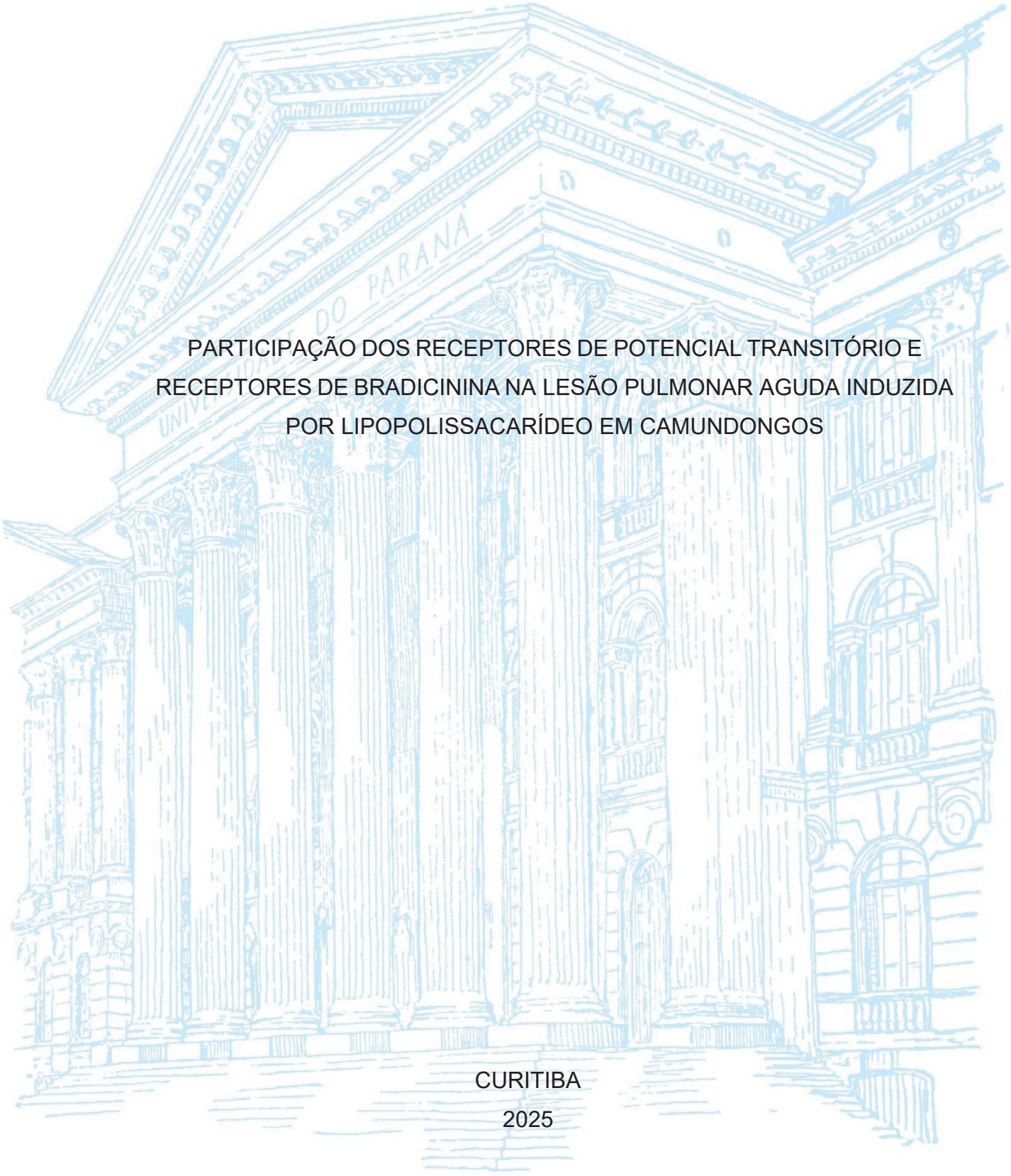
UNIVERSIDADE FEDERAL DO PARANÁ

MAYARA ALVES AMORIM



PARTICIPAÇÃO DOS RECEPTORES DE POTENCIAL TRANSITÓRIO E RECEPTORES DE BRADICININA NA LESÃO PULMONAR AGUDA INDUZIDA POR LIPOPOLISSACARÍDEO EM CAMUNDONGOS

CURITIBA

2025

MAYARA ALVES AMORIM

PARTICIPAÇÃO DOS RECEPTORES DE POTENCIAL TRANSITÓRIO E RECEPTORES DE BRADICININA NA LESÃO PULMONAR AGUDA INDUZIDA POR LIPOPOLISSACARÍDEO EM CAMUNDONGOS

Tese apresentada ao Programa de Pós-graduação em Farmacologia do Setor de Ciências Biológicas da Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutor em Farmacologia.

