sessa, 2012), which is the key factor in atherogenesis (Qaradakhi et al., 2016).

Studies have demonstrated that Ang-1–7 stimulates endothelial cells function restoration by increasing NO bioavailability (Pignone et al., 2007; Sampaio et al., 2007). In addition, Ang-1–7 downregulates adhesion molecules such as VCAM-1 and ICAM-1 in endothelium by preventing both the phosphorylation of p38 MAPK and the expression of NF-κB (Anton et al., 2007; Zhang et al., 2013; Liang et al., 2015). Moreover, Ang-1–7 induces proliferation of endothelial progenitor cells in the injured vascular tissue triggered by atherogenesis (Wang et al., 2010; Zhang et al., 2015c).

During the vascular inflammation, many cytokines and inflammatory cells are required to begin and maintain atherosclerosis progression. In this context, Ang-1–7 has been described to induce anti-inflammatory phenotypes which contribute to restrain vascular lipid accumulation (Yang et al., 2013; Jiang et al., 2014). Yang et al. (2015a) found that Ang-1–7 treatment reduced the oxidative stress and macrophage infiltration due to decreasing in Nox4 (a subunit of NADPH oxidase complex) and NF-κB in aorta from ApoE-KO (**Figure 2**; Yang et al., 2015a). Another interesting study revealed that Ang-1–7 administration induced a remarkable decrease in the expression of pro-inflammatory cytokines such as IL-6, TNF-α, and MCP-1 in both aortic plaque and macrophages from ApoE-KO (Yang et al., 2013). Furthermore, in the same mouse model, pretreatment with AVE0991, a MasR agonist, reduced activated $CD4^+$ T cells (Jawien et al., 2012a) and IL-12 (Jawien et al., 2012b). All these findings corroborate with an anti-inflammatory effect of Ang-1–7/MasR pathway in atherosclerosis.

In contrast to Ang-II-induced proliferative and hypertrophic effects, Ang-1–7 inhibits the migration and proliferation of vascular SMCs (Jiang et al., 2014; McKinney et al., 2014). This effect was described by Yang et al. (2013), showing that Ang-1–7 induces activation of MasR/ERK1,2/p38 and MasR/JAK/STAT pathways in vascular SMCs to mitigate the atherosclerotic plaque formation (Yang et al., 2013). Furthermore, Ang-1–7 has demonstrated a potential to negatively regulate the vascular fibrosis, as can be noticed by decreasing in matrix metalloproteases (MMP) MMP-2/MMP-9 in atherosclerotic plaques (Yang et al., 2013). Accordingly, Ang-1–7 treatment promoted a reduction in the neointimal layer growth by structural recovery of endothelium and showed atheroprotective properties attributed to its binding to both AT2R and MasR (Faria-Silva et al., 2005; Tesanovic et al., 2010). In addition, Ang-1–7 reduced atherosclerotic lesion formation by decrease in collagen accumulation through activation of AT2R (Dandapat et al., 2008). Conversely, it was described an increase in collagen content after Ang-1–7 administration, resulting in the increase of plaque stability (Yang et al., 2013). Similarly, the treatment with an Ang-1–7 antagonist, A779, induced a decline in plaque stability and reduction in collagen level (Yang et al., 2015b). Moreover, the heptapeptide can play a role as a β-arrestin-biased AT1R agonist without induce the Gq subunit activation, suggesting an additional antihypertrophic effect attributed to this peptide (Teixeira et al., 2017; Paz Ocaranza et al., 2020)

Interestingly, increase in plasmatic Ang-1–7 has been involved in regulation of lipid metabolism. It promoted a reduction in triglycerides and cholesterol levels, together with a decrease in adipose tissue mass as well as an improvement of glucose metabolism (Santos et al., 2010). The authors have suggested an involvement of adiponectin in the regulation of the glucose and lipid metabolism induced by Ang-1–7 (Santos et al., 2010). Curiously, the knocking out of MasR promoted opposing effects, once it augmented cholesterol and triglycerides levels and worsened the carbohydrate metabolism (Santos et al., 2008).

THE ROLE OF STATINS ON RAS COMPONENTS AND ATHEROSCLEROSIS

Some therapeutic strategies have been validated to positively modulate the RAS. The statins, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMGcoA-reductase) inhibitors, have emerged due to its pleiotropic properties demonstrating additional effects apart from those of decreasing cholesterol levels (Zhang et al., 2015c). Treatment with statins such as atorvastatin and rosuvastatin have promoted an upregulation of ACE2/Ang-1–7 axis, reducing the proliferation of vascular SMCs and intimal thickening, respectively (Li et al., 2000; Suski et al., 2014), effects that are closely related to atherogenesis. The mechanisms by which the statins act to promote these effects are still unclear; however, studies have revealed that HMGcoA-reductase inhibitors decrease the activation of NF-κB induced by TNF-α and Ang-II, factors responsible to stimulate the migration and proliferation of vascular wall (Ortego et al., 1999; Friedrich et al., 2006; Tristano et al., 2007; Suski et al., 2014). Furthermore, authors have demonstrated that atorvastatin induced an increase in ACE2 protein expression in heart and kidney from high cholesterol-fed rabbits and augmented the occupancy of histone H3 acetylation (H3-Ac) mark on ACE2 promoter region in heart, demonstrating direct or indirect ACE2 epigenetic upregulation (Tikoo et al., 2015).

The role of statins on RAS components also have been observed in clinical trials as showed by Schindler et al. (2014) that identified, for the first time, an increase of Ang-1–7 level

in hypercholesterolemic subjects after atorvastatin treatment. Altogether, those responses suggest an important role of statins on RAS components, including a decrease in Ang-II and, apparently, an upregulation in the ACE2/Ang-1–7 axis. This fact could be crucial to the atherosclerosis and cardiovascular diseases therapy.

CONCLUSION

In conclusion, here we briefly reviewed the role played by RAS components such as Ang-II, ACE2, and Ang-1–7 in atherosclerosis development. According to what is expected to components of RAS, Ang-II is considered to have pro-atherogenic effects while ACE2 and Ang-1–7 anti-atherogenic profiles. In addition to the direct pressure-related roles of these peptides, their effects on atherosclerosis involve modulation of endothelial function, oxidative stress, inflammation, cellular migration and proliferation, as well as plaque stability. Pharmacological strategies currently used to modulate the pressor effects of RAS components can offer beneficial outcomes in atherosclerosis. Moreover, we highlight the role played by statins, which have been identified to increase the RAS compensatory components (ACE2 and Ang-1–7), and induce an additional effect against the plaque formation. For this reason, the HMGcoA-reductase inhibitors should be considered when clinical decisions are made.

AUTHOR CONTRIBUTIONS

CB, TQ and MF-F conceived the manuscript and revised it critically. CB and NC prepared the figures. GS, NC, ML and DG drafted the manuscript. All authors contributed to the article and approved the submitted version.

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A Newly Isolated Carboxymethyl-Glucan (CM-G) Restores Depressed Baroreflex Sensitivity in Renovascular Hypertensive Rats

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This study was designed to investigate the effects of a newly synthesized carboxymethyl-glucan (CM-G) on blood pressure (BP), baroreflex sensitivity (BRS) and sympathetic vascular modulation in renovascular hypertensive rats. Male Wistar rats were divided into four groups: Sham (n = 10); 2K1C (subjected to renal artery clipping to induce renovascular hypertension, n = 10); Sham + CM-G (treated with CM-G, n = 7) and 2K1C + CM-G (treated with CM-G, n = 7). The daily treatment with CM-G (40 mg/kg) was performed for 2 weeks. Blood pressure, heart rate (HR), systolic BP variability, baroreflex sensitivity (BRS) and sympathetic vascular tone were evaluated. After six weeks of renal artery clipping, 2K1C rats exhibited arterial hypertension (171 ± 11 vs. 118 ± 4 mmHg, $p < 0.05$), impaired BRS (−1.30 ± 0.10 vs. −2.59 ± 0.17 bpm.mmHg-1, $p < 0.05$) and enhanced sympathetic activity as shown by the hexamethonium test (−60 ± 5 vs. −33 ± 2 ΔmmHg, $p < 0.05$) when compared to sham rats. Oral administration of CM-G in renovascular hypertensive rats reduced hypertension (126 ± 4 vs. 171 ± 11 mmHg, $p < 0.05$) and improved the BRS (−2.03 ± 0.16 vs. −1.30 ± 0.10 bpm.mmHg^{-1}, $p < 0.05$) in 2K1C rats when compared to placebo. Those effects seem to be caused by a reduction in sympathetic activity. The present study revealed for the first time that CM-G treatment reduces arterial hypertension and restores arterial baroreflex sensitivity via a reduction in the sympathetic tone in conscious renovascular hypertensive rats.