Orientadora: Profª. Drª. Eunice André.

CURITIBA

2025

DADOS INTERNACIONAIS DE CATALOGAÇÃO NA PUBLICAÇÃO (CIP)
UNIVERSIDADE FEDERAL DO PARANÁ
SISTEMA DE BIBLIOTECAS – BIBLIOTECA DE CIÊNCIAS BIOLÓGICAS

Amorim, Mayara Alves.
Participação dos receptores de potencial transitório e receptores de bradicinina na lesão pulmonar aguda induzida por lipopolissacarídeo em camundongos. / Mayara Alves Amorim. – Curitiba, 2025.
1 recurso on-line : PDF.

Tese (Doutorado) – Universidade Federal do Paraná, Setor de Ciências Biológicas. Programa de Pós-Graduação em Farmacologia.
Orientadora: Prof.ª Dra. Eunice André.

1. Pulmões - Inflamação. 2. Síndrome do Desconforto Respiratório. 3. Lesão Pulmonar. 4. Canal de Cátion TRPA1. 5. Canais de Cátion TRPV. 6. Síndrome respiratória aguda grave. I. André, Eunice, 1972-. II. Universidade Federal do Paraná. Setor de Ciências Biológicas. Programa de Pós-Graduação em Farmacologia. III. Título

Bibliotecária: Rosilei Vilas Boas CRB-9/939

ATA Nº355

ATA DE SESSÃO PÚBLICA DE DEFESA DE DOUTORADO PARA A OBTENÇÃO DO GRAU DE DOUTORA EM FARMACOLOGIA

No dia dez de outubro de dois mil e vinte e cinco às 09:00 horas, na sala Auditório, Departamento de Farmacologia, foram instaladas as atividades pertinentes ao rito de defesa de tese da doutoranda **MAYARA ALVES AMORIM**, intitulada: **PARTICIPAÇÃO DOS RECEPTORES DE POTENCIAL TRANSITÓRIO E RECEPTORES DE BRADICININA NA LESÃO PULMONAR AGUDA INDUZIDA POR LIPOPOLISSACARÍDEO EM CAMUNDONGOS**, sob orientação da Profa. Dra. EUNICE ANDRÉ. A Banca Examinadora, designada pelo Colegiado do Programa de Pós-Graduação FARMACOLOGIA da Universidade Federal do Paraná, foi constituída pelos seguintes Membros: EUNICE ANDRÉ (UNIVERSIDADE FEDERAL DO PARANÁ), NARA LINS MEIRA QUINTAO (UNIVERSIDADE DO VALE DO ITAJAÍ), GABRIELA TREVISAN DOS SANTOS (UNIVERSIDADE FEDERAL DE SANTA MARIA), MARIA FERNANDA DE PAULA WERNER (UNIVERSIDADE FEDERAL DO PARANÁ). A presidência iniciou os ritos definidos pelo Colegiado do Programa e, após exarados os pareceres dos membros do comitê examinador e da respectiva contra argumentação, ocorreu a leitura do parecer final da banca examinadora, que decidiu pela APROVAÇÃO. Este resultado deverá ser homologado pelo Colegiado do programa, mediante o atendimento de todas as indicações e correções solicitadas pela banca dentro dos prazos regimentais definidos pelo programa. A outorga de título de doutora está condicionada ao atendimento de todos os requisitos e prazos determinados no regimento do Programa de Pós-Graduação. Nada mais havendo a tratar a presidência deu por encerrada a sessão, da qual eu, EUNICE ANDRÉ, lavrei a presente ata, que vai assinada por mim e pelos demais membros da Comissão Examinadora.

CURITIBA, 10 de Outubro de 2025.

Assinatura Eletrônica
10/10/2025 16:05:56.0
EUNICE ANDRÉ
Presidente da Banca Examinadora

Assinatura Eletrônica
16/10/2025 17:04:24.0
NARA LINS MEIRA QUINTAO
Avaliador Externo (UNIVERSIDADE DO VALE DO ITAJAÍ)

Assinatura Eletrônica
14/10/2025 15:43:55.0
GABRIELA TREVISAN DOS SANTOS
Avaliador Externo (UNIVERSIDADE FEDERAL DE SANTA MARIA)

Assinatura Eletrônica
11/10/2025 06:11:29.0
MARIA FERNANDA DE PAULA WERNER
Avaliador Interno (UNIVERSIDADE FEDERAL DO PARANÁ)

TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação FARMACOLOGIA da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **MAYARA ALVES AMORIM**, intitulada: **PARTICIPAÇÃO DOS RECEPTORES DE POTENCIAL TRANSITÓRIO E RECEPTORES DE BRADICININA NA LESÃO PULMONAR AGUDA INDUZIDA POR LIPOPOLISSACARÍDEO EM CAMUNDONGOS**, sob orientação da Profa. Dra. EUNICE ANDRÉ, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de doutora está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

CURITIBA, 10 de Outubro de 2025.