Keywords: hypertension, sympathetic overactivity, baroreflex sensitivity, carboxymethyl-glucan

Abbreviations: ANG II, Angiotensin II; BRS, Baroreflex sensitivity; BP, Blood pressure; CM-G, Carboxymethyl-glucan; HR, Heart rate; LF, Low-frequency; MAP, Mean arterial pressure; PAP, Pulsatile arterial pressure; ROS, Reactive oxygen species; SBRS, Spontaneous baroreflex sensitivity; SAP, Systolic arterial pressure; 2K1C, Two-kidney one-clip.

INTRODUCTION

Baroreflex is essential for the short-term control of blood pressure (BP) and modulation of sympathetic activity (Malpas et al., 1997; Grassi et al., 1998; Julien, 2008). Convincing evidence from research with both animals and humans have reported a relationship between decreased arterial baroreflex sensitivity, sympathetic overactivity and arterial hypertension (Gao et al., 2002; Nagai et al., 2003; Salgado et al., 2007).

Our laboratory and other research groups have demonstrated that augmented oxidative stress, characterized by downregulation of the antioxidant capacity and/or increased pro-oxidant factors, depresses baroreflex sensitivity, promotes endothelial dysfunction, augments sympathetic activity and may contribute to the development and maintenance of arterial hypertension (Guimaraes et al., 2012; de Queiroz et al., 2015; Cavalcanti et al., 2016). The role of oxidative stress in baroreflex dysfunction is much more complex than a simple reflex dysfunction and needs to be further elucidated.

The two-kidney one-clip (2K1C) model of renovascular hypertensive is characterized by augmented angiotensin II, oxidative stress, inflammation, depressed baroreflex, sympathetic hyperactivity and endothelial dysfunction (Wang et al., 2005). The depressed arterial baroreflex sensitivity has also been reported in patients with renovascular hypertension (Gao et al., 2002).

For these reasons, it is reasonable to suggest that therapies or interventions with the potential to counteract the adverse effects of oxidative stress may be an important strategy for improving sympathetic baroreflex sensitivity and lower BP under hypertensive states (Guimaraes et al., 2012). In fact, recent studies from our research group have shown that an antioxidant therapy improves baroreflex sensitivity and BP control in hypertensive rats (Botelho-Ono et al., 2011; Guimaraes et al., 2012; Monteiro et al., 2012; Queiroz et al., 2012; Mendes-Junior et al., 2013). A recent meta-analysis suggested that baroreflex activation therapy could exert beneficial effects on BP in resistant hypertension. However, further experimental and clinical data are needed to confirm the application of baroreflex activation therapy under hypertensive conditions (Wallbach and Koziolek, 2017). Thus, we sought to identify antioxidant compounds that could be used as a potential anti-hypertensive strategy, mainly by modulating arterial baroreceptors.

Carboxymethyl-glucan (CM-G) is a water-soluble derivative of *Saccharomyces* spp. cell wall β(1-3)(1-6) glucan that appears to be translocated from the gastrointestinal (GI) tract into the systemic circulation by epithelial cells in the GI tract and their absorption kinetics differs among the different types of β-glucan (Vetvicka et al., 2015). Well-known by its antioxidant properties, CM-G reduces malondialdehyde levels in healthy men and it has an important role in the protection of biological membranes, probably by its main accepted mechanism that involves the capacity of CM-G to scavenge reactive oxygen species (Babincova et al., 2002; Araujo et al., 2015). Despite the free radical scavenging activity of CM-G, whether an oral treatment with CM-G could improve baroreflex sensitivity and ameliorate arterial hypertension in renovascular hypertensive rats remains unknown. Therefore, we assessed the ability of CM-G treatment to reduce arterial hypertension and restore baroreflex sensitivity in 2K1C hypertensive rats.

MATERIALS AND METHODS

Animals

Thirty-four male Wistar rats (270–330 g) were used for the experiments, collectively housed in cages (3–4 rat/cage), maintained in a temperature-controlled room and subjected to a 12:12-hour light-dark cycle with free access to standard chow diet (Labina®, Purina, Paulinea, SP, Brazil) and water. All experimental procedures were approved by the Animal Care and Use Committee (CEUA, protocol #0604/14) of the Federal University of Paraiba, Brazil.

General Experimental Protocol

The rats were randomly assigned into four groups: i) Sham (submitted to sham surgery, $n = 10$); ii) 2K1C (subjected to renal artery clipping to induce renovascular hypertension, $n = 10$); iii) Sham + CM-G (submitted to sham surgery + CM-G treatment, $n = 7$); iv) 2K1C + CM-G (submitted to renal artery clipping to induce renovascular hypertension + CM-G treatment, $n = 7$). In the sham groups, saline was administered as placebo for 2 weeks. In the CM-G groups, carboxymethyl-glucan at a dose of 40 mg/kg was administered daily for 2 weeks. Saline and/or CM-G administration was performed by oral gavage. The treatments started 28 days after surgery and lasted for 2 weeks (**Figure 1**). After 2 weeks of treatment with saline or CM-G, baseline BP, heart rate (HR) records, baroreflex sensitivity, sympathetic vascular tone and spectral analysis of systolic arterial pressure were evaluated in each group.

Renal Artery Clipping: Goldblatt (Two-Kidney, One Clip; 2K1C) Model Hypertension

In order to develop 2K1C renovascular hypertension, rats were anesthetized with ketamine and xylazine (75 and 10 mg·kg^{-1}, i.p., respectively) and a midline abdominal incision was made. The right renal artery was exposed and isolated over a short segment by blunt dissection. A silver clip (0.20 mm internal diameter) was placed around the artery. The wound was closed and sutured. Sham-operated rats underwent a similar procedure but without permanent renal artery clipping, to serve as a control.

Blood Pressure and Heart Rate Recordings

Six weeks after silver clip implantation in the renal artery or sham surgery, the rats were anesthetized with ketamine and xylazine (75 and 10 mg/kg, i.p., respectively) to insert polyethylene catheters in the femoral vein and artery for drug injection and arterial pressure recordings, respectively. BP and heart rate (HR) measurements were recorded 24h after catheter implantation in conscious rats using a pressure transducer coupled to an acquisition system (PowerLab; ADInstruments, CastleHill, NSW,

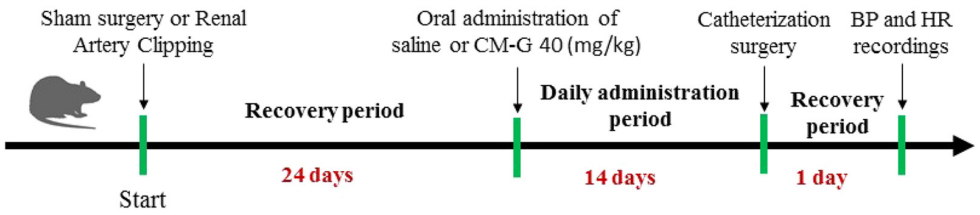

FIGURE 1 | Experimental design performed in the study.

Australia) connected to a computer running LabChart 5.0 software (ADInstruments).

Baroreflex Sensitivity Test

After 50 min of BP and HR baseline recordings, baroreflex was evaluated using classical vasoactive drugs. Phenylephrine (8μg/Kg) and sodium nitroprusside (25μg/Kg) were given as an intravenous bolus injection to each group as previously reported (Braga et al., 2008; Guimaraes et al., 2012). Reflex changes in HR produced by vasoactive drug administration were quantified and plotted as changes in HR over changes in mean arterial pressure (ΔHR/ΔMAP). Data were analyzed by linear regression using Prism 6 (GraphPad Software, Inc., SanDiego, CA, United States); the slope of linear regression yield the baroreflex gain for each animal. In addition, during the baseline recordings without any intervention, spontaneous baroreflex sensitivity (SBRS) was calculated through the sequence method by computer software CardioSeries (v. 2.4).

Evaluation of Sympathetic Tonus on the Vascular System

One hour after the baroreflex sensitivity test, the sympathetic vascular tone was evaluated by an intravenous injection of the ganglionic blocker hexamethonium (30 mg/kg, Sigma-Aldrich, São Paulo, SP, Brazil). The sympathetic tonus was calculated by the difference between the mean arterial pressure after the blockade and at the baseline.

Power Spectral Analysis of Systolic Arterial Pressure Signals

A beat-by-beat time series of systolic arterial pressure (SAP) was extracted from baseline cardiovascular recordings (10 min epochs) of the pulsatile arterial pressure from rats in each group and the overall variability of those series was assessed using Fast Fourier Transform (FFT) spectral analysis (Cardioseries Software v2.4; www.danielpenteado.com). The spectra were integrated and the low-frequency component (LF, 0.2–0.75 Hz) was evaluated. LF from systolic arterial pressure is an index for sympathetic modulation.

Determination of Kidneys and Heart Weight

Heart and kidneys were collected and weighed. Total organ mass (mg) was normalized by the body weight (g) giving an organ weight/body weight ratio index (ow/bw).

Statistical Analysis

Results are expressed as mean ± SEM. Data were analyzed by *t*-test or one-way or two-way ANOVA followed by Tukey's post hoc when appropriate. Statistical analyses were performed using Prism 6 (GraphPad Software® . Inc., La Jolla, CA, United States) and the differences were considered significant when $p < 0.05$.

RESULTS

Body and Organs Weights

As shown in **Table 1**, the right clipped kidneys from both 2K1C groups presented a reduction in the kidney mass index when compared to the right kidneys from the sham-operated rats. In addition, the left non-clipped kidney mass index was increased in both 2K1C groups when compared to the left kidney from sham-operated rats as an expected compensatory functional effect. Absolute values for organ weights are also provided in **Table 1**.

CM-G Treatment Reduces Blood Pressure in 2K1C Rats

Figure 2A shows original tracings of pulse arterial pressure (PAP), MAP and HR from one representative animal from each group. 2K1C rats exhibited high BP levels after six weeks of renal artery clipping in comparison to sham rats [171 ± 11 *vs.* 118 ± 4 mmHg, $p < 0.05$; (**Figure 2B**)]. Oral CM-G treatment for 2 weeks (40 mg/kg/day) in 2K1C rats effectively reduced MAP when compared to non-treated hypertensive rats [126 ± 4 *vs.* 171 ± 11 mmHg; $p < 0.05$; (**Figure 2B**)]. Regarding sham conditions, oral CM-G administration did not alter BP between groups. Lastly, HR was not different between groups ($p > 0.05$; **Figure 2C**).

CM-G Treatment Restores Depressed Baroreflex Sensitivity in 2K1C Rats

Original tracings from one representative animal from each group showing the changes in BP and HR in response to administration of vasoactive drugs are illustrated in **Figure 3A**. 2K1C hypertensive rats presented a reduction in baroreflex gain when compared to the sham group (−1.30 ± 0.10 *vs.* −2.59 ± 0.17 bpm.mmHg^{-1}, $p < 0.05$; **Figures 3B,C**). Daily CM-G treatment in 2K1C hypertensive rats restored the depressed baroreflex sensitivity when compared to non-treated hypertensive rats [−2.03 ± 0.16 *vs.* −1.30 ± 0.10

TABLE 1 | Absolute body weight (BW) and organ weight in sham rats or those treated with Carboxymethyl-glucan (CM-G).

Experimental groups	Initial BW (g)	Final BW (g)	Right kidney weight/BW (mg/g)	Left kidney weight/BW (mg/g)	Heart weight/BW (mg/g)	Right kidney weight (g)	Left kidney weight (g)
Sham + saline	169.1 ± 6.2	239.3 ± 5.1*	4.3 ± 0.14*	4.2 ± 0.13*	3.6 ± 0.24	1.0 ± 0.03	1.0 ± 0.04*
Sham + CM-G	194 ± 3.6	326.5 ± 8.8	3.8 ± 0.10	3.8 ± 0.1	3.8 ± 0.19	1.2 ± 0.06	1.2 ± 0.05
2K1C + saline	181.2 ± 5.2	272.5 ± 13.7	3.37 ± 0.22#	4.8 ± 0.17	4.3 ± 0.39	0.91 ± 0.07#	1.3 ± 0.05
2K1C + CM-G	184.8 ± 6.3	316.6 ± 9.8	2.6 ± 0.53#	4.5 ± 0.28	3.7 ± 0.10	0.83 ± 0.17#	1.4 ± 0.08

** $p < 0.05$ vs. all other groups. #$p < 0.05$ vs left side from the same group. Ratios were calculated by dividing the organ weight by the final body weight per animal. (Mean values with their standard errors)*

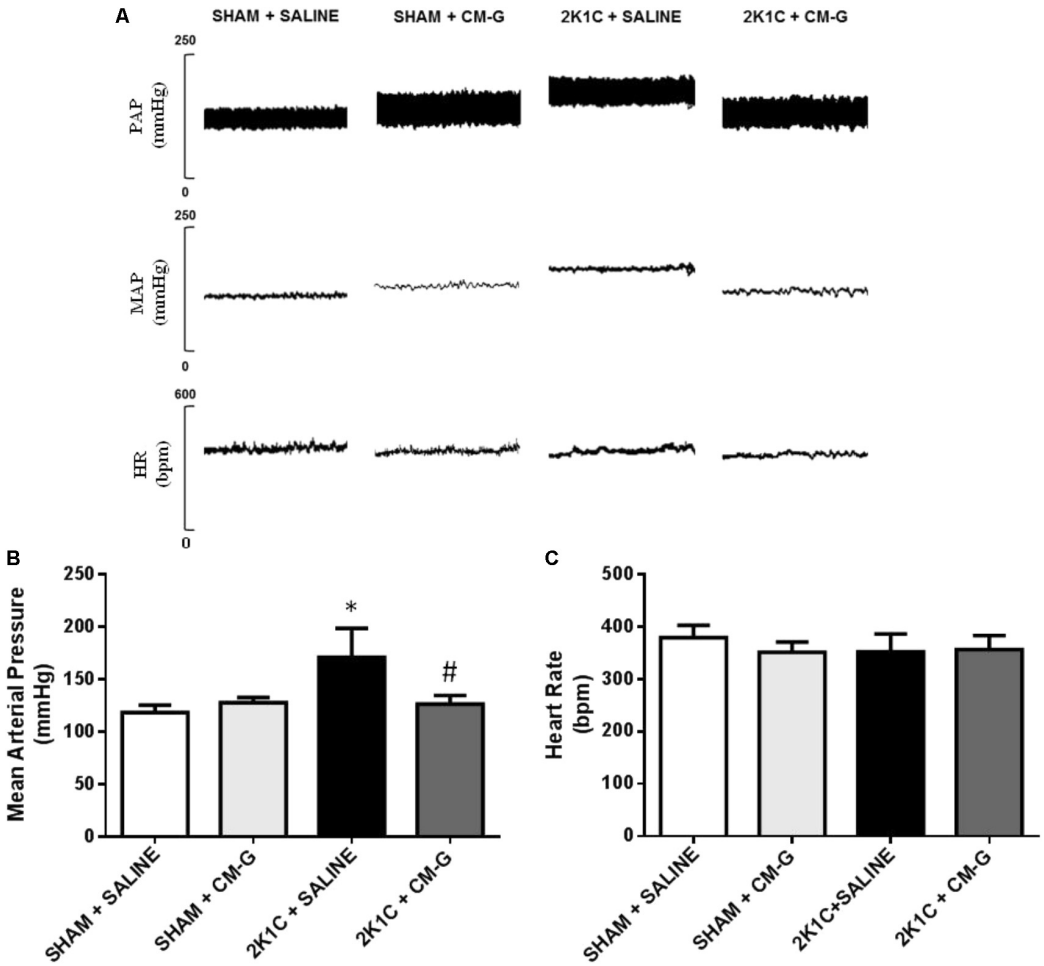

FIGURE 2 | Oral administration of carboxymethyl-glucan (CM-G) reduces blood pressure in renovascular hypertensive rats **(A)** Original tracings from one representative animal from each group (Sham + Saline, Sham + CM-G, 2K1C + Saline, 2K1C + CM-G) showing pulsatile arterial pressure (PAP), mean arterial pressure (MAP) and heart rate (HR). **(B)** Effects of oral treatment for 2 weeks on MAP in 2K1C and sham rats. **(C)** Effects of oral treatment for 2 weeks on HR in 2K1C and sham rats. * $P < 0.05$ 2K1C + SALINE *vs* SHAM + SALINE; #$P < 0.05$ 2K1C + CM-G *vs* 2 K1C + SALINE.

bpm.mmHg^{-1}; $p < 0.05$; (**Figures 3B,C**)]. Similar to baseline BP results, the CM-G treatment in sham rats did not alter baroreflex sensitivity ($p > 0.05$, **Figures 3B,C**). Regarding the spontaneous baroreflex sensitivity (SBRS), 2K1C rats exhibited reduced SBRS when compared to sham rats (0.58 ± 0.08 *vs.* 1.48 ± 0.04 ms.mmHg^{-1}, $p < 0.05$) and oral CM-G treatment improved the SBRS in 2K1C rats when compared to non-treated hypertensive rats (1.32 ± 0.03 vs. 0.58 ± 0.08 ms.mmHg^{-1}; $p < 0.05$). No changes in SBRS were observed in the sham rats.