Assinatura Eletrônica
10/10/2025 16:05:56.0
EUNICE ANDRÉ
Presidente da Banca Examinadora

Assinatura Eletrônica
16/10/2025 17:04:24.0
NARA LINS MEIRA QUINTAO
Avaliador Externo (UNIVERSIDADE DO VALE DO ITAJAÍ)

Assinatura Eletrônica
14/10/2025 15:43:55.0
GABRIELA TREVISAN DOS SANTOS
Avaliador Externo (UNIVERSIDADE FEDERAL DE SANTA MARIA)

Assinatura Eletrônica
11/10/2025 06:11:29.0
MARIA FERNANDA DE PAULA WERNER
Avaliador Interno (UNIVERSIDADE FEDERAL DO PARANÁ)

NOTA EXPLICATIVA

Esta tese é apresentada em formato alternativo – artigos publicados e submetidos para publicação – de acordo com as normas do Programa de Pós-Graduação em Farmacologia da Universidade Federal do Paraná, constando de uma revisão de literatura, objetivos do trabalho e dois artigos científicos abordando os experimentos realizados, com resultados e discussão, além da conclusão final.

AGRADECIMENTOS

Ao meu bom Deus e à Santa Rita de Cássia, por me concederem força, sabedoria, fé e paciência em todos os momentos, guiando-me e permitindo que este sonho se tornasse realidade.

À minha orientadora e verdadeira mãe científica, Dra. Eunice André, agradeço profundamente por todo apoio durante essa jornada. Obrigada por depositar total confiança em meu trabalho e contribuir para o meu crescimento pessoal e profissional.

Aos meus pais, Ivanir e Gilberto, e aos demais familiares que deram todo o suporte, desde o início dos meus estudos, acreditando sempre em meu potencial.

Ao meu namorado Eugênio por compartilhar os momentos alegres e tristes, com infinita confiança e amor. E aos meus grandes amigos que me deram muita força, Janiana, Vitor, Juliana, Arianne e Débora, meu sincero agradecimento pela amizade, acolhimento e por partilharem comigo tantos momentos marcantes durante essa trajetória.

À banca examinadora, por aceitarem avaliar este trabalho, à Universidade Federal do Paraná, ao Departamento de Farmacologia e a todos os professores e funcionários do programa.

Ao biotério e aos animais que cederam suas vidas em prol da ciência. Ao Centro de Tecnologia Avançadas em Fluorescência que disponibilizou os microscópios para coleta das imagens que foram essenciais para a minha pesquisa científica.

À CAPES, pelo apoio do suporte técnico e financeiro para a realização deste trabalho.

E a todos que, de alguma forma, contribuíram para a realização deste trabalho e para minha formação.

A todos minha profunda gratidão!

“A ciência será sempre uma busca, jamais uma descoberta.
É uma viagem, nunca uma chegada.”