CM-G Treatment Reduces Sympathetic Tone in 2K1C Rats

Representative tracings of PAP and MAP after hexamethonium application are shown in **Figure 4A**. After ganglionic blockage,

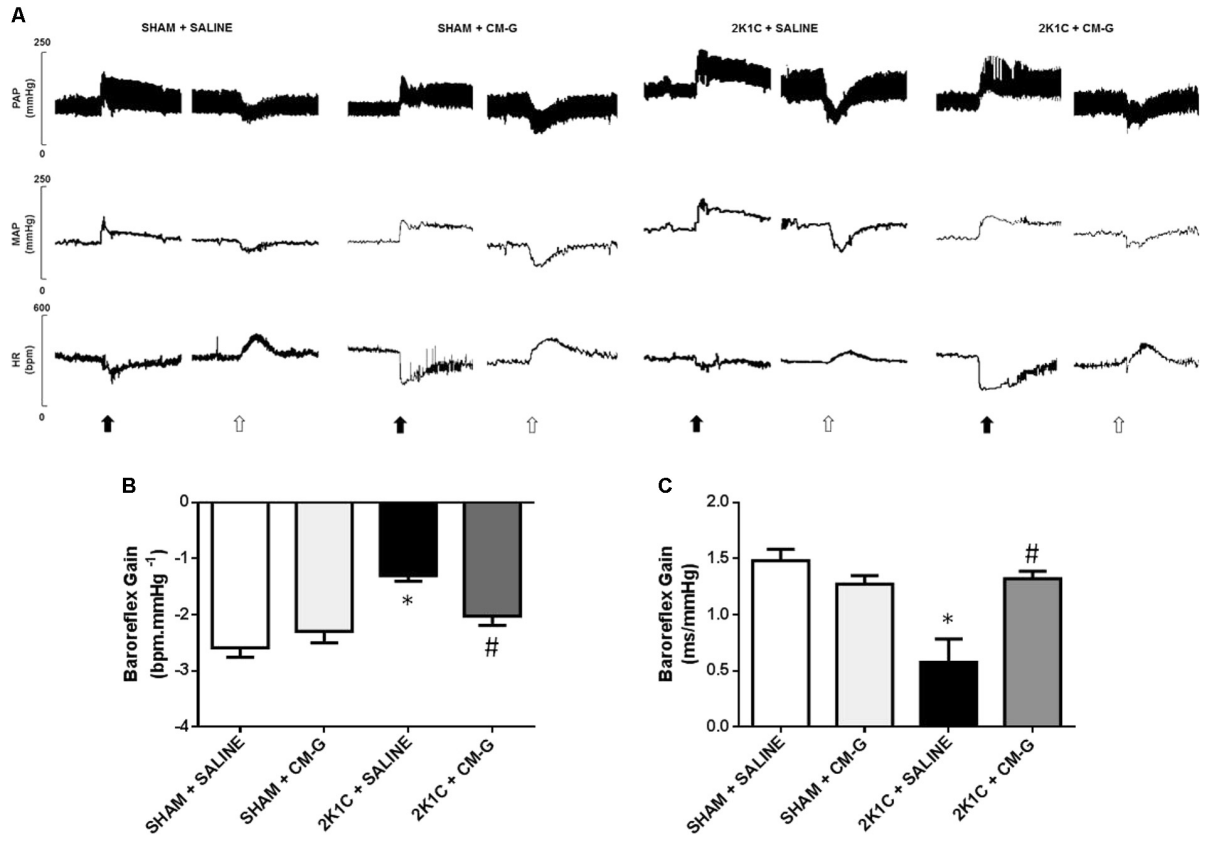

FIGURE 3 | Oral administration of carboxymethyl-glucan (CM-G) restores baroreflex sensitivity in renovascular hypertensive rats **(A)** Original tracings from one representative animal from each group (Sham + Saline, Sham + CM-G, 2K1C + Saline, 2K1C + CM-G) showing changes in pulse arterial pressure (PAP), mean arterial pressure (MAP) and heart rate (HR) in response to phenylephrine (8 μg/Kg, i.v., black arrows) and sodium nitroprusside (25 μg/Kg, i.v., open arrows). **(B)** Effects of oral administration of CM-G for 2 weeks on pharmacologically evoked baroreflex sensitivity. **(C)** Effects of oral administration of CM-G for 2 weeks on spontaneous baroreflex sensitivity. * $P < 0.05$ 2K1C + SALINE *vs* SHAM + SALINE; $^{\#}P < 0.05$ 2K1C + CM-G *vs* 2 K1C + SALINE

the 2K1C hypertensive rats showed a greater fall in blood pressure [ΔMAP, −60 ± 5 vs. −33 ± 2 mmHg, $p < 0.05$; (**Figure 4B**)] when compared to the sham rats. Oral CM-G treatment in 2K1C rats for 2 weeks attenuated the fall in blood pressure when compared to non-treated 2K1C rats [−35 ± 3 vs. −60 ± 5 mmHg; $p < 0.05$; (**Figure 4B**)], suggesting a reduction in sympathetic tone. The percentage changes in blood pressure among groups after hexamethonium is illustrated in **Figure 4C**. CM-G treatment had no effect on the sympathetic tone in sham rats when compared to non-treated sham rats.

CM-G Treatment Reduces Neurogenic Sympathetic Vasomotor Activity in 2K1C Rats

Renovascular hypertensive rats exhibited an increase in the magnitude of oscillatory components in the low-frequency (LF) range of SAP when compared to the sham group [9.16 ± 0.52 vs. 3.32 ± 0.38 $mmHg^2$, $p < 0.05$; (**Figure 4D**)]. Oral treatment with CM-G for 2 weeks reduced LF bands in 2K1C rats in comparison to non-treated renovascular hypertensive rats [6.23 ± 0.46 vs. 9.16 ± 0.52 $mmHg^2$; $p < 0.05$, (**Figure 4D**)].

DISCUSSION

We have demonstrated that oral CM-G treatment for 2 weeks reduced arterial hypertension and restored depressed baroreflex sensitivity in conscious renovascular hypertensive rats. These benefits seem to be related to a reduction in sympathetic tone.

The pre-clinical and clinical use of CM-G has been proposed as part of a combination therapy for a variety of diseases mainly because of its strong capability to scavenge reactive oxygen species (ROS) (Magnani et al., 2011; Araujo et al., 2015). For example, it has been reported that oral administration of CM-G helps to protect DNA against damage in prostate cancer patients (Magnani et al., 2011) and reduced the malondialdehyde serum levels in healthy individuals (Araujo et al., 2015). In addition, it has been reported that the daily oral treatment with CM-G (20 mg/kg) for eight days reduced interleukin 8 (IL-8), improved vascular response to nitric oxide and exhibited anti-aggregation activity. This suggests that CM-G could have a beneficial effect on the vascular system (Bezerra et al., 2017).

Considering the antioxidant capacity of the CM-G, we may suggest that our treatment with CM-G might have reduced BP in the 2K1C rats, secondary to the capability of scavenging reactive