- Karl Popper

RESUMO

A síndrome do desconforto respiratório agudo (SDRA) é uma condição grave caracterizada por inflamação pulmonar intensa, aumento da permeabilidade vascular, edema alveolar e comprometimento das trocas gasosas, resultando em hipoxemia e insuficiência respiratória. Em modelos experimentais, a lesão pulmonar aguda (LPA) reproduz os principais eventos fisiopatológicos da SDRA, permitindo investigar seus mecanismos celulares e moleculares. Nesse contexto, os receptores de potencial transitório (TRP) constituem uma família de canais iônicos amplamente expressos em células neuronais e não neuronais do pulmão, incluindo epiteliais, musculares lisas e imunes, sendo reconhecidos como moduladores de reflexos protetores das vias aéreas e como mediadores da inflamação. Paralelamente, receptores de bradicinina B_1 e B_2 desempenham papel central na resposta inflamatória pulmonar, podendo interagir funcionalmente com canais TRP. Nesta tese, investigamos a participação dos canais TRP do tipo vaniloide 1 (TRPV1), anquirina 1 (TRPA1) e vaniloide 4 (TRPV4), bem como dos receptores de bradicinina B_1 e B_2, na lesão pulmonar aguda (LPA) induzida por lipopolissacarídeo (LPS) em camundongos. Nesse sentido, camundongos C57BL/6 foram submetidos à instilação intratraqueal de LPS em diferentes doses e tempos para caracterização do modelo de LPA. Para investigar o papel dos receptores, animais foram tratados com antagonistas seletivos de TRPV1, TRPA1, TRPV4 e dos receptores B_1 e B_2 de bradicinina. A resposta inflamatória foi avaliada por análise do lavado broncoalveolar (LBA), incluindo a quantificação de células inflamatórias, proteínas totais e citocinas, além da avaliação histopatológica do parênquima pulmonar. Adicionalmente, foram realizadas análises de deposição de colágeno e de expressão dos canais TRP por imunohistoquímica, bem como a investigação dos efeitos do bloqueio farmacológico sobre essas alterações. Nossos resultados demonstraram que o LPS promoveu inflamação pulmonar caracterizada por aumento de citocinas, infiltrado celular no LBA, extravasamento plasmático, deposição de colágeno, edema pulmonar e alterações histológicas compatíveis com LPA. Verificamos que os canais TRPV1, TRPA1 e TRPV4 contribuem para a amplificação dessa resposta inflamatória, uma vez que sua inibição farmacológica reduziu a expressão de marcadores inflamatórios, o acúmulo celular no LBA e a intensidade do dano tecidual. Além disso, a ativação dos receptores B_1 e B_2 aumentou a atividade dos TRP, enquanto sua modulação farmacológica atenuou a lesão pulmonar induzida por LPS. Coletivamente, nossos achados indicam que a sinalização coordenada entre os canais TRPV1/TRPA1/TRPV4 e receptores de bradicinina B_1/B_2 constitui um eixo patofisiológico relevante na LPA induzida por LPS. A elucidação dessa via integrativa não apenas aprofunda a compreensão dos mecanismos moleculares subjacentes à inflamação pulmonar aguda, como também destaca potenciais alvos terapêuticos para o desenvolvimento de estratégias farmacológicas mais eficazes no controle da progressão e gravidade da doença.

Palavras-chave: inflamação; lesão pulmonar; TRPV1; TRPA1; TRPV4; bradicinina.

ABSTRACT

Acute respiratory distress syndrome (ARDS) is a severe clinical condition characterized by intense pulmonary inflammation, increased vascular permeability, alveolar edema, and impaired gas exchange, resulting in persistent hypoxemia and respiratory failure. In experimental models, acute lung injury (ALI) reproduces the main pathophysiological features of ARDS, allowing the investigation of underlying cellular and molecular mechanisms. In this context, transient receptor potential (TRP) channels constitute a family of ion channels widely expressed in neuronal and non-neuronal cells of the lung, including epithelial, smooth muscle, and immune cells, being recognized as modulators of airway protective reflexes and mediators of inflammation. In parallel, bradykinin B_1 and B_2 receptors play a central role in the pulmonary inflammatory response and may functionally interact with TRP channels. In this thesis, we investigated the participation of transient receptor potential channels of the vanilloid 1 (TRPV1), ankyrin 1 (TRPA1), and vanilloid 4 (TRPV4) subtypes, as well as bradykinin B_1 and B_2 receptors, in lipopolysaccharide (LPS)-induced acute lung injury (ALI) in mice. In this sense, C57BL/6 mice were subjected to intratracheal instillation of LPS at different doses and time points to characterize the ALI model. To investigate the role of the receptors, animals were treated with selective antagonists of TRPV1, TRPA1, TRPV4, and bradykinin B_1 and B_2 receptors. The inflammatory response was evaluated by analysis of bronchoalveolar lavage fluid (BALF), including quantification of inflammatory cells, total proteins, and cytokines, in addition to histopathological assessment of lung parenchyma. Furthermore, analyses of collagen deposition and TRP channel expression by immunohistochemistry were performed, as well as investigation of the effects of pharmacological blockade on these alterations. Our results demonstrated that LPS promoted pulmonary inflammation characterized by increased cytokines, cellular infiltrate in BALF, plasma extravasation, collagen deposition, pulmonary edema, and histological changes compatible with ALI. We verified that TRPV1, TRPA1, and TRPV4 channels contribute to the amplification of this inflammatory response, since their pharmacological inhibition reduced the expression of inflammatory markers, cellular accumulation in BALF, and the intensity of tissue damage. Moreover, activation of B_1 and B_2 receptors enhanced TRP channel activity, whereas their pharmacological modulation attenuated LPS-induced lung injury. Collectively, our findings indicate that coordinated signaling between TRPV1/TRPA1/ TRPV4 channels and bradykinin B_1/B_2 receptors constitutes a pathophysiological axis relevant to LPS-induced ALI. Elucidation of this integrative pathway not only deepens the understanding of the molecular mechanisms underlying acute pulmonary inflammation but also highlights potential therapeutic targets for the development of more effective pharmacological strategies to control disease progression and severity.

Keywords: inflammation; lung injury; TRPV1; TRPA1; TRPV4; bradykinin.

LISTA DE FIGURAS

LISTA DE FIGURAS DOS ARTIGOS

MANUSCRITO ORIGINAL 1

MANUSCRITO ORIGINAL 2

LISTA DE TABELAS

LISTA DE ABREVIATURAS OU SIGLAS

4-HNE – 4-hidroxinonenal
12-LOX – 12-lipoxigenase
AECC – American European Consensus Conference
B_1 – Receptor de bradicinina subtipo 1
B_2 – Receptor de bradicinina subtipo 2
BK – Bradicinina
CGRP – Peptídeo relacionado ao gene de calcitocina
COVID-19 – Coronavírus 19
COX – Cicloxigenase
DPOC – Doença pulmonar obstrutiva crônica
ERO – Espécies reativas de oxigênio
et al. – E outros
FIG – Figura
FiO2 – Fração inspirada de oxigênio
IL – Interleucina
IL-4 – Interleucina 4
IL-6 – Interleucina 6
IL-10 – Interleucina 10
IL-1β – Interleucina 1 beta
LBA – Lavado broncoalveolar
LPS – Lipopolissacarídeo
LPA – Lesão pulmonar aguda
MAPK – Proteína quinase ativada por mitógeno
MERS – Síndrome respiratória do Oriente Médio
NO – Óxido nítrico
NF-$_K$B – Fator nuclear kappa B
PaO_2 – Pressão parcial de oxigênio
PGE2 – Prostaglandina E2
PEEP – Pressão expiratória final positiva
PKA – Proteína quinase A

PKC – Proteína quinase C
PLC – Fosfolipase C
SARS – Síndrome respiratória aguda grave
SDRA – Síndrome do desconforto respiratório agudo
TLRs – Receptor toll-like
TNF – Fator de Necrose Tumoral
TRP – Receptores de potencial transitório
TRPA – Receptores de potencial transitório anquirina
TRPA1 – Receptores de potencial transitório do tipo 1
TRPC – Receptores de potencial transitório canônica
TRPM – Receptores de potencial transitório melastatina
TRPML – Receptores de potencial transitório mucolipina
TRPP – Receptores de potencial transitório policistina
TRPV – Receptores de potencial transitório vanilóide
TRPV1 – Receptores de potencial transitório vanilóide 1
TRPV4 – Receptores de potencial transitório vanilóide 4

LISTA DE ABREVIATURAS OU SIGLAS DOS ARTIGOS

ALI	– Acute lung injury
ANOVA	– Analysis of variance
ARDS	– Acute respiratory distress syndrome
ARRIVE	– Animal research: reporting of in vivo experiments
B_1	– Bradykinin B_1 receptor
B_2	– Bradykinin B_2 receptor
BALF	– Bronchoalveolar lavage fluid
BSA	– Bovine Serum Albumin
COPD	– Chronic obstructive pulmonary disease
DAB	– 3, 3'-diaminobenzidine
DALBK	– Des-Arg 10 -Lys-bradykinin
DMSO	– Dimethyl sulfoxide
ELISA	– Enzyme-Linked Immunosorbent Assay
H&E	– Hematoxylin and Eosin
ILs	– Interleukins
IL-1β	– Interleukin-1 beta
IL-6	– Interleukin-6
IL-10	– Interleukin-10
IL-17	– Interleukin-17
IL-23	– Interleukin-23
INF-γ	– Interferon gamma
i.p.	– Intraperitoneal
I.t.	– Intratracheal
LPS	– Lipopolysaccharide
PKC	– Protein kinase C
PBS	– Phosphate buffer saline
SEM	– Standard error of the mean
SP	– Substance P
TNF-α	– Tumor necrosis factor alpha
TGF-β	– Transforming Growth Factor Beta
TRP	– Transient receptor potential

TRPA1 – Transient receptor potential ankyrin type 1

TRPV1 – Transient receptor potential vanilloid type 1

TRPV4 – Transient receptor potential vanilloid type 4

VEH – Vehicle

VEH_1 – Vehicle 1

W/D ratio – Wet-to-Dry weight ratio

LISTA DE SÍMBOLOS

°C	– Grau Celsius
α	– Alfa
β	– Beta
±	– Mais ou menos
>	– Maior
<	– Menor
≤	– Menor ou igual
%	– Por cento
Ca^{2+}	– Cálcio
Mg^{2+}	– Magnésio
µg	– Micrograma
µg/kg	– Micrograma por quilograma
µg/mL	– Micrograma por mililitro
µL	– Microlitro
µmol/kg	– Micromol por quilograma
kg	– Quilograma
g	– Grandeza de aceleração da centrífuga
mg	– Miligrama
mg/g	– Miligrama por grama
mg/kg	– Miligrama por quilograma
mg/mL	– Miligrama por mililitro
mL	– Mililitro
NaCl	– Cloreto de sódio
nmol	– Nanomol
nmol/kg	– Nanomol por quilograma
pH	– Potencial hidrogeniônico
vs.	– Versus
γ	– gama