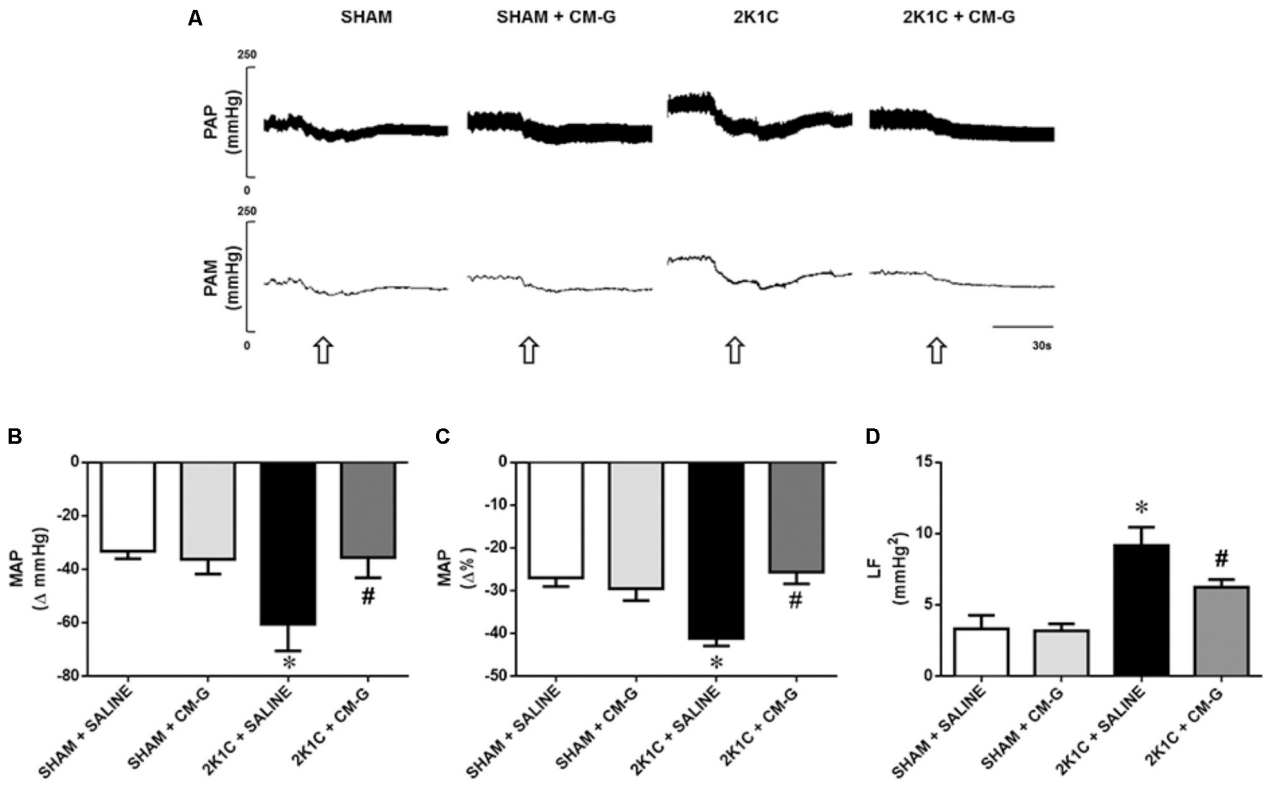

FIGURE 4 | Oral administration of carboxymethyl-glucan (CM-G) reduces sympathetic tone in renovascular hypertensive rats **(A)** Original tracings from one representative animal from each group (Sham + Saline, Sham + CM-G, 2K1C + Saline, 2K1C + CM-G) showing changes in pulse arterial pressure (PAP) and mean arterial pressure (MAP) in response to ganglionic blocker with hexamethonium (30 μg/Kg, i.v., open arrows). **(B)** Effects of oral administration of CM-G for 2 weeks on the evaluation of delta change of the MAP after blockade with hexamethonium. **(C)** Data now presented as percentage changes in MAP after blockade with hexamethonium. (d) Values of low frequency component (LF) of each group showing that oral administration of carboxymethyl-glucan (CM-G) reduces neurogenic sympathetic vasomotor activity in renovascular hypertensive rats. * $P < 0.05$ 2K1C + SALINE *vs* SHAM + SALINE; $^{\#}P < 0.05$ 2K1C + CM-G *vs* 2 K1C + SALINE.

oxygen species. In agreement with other studies that used acute antioxidant treatment for reducing BP in hypertensive rats (Costa et al., 2009; Guimaraes et al., 2012; Mengal et al., 2016), our study showed that CM-G treatment may be a relevant strategy to ameliorate BP in renovascular hypertension. However, we point out that CM-G treatment reduced blood pressure after 2 weeks of treatment, while most studies using antioxidant intervention with similar dose found reduction in BP only after 3 or 4 weeks of treatment (Alves et al., 2015; de Queiroz et al., 2015; Mengal et al., 2016). A recent study examined the antioxidant effects of green tea demonstrated that 1-week administration of the tea reduced blood pressure and sympathoexcitation in hypertensive rats (Garcia et al., 2017). Similarly, daily treatment with quercetin (25 mg/kg, for 1 week) reduced blood pressure in spontaneously hypertensive rats (Monteiro et al., 2012).

One of the key mechanisms in controlling blood pressure in health and disease is the baroreflex (Grassi et al., 1998). Baroreceptors located in the carotid sinuses and aortic arch detect changes in blood pressure and trigger reflex autonomic adjustments that buffer alterations in blood pressure (Malpas et al., 1997; Kanbar et al., 2007; Salgado et al., 2007). In pathological conditions such as hypertension, there is impairment in the autonomic control of blood pressure resulting in changes in baroreflex sensitivity (Gao et al., 2002; Nagai et al., 2003; Martinka et al., 2005).

The 2K1C model increased levels in ANG-II circulation, which might act in specific areas of the brainstem, augmenting oxidative stress and promoting autonomic dysfunction and reduction of baroreflex sensitivity (Heitzer et al., 1999). Our results showed that CM-G treatment improved the baroreflex sensitivity in animals with renovascular hypertension. Considering the well-known efficacy of antioxidant therapies for improving of baroreflex sensitivity in ANG-II-dependent hypertension models (Botelho-Ono et al., 2011; Queiroz et al., 2012; Mendes-Junior et al., 2013; Alves et al., 2015), it is probable that the improvement of baroreflex sensitivity was a result of the antioxidant therapy promoted by the oral CM-G treatment. In fact, previous studies have demonstrated that the antioxidant therapy had no effect on baroreflex function in normotensive animals (Li et al., 1996; Pickering et al., 2008), but improved the baroreflex in hypertensive rats (Guimaraes et al., 2012; Monteiro et al., 2012; Mendes-Junior et al., 2013). This suggests that antioxidant administration in the absence of oxidative stress has no influence on baroreflex sensitivity.

Additionally, CM-G treatment plays an important role in reducing sympathetic tone in rats with renovascular

hypertension. Reactive oxygen species (ROS) in the brainstem have a potential role in the modulation of sympathetic activity and BP in hypertensive rats, suggesting that oxidative stress can contribute to higher sympathetic activity and hypertension (Malpas, 2010; Chan and Chan, 2014). Given this, further study is needed to see whether the reduced sympathetic overactivity was a consequence of the re-establishment of baroreflex sensitivity or if CM-G treatment could act as antioxidant therapy directly on the neuronal network involved in sympathetic control (Huber and Schreihofer, 2010). One possible limitation of our study is that the sympathetic modulation of blood pressure was evaluated one hour after the vasoactive drugs protocol. One could argue that using vasoactive drugs to evaluate baroreflex could trigger vasopressin release. However, it is important to note that vasopressin release is far more sensitive to changes in plasma osmolarity than to changes in blood volume. Therefore, repeated doses of the vasoactive drugs would be needed to trigger effective changes in vasopressin release, which was not the case. The use of a single bolus injection of vasoactive drugs in very small volumes/concentrations for the baroreflex sensitivity test allows a considerable margin of safety regarding the intervention of other mechanisms such as osmolality changes and release of vasopressin.

In order to further assess the idea that CM-G treatment reduces arterial hypertension, we performed the spectral analysis of systolic blood pressure. Our findings indicated that CM-G treatment significantly reduces the LF component of the spectral analysis. The LF component is a well-accepted index of sympathetic modulation (Inoue et al., 1991). Therefore, although we were not able to perform direct recordings of the sympathetic activity, which would be the gold standard for sympathetic evaluation, both the hexamethonium and the spectral analysis data suggest that CM-G treatment reduces sympathetic activity in renovascular hypertensive rats.

Further studies investigating the underlying mechanisms involved in CM-G treatment and reduction of arterial hypertension, improvement of the baroreflex sensitivity and the involvement of the sympathetic tone will be needed. Our experience leads to the following suggestions. First, it is important knowing if CM-G alters antioxidant enzymes, such as superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase activities, in the vascular or brainstem level. Second, we need to understand if CM-G can improve other control mechanisms of arterial blood pressure, such as the peripheral chemoreflex or renin-angiotensin-aldosterone system.

In summary, we reported that acute oral CM-G treatment reduced arterial hypertension and restored baroreflex sensitivity via reduction of the sympathetic tone in renovascular hypertensive rats. In fact, the precise site of action where CM-G therapy produces its beneficial effects in order to ameliorate hypertension remains unknown. However, we have identified one more antioxidant compound with baroreceptor modulation properties that could be used in the future as an additional therapy for renovascular hypertension.

AUTHOR CONTRIBUTIONS

AC-G and VB designed the experiments. AC-G and DG performed and analyzed the experiments. AC-G, DG, JB, and VB wrote the manuscript. AC-G, DG, JB, BK, RC-G, JC, MM, and VB reviewed the manuscript.

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CAPÍTULO 42

PROBIÓTICOS COMO ALTERNATIVA AO TRATAMENTO DA HIPERTENSÃO ARTERIAL: UMA REVISÃO

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RESUMO: Hipertensão Arterial (HA) é um grave problema de saúde pública, visto que existe uma alta prevalência de pessoas diagnosticadas com essa patologia. Sendo de caráter multifatorial, essa desordem consiste como principal fator de risco para doenças cardiovasculares (DCVs). Recentemente, a disbiose intestinal tem sido relacionada a hipertensão. A fim de reverter isso, alguns trabalhos científicos têm investigado os efeitos benéficos devido à suplementação de probióticos frente a hipertensão. Diante disso, esta revisão tem por objetivo elucidar estudos que relacionam o uso de probióticos sobre a condição hipertensiva. Para isso, foi realizado buscas em plataformas *online* nacionais e internacionais acerca do tema proposto. Observou-se que suplemento diário de probióticos consegue melhorar a pressão arterial (PA) por meio da redução do estresse oxidativo, diminuição da atividade da enzima ECA, diminuição da inflamação vascular, redução da hipertrofia cardíaca, reversão disautonomia cardíaca e produção de peptídeos bioativos com efeito anti-hipertensivo. À vista disso, é notório que os probióticos configuram um alvo importante na

busca de estratégias terapêuticas não medicamentosas e eficientes para a HA. Sendo uma opção a ser considerada por indivíduos hipertensos que tomam mais de dois ou três medicamentos, visto que a utilização de probióticos pode ajudar a reduzir a quantidade de medicamentos utilizados para redução da PA.
Palavras-chave: Bactérias lácticas. Doença Cardiovascular. Pressão Arterial.

INTRODUÇÃO

Doenças Cardiovasculares (DCV) são desordens que envolvem o funcionamento do coração e dos vasos sanguíneos, incluindo doença coronariana, cerebrovascular, arterial periférica, cardíaca reumática, cardiopatia congênita, trombose venosa profunda e embolia pulmonar. Em 2015, das 17,7 milhões de mortes em indivíduos com menos de 70 anos, 32% foram relacionadas a complicações decorrentes de DCVs, ocorrendo 82% em países de baixa e média renda (BRASIL, 2017).

A HA é o principal fator de risco para as DCVs, tanto em países desenvolvidos como em desenvolvimento, visto que é uma condição presente em metade das pessoas que morrem por essas doenças. Assim, pode-se afirmar que a HA causa um grande impacto na saúde pública (CALZEIRA et al., 2018). No Brasil, a HA atinge 32,5% (36 milhões) de indivíduos adultos, mais de 60% dos idosos, contribuindo direta ou indiretamente para 50% das mortes por (DCV) (MALACHIAS et al., 2016a).

Alguns fatores de riscos estão associados a essa enfermidade como fatores hereditários, hábitos alimentares não saudáveis e sedentarismo. Esses últimos, reforçam a importância de mudanças no estilo de vida e medidas

nutricionais como tratamento não medicamentoso para a HA, os quais envolvem principalmente o estímulo a prática de atividades físicas e mudanças dietéticas (MALACHIAS et al., 2016b).

Sabe-se que a hipertensão não está apenas relacionada a disfunção autonômica, disfunção endotelial e ao desbalanço de sal como é relatado há anos na literatura, mais também à disbiose intestinal (YANG et al., 2015a). A disbiose tem sido relacionada ao desenvolvimento e progressão de inúmeras doenças relacionadas à dieta, incluindo as doenças cardiovasculares (DE LIMA MARKUS; SOUZA, 2018)

Na estratégia de reverter o quadro de disbiose ocasionada pela HA, o uso de probióticos tornou-se uma excelente alternativa. Estes são definidos como microrganismos vivos e de classificação gram-positiva, que quando inseridos na dieta em quantidades adequadas, afetam beneficamente o organismo do hospedeiro sendo capazes de prevenir ou tratar patologias (DE SOUZA, 2017).

Os probióticos possuem diversos efeitos benéficos quando inseridos na alimentação habitual, dentre eles pode-se destacar o equilíbrio na microbiota intestinal, favorecendo o controle das infecções intestinais; o estímulo da motilidade intestinal; alívio de intolerância aos açúcares como a lactose; fortalecimento do sistema imunológico e efeitos anticarcinogênicos (DE LIMA, 2019).

Os benefícios dos probióticos se estendem para a modulação da PA mediante atuação em diversos mecanismos de controle, contribuindo, assim, para a reversão da disautonomia cardíaca, atenuação da variabilidade da PA e frequência cardíaca, e melhora da sensibilidade do barorreflexo (VASQUEZ et al., 2019).

Diante disso, o presente trabalho tem como objetivo realizar uma revisão bibliográfica acerca dos impactos dos probióticos sobre a HA, demonstrando também as evidências da influência dos componentes da microbiota intestinal na condição hipertensiva.

METODOLOGIA

O presente trabalho trata-se de uma pesquisa bibliográfica, limitada a estudos publicados entre 2015 e 2019, que abrangem a relação entre probióticos e o processo hipertensivo. Para a elaboração desta revisão foi realizado um levantamento na literatura nacional e internacional utilizando plataformas *online* como *Pubmed, Scielo, Google Acadêmico, Science Direct, National Center for Biotechnology Information* (NCBI). Os descritores utilizados para pesquisa foram: hipertensão arterial, microbiota intestinal, probióticos, Bactérias ácido-lácticas, *Lactobacillus* sp.; *Bifidobacterium sp.* Foram filtrados artigos em português, inglês e espanhol.

Os critérios de inclusão para construção deste trabalho foram artigos originais e de revisão que abordavam as características gerais da hipertensão arterial e sua relação com probióticos. Além disso, foi evidenciado os mecanismos pelos quais as bebidas probióticas promovem uma melhora nesta desordem. As informações foram obtidas e analisadas para corresponder a proposta do trabalho.

RESULTADOS E DISCUSSÃO

Hipertensão Arterial

A hipertensão arterial (HA) é o aumento anormal da pressão que o sangue exerce nas paredes das artérias. O indivíduo hipertenso apresenta persistentemente uma pressão arterial sistólica (PAS) acima de 140 mmHg e/ou pressão arterial diastólica (PAD) acima de 90 mmHg (MALACHIAS, et al., 2016a)

Durante o desenvolvimento da hipertensão, o controle cardiovascular autonômico torna-se prejudicado, com uma redução parassimpática e um aumento na atividade simpática direcionada aos vasos periféricos e coração. Consequentemente, a hiperatividade simpática promove alterações cardiometabólicas complexas (GRASSI; RAM, 2016). Em suma, a hiperativação simpática promove um aumento do débito cardíaco, volume de sangue bombeado pelo coração por minuto, retenção de água e da resistência vascular periférica. Esses fatores desempenham um papel fundamental na fisiopatologia da HA (SILVA, 2018).

A hipertensão pode levar ao dano renal, o que inviabiliza o rim de manter o balanço hidroeletrolítico, produzir hormônios, regular a osmolaridade, purificar as toxinas do organismo e outras substâncias derivadas de metabólitos celulares presentes na circulação (ANTUNES, 2015). Além disso, o prejuízo no funcionamento renal também contribui para o processo hipertensivo, já que os rins são responsáveis pela produção de renina, que leva à ativação do sistema renina-angiotensina-aldosterona (SRAA), cujas ações contribuem para

a elevação da PA. Assim, a HA está diretamente relacionada à superativação do SRAA (HALL; GUYTON, 2017).

Níveis aumentados de espécies reativas de oxigênio (EROs) também são relacionados à hipertensão, desencadeando vias de sinalização proliferativas que contribuem para a disfunção endotelial e remodelação cardíaca e vascular, sendo eles, fatores de riscos para as doenças cardiovasculares (PEREIRA et al., 2018).

Nessa perspectiva, desenvolver meios que possibilitam novos tratamentos para o controle da PA diminuem significativamente as chances de um indivíduo desenvolver complicações cardiovasculares (ETTEHAD et al., 2016) e a compreensão da relação entre microbiota e hipertensão é um dos caminhos promissores.

Microbiota Intestinal Humana

O intestino é um órgão que apresenta diversas funções, dentre elas o reconhecimento, seleção, regulação e absorção de nutrientes. O trato gastrointestinal (TGI) tem um sistema nervoso próprio, denominado sistema nervoso entérico. Este é especialmente importante no controle dos movimentos e da secreção gastrointestinal, inicia-se no esôfago e se estende até o ânus, sendo composto por cerca de 100 milhões de neurônios. O sistema nervoso entérico é composto basicamente pelo plexo externo, disposto entre as camadas musculares longitudinal e circular, denominado plexo mioentérico ou plexo de Auerbach; e plexo interno, denominado plexo submucoso ou plexo de Meissner, localizado na submucosa. Embora, este sistema nervoso do TGI funcione de

forma independente, pode ocorrer influências do sistema nervoso simpático e parassimpático (HALL; GUYTON, 2017).

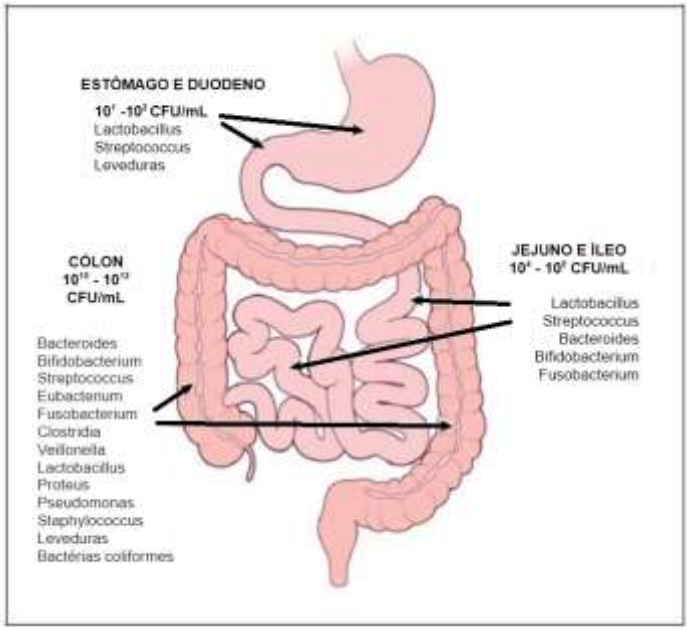
O sistema nervoso simpático e o intestino possuem uma relação bastante adaptativa, quando outros órgãos necessitam de um maior fluxo de sangue o sistema simpático limita o fluxo sanguíneo esplâncnico. A estimulação simpática promove vasoconstrição das veias intestinais e mesentéricas de grande calibre, em consequência o volume de sangue é minimizado (HALL; GUYTON, 2017). Além disso, a hiperestimulação simpática, por meio da redução na perfusão sanguínea do TGI, acaba influenciando na microbiota intestinal.