SUMÁRIO

1. INTRODUÇÃO

1.1 SÍNDROME DO DESCONFORTO RESPIRATÓRIO AGUDO (SDRA)

O primeiro relato sobre a síndrome do desconforto respiratório agudo (SDRA) ou síndrome da angústia foi publicado em 1967 por Ashbaugh et al., que a descreveram como o estágio final da lesão pulmonar aguda (LPA), sendo considerada a forma clínica mais grave dessa condição (Matthay et al., 2024).

A SDRA é definida como um distúrbio agudo que se desenvolve dentro de sete dias após um evento clínico inicial, caracterizando-se por infiltrados pulmonares bilaterais, hipoxemia grave de difícil correção e alteração acentuada da mecânica pulmonar, na ausência de evidências de edema pulmonar de origem cardiogênica (Estenssoro e Dubin, 2016; Huppert et al., 2019). Durante sua evolução, observa-se um processo inflamatório intenso, acompanhado por lesão e edema pulmonar, que comprometem significativamente as trocas gasosas, culminando em hipóxia profunda (para revisão, ver Diamond et al., 2024; Matthay et al., 2019).

Em 1971, foram propostos os primeiros critérios diagnósticos para a SDRA, os quais incluíam: início agudo da insuficiência respiratória, complacência pulmonar reduzida, presença de infiltrados alveolares difusas em radiografia de tórax e cianose refratária à oxigenoterapia (Cepkova e Matthay, 2006).

Dada a complexidade do diagnóstico e com o objetivo de padronizar e facilitar a identificação da síndrome, foi publicada em 2012 a definição de Berlim. Essa classificação inclui parâmetros como: tempo de instalação (até sete dias após o insulto clínico), achados radiográficos (infiltrados bilaterais não explicados por derrame pleural, atelectasia ou nódulos), origem do edema (excluindo causas cardíacas ou hipervolemia) e graus de alteração na oxigenação (Force et al., 2012; Matthay et al., 2024). Além da radiografia de tórax, exames de tomografia computadorizada também são amplamente utilizados para avaliar a extensão e a distribuição das lesões pulmonares, fornecendo informações mais detalhadas sobre o acometimento estrutural do parênquima pulmonar (Matthay et al., 2024).

A relação entre a pressão parcial de oxigênio no sangue arterial (PaO_2) e a fração de oxigênio no ar inspirado (FiO_2) é um parâmetro essencial para o diagnóstico da SDRA, visto que pacientes acometidos apresentam uma razão PaO_2/FiO_2 inferior a 300 mmHg. Com base nesse valor, a síndrome é classificada em três níveis de gravidade: leve (PaO_2/FiO_2 entre 200 e 300 mmHg), moderada (entre 100 e 200 mmHg) e grave (inferior a 100 mmHg) (Diamond et al., 2024; Matthay et al., 2024).

Para o diagnóstico da SDRA, além da avaliação clínica, física e radiológica, também são realizados exames laboratoriais e, quando possível, análises histopatológicas. Entre os exames laboratoriais comumente solicitados estão: hemograma completo, testes de coagulação, tempo de trombina, dosagem de enzimas cardíacas, além da avaliação dos níveis séricos de magnésio, cálcio ionizado, fósforo e lactato, entre outros (Diamond et al., 2024; Matthay et al., 2024).

Esses exames auxiliam na detecção de disfunções orgânicas secundárias à gravidade da síndrome, uma vez que a SDRA pode evoluir para falência de múltiplos órgãos, incluindo insuficiência renal, hepática e hematopoiética. No exame histológico, observam-se achados característicos da LPA, como edema alveolar e intersticial, hemorragia alveolar, presença de membranas hialinas, congestão capilar pulmonar e lesão de pneumócitos (Diamond et al., 2024; Matthay et al., 2024).

Quanto à etiologia, a SDRA pode resultar de lesões pulmonares diretas ou indiretas. A forma direta, também denominada SDRA primária, é causada por insultos que afetam diretamente os pulmões, como pneumonia, aspiração de conteúdo gástrico, quase afogamento e contusão pulmonar. Já a forma indireta, conhecida como SDRA secundária, decorre de processos sistêmicos que comprometem secundariamente o tecido pulmonar, como sepse, politraumatismo, transfusões maciças de hemoderivados, entre outros (Kaku et al., 2020; Matthay et al., 2019).

As causas mais comuns incluem sepse, pneumonia, trauma torácico, aspiração, pancreatite, consumo crônico de álcool, tabagismo e cirurgias cardiovasculares. Em casos de pneumonia ou sepse, a etiologia geralmente é evidente; no entanto, em outras situações, é necessário realizar uma investigação clínica mais aprofundada, incluindo anamnese detalhada com o paciente ou familiares, a fim de identificar exposições recentes e elucidar o agente causal (Confalonieri et al., 2017; Kaku et al., 2020).

Infecções bacterianas e virais associadas ao desenvolvimento de pneumonia estão, de fato, entre os principais fatores desencadeantes da SDRA, com picos

observados durante pandemias, como as causadas por vírus da influenza e por coronavírus emergentes, além dos responsáveis pela síndrome respiratória do oriente médio (MERS) e síndrome respiratória aguda grave (SARS). Recentemente, a SDRA foi amplamente observada em pacientes com coronavírus 19 (COVID-19), estando associada a uma intensa resposta inflamatória sistêmica caracterizada por tempestades de citocinas e cininas, o que contribui para a piora do quadro clínico (Meyer et al., 2021).

Outras condições que aumentam a suscetibilidade ao desenvolvimento da SDRA incluem obesidade, tabagismo, hipoalbuminemia, quimioterapia, exposição a poluentes atmosféricos e o uso de ventilação mecânica com volumes correntes elevados e pressões inspiratórias excessivas (Reilly et al., 2019).

As estimativas da incidência de SDRA corresponde a 82 casos por 100.000 habitantes, com uma taxa de mortalidade em torno de 43% (Bellani et al., 2016; Diamond et al., 2024). Vale destacar que, na maioria dos casos, o óbito não ocorre por insuficiência respiratória isolada, mas sim como consequência da sepse e da falência de múltiplos órgãos (Matthay et al., 2019; Meyer et al., 2021).

Além disso, estima-se ainda que cerca de 75% dos casos sejam classificados como moderados ou graves (Diamond et al., 2024). A SDRA tem alta incidência entre pacientes de unidade de terapia intensiva, além disso essa condição depende da idade, aumentando de 16/100.000 pessoas-ano para indivíduos de 15 a 19 anos para 306/100.000 pessoas-ano para indivíduos de 75 a 84 anos (Hendrickson et al., 2021; Papazian et al., 2021).

A incidência de SDRA também depende do gênero, os homens têm maior probabilidade de desenvolver SDRA (62%) do que as mulheres (38%) (McNicholas et al., 2019). No entanto, quando a síndrome acomete mulheres, a taxa de mortalidade é proporcionalmente mais elevada em comparação aos homens (McNicholas et al., 2019; Meyer et al., 2021).

Diversos pacientes que sobreviveram à SDRA relatam sequelas prolongadas, como perda de massa muscular e distúrbios neuropsiquiátricos, incluindo disfunção cognitiva, ansiedade, depressão e transtorno do estresse pós-traumático. Apesar dessas manifestações extrapulmonares, a função respiratória tende a se recuperar de forma quase completa na maioria dos casos (Sweeney e McAuley, 2016).

A SDRA pode acometer tanto crianças quanto adultos, e, independentemente da causa, ainda não há uma terapia farmacológica específica para a condição, o que

contribui para sua alta morbidade e mortalidade. As estratégias terapêuticas atualmente disponíveis incluem principalmente o suporte ventilatório, sendo a ventilação mecânica a principal intervenção utilizada. Tanto a ventilação invasiva quanto a não invasiva visam assegurar uma adequada troca gasosa, ao mesmo tempo em que se busca minimizar o risco de lesão pulmonar induzida pela ventilação e de comprometimento hemodinâmico decorrente do aumento das pressões intratorácicas (Boyle et al., 2013; Qadir et al., 2021).

A ventilação com baixos volumes correntes, associada ao uso de pressão expiratória final positiva (PEEP) em níveis moderados, é amplamente utilizada na prática clínica como uma estratégia de proteção pulmonar. Além disso, abordagens complementares como o manejo hemodinâmico e o fornecimento adequado de oxigênio também são essenciais no suporte ao paciente com SDRA (Cepkova e Matthay, 2006).

Diversas terapias farmacológicas têm sido investigadas, incluindo o uso de vasodilatadores inaláveis, como o óxido nítrico (NO) (Gebistorf et al., 2016) e as prostaciclinas (Haeberle et al., 2023), bem como vasodilatadores sistêmicos, como a prostaglandina E1 (Fuller et al., 2015).