O TGI humano contém mais de 1000 espécies **(Figura 1)** de bactérias conhecidas com mais de 3 milhões de genes, o que equivale a 150 vezes mais do que genes humanos (PINTO, 2016). A **Figura 1** esquematiza a distribuição das bactérias de diferentes gêneros ao longo do TGI, no estômago e duodeno encontram-se microrganismos dos gêneros *Lactobacillus* spp., *Streptococcus* spp., já no jejuno e íleo são regiões colonizadas por *Lactobacillus* spp., *Streptococcus* spp., *Bacterioides* spp., *Fusobacterium* spp. e *Bifidobacterium* spp., o intestino grosso é a área do TGI onde ocorre uma maior concentração de microrganismos (CRESCI; BAWDEN, 2015).

Bactérias dos gêneros *Bifidobacterium* spp., *Clostridium* spp., *Lactobacillus* spp., *Enterococcus* spp., *Eubacterium* spp., *Fusobacterium* spp., *Peptostreptococcus* spp. e *Ruminococcus* spp. são responsáveis por manter uma relação de simbiose com o hospedeiro, ou seja, ambas as partes se beneficiam para estabelecer um equilíbrio intestinal, favorecendo funções fisiológicas como fermentação de substratos energéticos; fortalecimento do sistema imune; crescimento de bactérias patogênicas; regulação e desenvolvimento intestinal; e

produção de vitaminas essenciais, como vitamina K e biotina (ALVES et al., 2018; SILVA, 2018).

Figura 1. Distribuição dos microrganismos pelo trato gastrointestinal humano. CFU, Unidades formadoras de colônias.



Fonte: Adaptado de Cresci e Bawden (2015).

O desenvolvimento da microbiota humana começa desde o nascimento (SANTOS, 2018). No momento do parto normal, algumas espécies como *Escherichia coli*, *Streptococcus* sp., *Lactobacillus* sp. e *Staphylococcus* sp., bactérias da flora vaginal, acabam entrando em contato com o recém-nascido, influenciando positivamente no desenvolvimento da microbiota intestinal. A amamentação,

também possui efeitos benéficos na formação da microbiota, bactérias do gênero *Bifidobacterium* sp*.* e *Lactobacillus* sp*.* permitem a estabilidade da simbiose intestinal e no fortalecimento do sistema imunológico. Outros fatores como a dieta, genótipo e uso de antibióticos, similarmente possuem atuação no desenvolvimento e homeostase da microbiota (ALVES et al., 2018; DA SILVA QUIRINO, 2019; CRESCI; BAWDEN, 2015).

A disbiose intestinal ocorre quando há um desequilíbrio entre as bactérias benéficas e patogênicas da microbiota intestinal, trata-se de um distúrbio cada vez mais relevante, que tem sido relacionado a causa ou desenvolvimento de doenças crônicas não transmissíveis como a HA (DE MELO; OLIVEIRA, 2018). O uso de probióticos, suplementos de microrganismos vivos que auxiliam na simbiose da microbiota intestinal e na constituição da barreira intestinal, têm mostrado ser uma alternativa favorável na prevenção de doenças crônicas como a hipertensão.

Disbiose Intestinal e a Hipertensão Arterial

A microbiota intestinal tem um papel fundamental na manutenção da pressão arterial e a disbiose pode resultar em hipertensão. Yang et al. (2015a), relataram que as alterações na microbiota intestinal podem ser decorrentes de diversos fatores de risco para a hipertensão como a predisposição genética e o desequilíbrio nutricional.

Mell et al. (2015), evidenciaram uma ligação entre a microbiota intestinal e hipertensão através de uma pesquisa utilizando ratos Dahl sensíveis ao sal (modelo genéticos de hipertensão) e ratos Dahl resistentes ao sal (controle genético

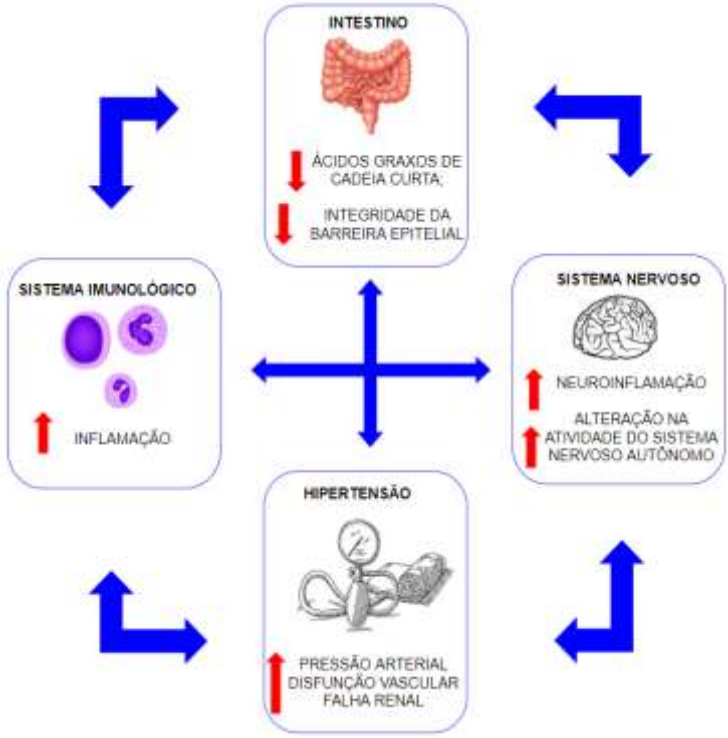
normotenso), onde análises da microbiota cecal confirmaram a existência de diferenças significativas entre a microbiota dos ratos hipertensos e normotensos utilizados no estudo. De modo semelhante, Yang et al. (2015a), publicaram um trabalho relatando que a composição microbiana intestinal de ratos espontaneamente hipertensos (SHR) eram diferentes em quantidade e diversidade de microrganismos quando comparada aos ratos normotensos Wistar-Kyoto. Esses achados sugerem que na hipertensão há um quadro de disbiose intestinal.

Na hipertensão, **Figura 2**, há uma redução do número de bactérias produtoras de ácidos graxos de cadeia curta, um dos produtos das bactérias que compõem a microbiota intestinal, além disso há a redução no fluxo sanguíneo intestinal devido à super estimulação do sistema nervoso simpático e, dessa forma, o desenvolvimento da disbiose intestinal. O fato é que o desequilíbrio microbiano causado pela hipertensão, leva a perda da integridade da barreira epitelial, desenvolvendo assim, uma inflamação intestinal e redução dos ácidos graxos de cadeia curta. Essas alterações na microbiota comprometem a regulação da PA e, em consequência, causam danos aos rins. Com as funções renais comprometidas, acumula-se altos níveis de toxinas urêmicas que atingem o intestino causando alterações na composição das bactérias, ocasionando a translocação de endotoxinas para a circulação sanguínea. Essas alterações associadas com o sistema imunológico neural e o intestinal, fortalece o *feedback* positivo que induz a disbiose e as doenças cardiorrenais (FELIZARDO et al., 2019).

A **Figura 2**, mostra que o aumento da permeabilidade intestinal pode resultar em translocação de bactérias do lúmen intestinal (interior do intestino) para a circulação, refletindo, na

desregulação do sistema imunológico local e sistêmico e colaborando para o quadro de disbiose e doenças cardiorrenais. Adicionalmente, a disbiose intestinal está interligada com a mudança da atividade do sistema nervoso autônomo e do tônus vascular (FELIZARDO et al., 2019).

Figura 2. Relação entre hipertensão, disbiose intestinal, inflamação e atividade nervosa autonômica.



Fonte: Adaptado de Felizardo et al. (2019).

Assim sendo, fica claro que reverter o quadro de disbiose intestinal pode ter efeitos benéficos frente a HA e desenvolver

procedimentos para manutenção da simbiose intestinal pode prevenir essa desordem. Alternativas simples e viáveis para a regulação da microbiota frente a HA, como a suplementação de probióticos que podem estar facilmente inseridos em alimentos impactam diretamente na saúde pública.

Uso de Probióticos na Hipertensão

Probióticos podem ser definidos como organismos vivos administrados em quantidades adequadas para conferir benefícios à saúde do hospedeiro. Esses efeitos benéficos são resultados da ativação de vários mecanismos, tais como a liberação de metabólitos antibacterianos, indução da produção de defensinas pelo hospedeiro, atividade de enzimas probióticas no lúmen do intestino, inibição da aderência bacteriana, competição por nutrientes e a melhora da resposta imunológica (ROBLES-VERA et al., 2017).

A manipulação da microbiota intestinal pode levar ao desenvolvimento de novas terapias para problemas cardiovasculares. Alguns estudos vêm evidenciando o potencial de probióticos para aumentar a absorção de drogas, destacamos aqui a suplementação com *Lactobacillus plantarum* IS-10506 em coelhos para aumentar a concentração plasmática de amlodipina, um antagonista de diidropiridinas usado no tratamento da angina e hipertensão (SAPUTRI et al. 2018).

As espécies de bactérias do ácido lático (BAL) mais comumente empregadas na suplementação probiótica são: *L. acidophilus, L. plantarum, L. casei, L. bulgaricus* e *Streptococcus thermophilus*. Gómez-Guzmán et al. (2015), demonstraram o efeito das bactérias *Lactobacillus fermentum*

CECT5716 (LC40), *L. coryniformis* CECT5711 (K8) e *L. gasseri* CECT5714 (LC9) na redução progressiva da PAS, através da redução do estresse oxidativo vascular e diminuição da inflamação vascular na aorta de ratos SHR.

Além disso, as BAL podem ser geneticamente modificadas a fim de aumentar o potencial terapêutico. Considerando que o sistema renina-angiotensina é determinante na regulação da PA, Yang et al. (2015b), usaram metodologias de engenharia genética para produzir uma linhagem de *Lactobacillus plantum* que expressam o peptídeo inibidor da enzima de conversão da angiotensina (ECA), produtora de ang II, reduzindo assim os níveis de PA e os efeitos resultantes da hipertensão em modelos animais SHR.

Yap et al. (2016), relataram que após oito semanas de administração de *Lactobacillus casei* C1 em animais SHR houve uma melhora na pressão arterial sistólica semanal (PAS), pressão arterial média (PAM), pressão arterial diastólica (PAD) e a função de reatividade aórtica. Níveis elevados de glutationa e óxido nítrico no soro dos animais SHR tratados, propõe que a bactéria exerce uma proteção vascular através de funções antioxidantes.

Toral et al. (2018), observaram que o tratamento com Lactobacillus fermentum CECT5716 foi capaz de prevenir a disbiose intestinal, a disfunção endotelial e a hipertensão em animais tratados com tacrolimus, droga que aumenta a pressão arterial sistólica e prejudica o relaxamento dependente do endotélio pela acetilcolina. O efeito anti-hipertensivo desse probiótico incluiu à redução do estresse oxidativo vascular, por meio da regulação negativa de nicotinamida adenina dinucleotídeo fosfato (NADPH) oxidase 2 (NOX2) e prevenção do desacoplamento da óxido nítrico sintase endotelial (eNOS),

além de efeitos anti-inflamatórios como a diminuição de linfócitos T-helper 17 e aumento da polarização das células Treg nos linfonodos mesentéricos.

Diante disso, os estudos acima comprovam que o uso de probióticos promove o equilíbrio microbiológico intestinal, que por sua vez, apresenta efeitos promissores no controle da HA, sendo considerados uma interessante possibilidade de tratamento complementar.

Efeitos Benéficos dos Alimentos Probióticos Frente a Hipertensão

Na perspectiva de alimentos que podem ser usados como complemento no tratamento da HA, o kefir apresentou ser uma excelente bebida rica em probiótico com efeito anti-hipertensivo. O kefir, caracteriza-se como uma bebida ácida que pode ser produzida com leite ou água, podendo ser ou não saborizada com frutas.

Uma análise microbiológica realizada em bebidas à base de kefir revelou a presença de bactérias do gênero *Acetobacter aceti*, *Acetobacter sp.*, *Lactobacillus delbrueckii delbrueckii*, *Lactobacillus fermentum*, *Lactobacillus fructivorans*, *Enterococcus faecium*, *Leuconostoc* spp., *Lactobacillus kefiranofaciens* e leveduras do gênero *Candida famata* e *Candida krusei* (KLIPPEL et al., 2016).

Segundo Barboza (2016), o kefir pode prevenir a lesão renal induzida por isquemia reperfusão, provavelmente por meio da redução do estresse oxidativo e apoptose, indicando que a utilização do kefir como um adjuvante não farmacológico possui potencial terapêutico para retardar a progressão das complicações renais e cardiovasculares.

Tratamento à base de Kefir por 60 dias foi capaz de melhorar a função endotelial na SHR, restaurando parcialmente o desequilíbrio de espécies reativas de oxigênio e óxido nítrico, além do recrutamento de células progenitoras endoteliais (FRIQUES et al., 2015).

Posteriormente, Monteiro (2017) demonstrou que a suplementação com kefir durante 60 dias em ratos com hipertensão renovascular (2R1C) promoveu redução da pressão arterial, melhora na disfunção endotelial e no estresse oxidativo, como também diminuição da atividade da ECA. Em consonância com o estudo supracitado, Silvia-Cutini (2019), mostraram que o tratamento com kefir promove a redução da pressão sanguínea, redução da hipertrofia cardíaca e recuperação da função cardíaca por meio da melhoria na contratilidade devido à modulação de proteínas transportadoras de cálcio e redução da atividade simpática.

Em 2015, Ahrén e sua equipe, analisaram que mirtilos fermentados por *Lactobacillus plantarum* DSM 15313 possuíam efeito anti-hipertensivo. De acordo com sua pesquisa, após quatro semanas de alimentação com mirtilos fermentados, ratos saudáveis tiveram uma redução significativa na PA em comparação aos ratos controle, e os animais com hipertensão induzida por N^{ω}-nitro-arginina-metil-ester (L-NAME) apresentaram a PA reduzia depois de duas semanas de suplementação.

Lollo et al. (2015), mostraram que a ação proteolítica da *Saccharomyces cerevisiae* nas proteínas do leite foi capaz de gerar peptídeos bioativos que diminuíram a hipertensão mediante a inibição da ECA. Os principais gêneros de organismos probióticos produtores de peptídeos bioativos anti-hipertensivo a partir do leite são as bactérias: *Lactobacillus,*

Lactococcus e *Bifidobacterium* e as leveduras: *Candida, Galactomyces, Geotrichum, Kluyveromyces, Pichia, Saccharomyces* e *Schizosaccharomyces* (AHTESH; STOJANOVSKA; APOSTOLOPOULOS, 2018.).

Diante do exposto, é notório que a implementação de alimentos ricos em probióticos promove efeitos importantes na melhora da HA. A redução de espécies reativas de oxigênio, a melhor disponibilidade de óxido nítrico, a inibição da ECA e a melhora na disfunção endotelial são mecanismos pelos quais os probióticos reduzem a PA.

CONSIDERAÇÕES FINAIS

Com isso, fica evidente que a microbiota intestinal é um regulador da homeostase humana e a disbiose intestinal pode estar relacionada ao desenvolvimento de doenças crônicas como a hipertensão. Dessa forma, compreender a relação entre a microbiota intestinal e a hipertensão é extremamente importante para a saúde pública.

Com base nos trabalhos apresentados nesta revisão, o suplemento diário de probióticos consegue melhorar a pressão arterial por meio da redução do estresse oxidativo, diminuição da atividade da enzima ECA, diminuição da inflamação vascular, redução da hipertrofia cardíaca, reversão disautonomia cardíaca e produção de peptídeos bioativos com efeito anti-hipertensivo. À vista disso, é notório que os probióticos configuram um alvo importante na busca de estratégias terapêuticas não medicamentosas e eficientes para a HA.

Por conseguinte, identificar quais linhagens de bactérias probióticas apresentam efeito anti-hipertensivo, pode

revolucionar as terapias complementares. Sendo uma opção a ser considerada por pessoas hipertensas que tomam mais de dois ou três medicamentos, visto que a utilização de probióticos pode ajudar a reduzir a quantidade de medicamentos utilizados para redução da PA.

Em relação à aplicabilidade, o uso de probióticos na terapêutica é bastante promissor e exequível, uma vez que os mesmos podem ser facilmente incorporados em excipientes farmacêuticos, alimentos e bebidas. Dessa forma, abrem-se possibilidades não apenas para a indústria farmacêutica, mas também para indústria alimentícia que poderá ofertar os probióticos em iogurtes, queijos e leites fermentados.

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Autoria na elaboração de capítulo de livro intitulado “Avaliação dos efeitos da suplementação com *Lactobacillus acidophilus* (LA – 05) nos parâmetros cardiovasculares de animais normotensos e hipertensos” publicado na revista “Aqui se faz ciência!” com ISBN 978-65-00-06195-6 em 2020. Um trabalho que aborda os mais variados temas da ciência no Brasil para fornecer informações à sociedade de maneira mais objetiva e clara.

Autoria na elaboração de capítulo de livro intitulado “Nitrato orgânico 2-nitrato-1,3- dibutoxipropano melhora parâmetros cardiovasculares de animais hipertensos sem causar tolerância” publicado na revista “Farmácia: tecnologia a serviço da saúde” com ISBN 978-85-53005-25-3 (on-line) no Congresso Internacional de Saúde e Meio Ambiente (CINASAMA) em 2020.

Coautoria na elaboração de capítulo de livro intitulado “Novos componentes do sistema renina-angiotensina: implicações, efeitos e perspectivas” publicado na revista “Biotecnologia: tecnologia a serviço da saúde” com ISBN 978-85-53005-27-7 (on-line) no Congresso Internacional de Saúde e Meio Ambiente (CINASAMA) em 2020.