Agentes anti-inflamatórios também foram explorados, como os glicocorticoides (Zhang et al., 2023), pentoxifilina (Nazemi et al., 2024). Entre as abordagens antioxidantes, destacam-se a N-acetilcisteína (Zhang et al., 2017). Outros tratamentos incluem agentes anticoagulantes (Cepkova e Matthay, 2006), agonistas β2-adrenérgicos (Matthay et al., 2011), surfactantes exógenos (Dushianthan et al., 2023) e agentes imunomoduladores como a interleucina-10 (IL-10) (Shih et al., 2023).

No entanto, essas terapias apresentam eficácia limitada por não reduzirem de forma consistente a mortalidade, mostrarem resultados variáveis entre estudos e fases da doença, e carecerem de evidências clínicas robustas que justifiquem seu uso na SDRA (Cepkova e Matthay, 2006; Peck e Hibbert, 2019).

Outras abordagens terapêuticas incluem o manejo mais eficiente de fluidos, o uso de antibióticos de amplo espectro e suporte nutricional adequado. Ainda assim, os índices de mortalidade associados à SDRA permanecem elevados, e nenhuma terapia farmacológica atual demonstrou benefícios consistentes na sobrevida dos pacientes (Cepkova e Matthay, 2006; Qadir et al., 2021).

Dessa forma, torna-se evidente a necessidade de um entendimento mais aprofundado dos mecanismos fisiopatológicos envolvidos na SDRA. Tal

conhecimento é fundamental para o desenvolvimento de novas abordagens terapêuticas eficazes que possam, de fato, impactar positivamente o prognóstico e a taxa de sobrevida dos pacientes acometidos por essa síndrome.

1.2 LESÃO PULMONAR AGUDA (LPA) E SUA RELAÇÃO COM SDRA

A SDRA e a LPA representam diferentes graus de severidade dentro de um mesmo espectro clínico, caracterizado por edema pulmonar rico em proteínas decorrente do aumento da permeabilidade da membrana alveolar. Esse processo culmina no aparecimento de opacidades bilaterais difusas, hipoxemia grave e, consequentemente, insuficiência respiratória aguda (Matuschak et al., 2010).

A principal distinção entre LPA e SDRA está relacionada à gravidade da hipoxemia, que é avaliada pela razão entre PaO_2 e FiO_2. A definição proposta pelo consenso da *American-European Consensus Conference* (AECC) considera LPA quando a razão PaO_2/FiO_2 é ≤300 mmHg, enquanto valores ≤200 mmHg caracterizam SDRA (Raghavendran et al., 2011).Com a padronização mais recente, estabelecida pelos Critérios de Berlim, o termo LPA foi descontinuado, e a SDRA passou a ser classificada nas categorias leve, moderada e grave, desde que outros critérios também estejam presentes (imagens radiológicas compatíveis e ausência de edema de origem cardíaca) (Force et al., 2012).

Portanto, é importante ressaltar que todo paciente com SDRA, necessariamente, apresenta LPA; entretanto, nem todo paciente com LPA progride para SDRA (Amato et al., 2007). Os critérios diagnósticos estão resumidos na Tabela 2, permitindo melhor compreensão e diferenciação entre os estágios dessa síndrome.

	TEMPO DE INSTALAÇÃO	RAIO X DE TÓRAX	POAP	OXIGENAÇÃO
LPA	Início agudo	Infiltrados bilaterais no raio X frontal	PAPO ≤18 mmHg quando medida ou ausência de hipertensão atrial esquerda	PaO2 ≤300 mmHg independente do nível do PEEP
SDRA				PaO2 ≤200 mmHg independente do nível do PEEP

Tabela 1. Critérios diagnósticos para LPA e SDRA. Fonte: Bernards et al., 1994 - adaptada pelo autor.

1.3 FISIOPATOLOGIA DA SDRA: PAPEL DOS MEDIADORES INFLAMATÓRIOS

Os pulmões são altamente sensíveis a processos inflamatórios, e a resposta inflamatória desempenha um papel central na fisiopatologia da SDRA. Diante de uma agressão, seja de origem pulmonar ou extrapulmonar, ocorre inicialmente a ativação dos macrófagos alveolares, o que resulta no recrutamento de neutrófilos e de macrófagos circulantes para o tecido pulmonar, mediado pela liberação de citocinas inflamatórias. Essas citocinas promovem lesão tanto do epitélio alveolar quanto do endotélio capilar, intensificando o processo inflamatório (Huang et al., 2018; Short et al., 2014).

Como consequência, há extravasamento de proteínas e fluido do compartimento intravascular para o espaço intersticial e, posteriormente, para os alvéolos. Esse acúmulo de líquido pode ultrapassar a capacidade de drenagem linfática pulmonar, culminando em edema intersticial e alveolar (Raghavendran et al., 2011; Ware e Matthay, 2005). A destruição da barreira alvéolo-capilar favorece a formação de membranas hialinas nas superfícies alveolares. O edema pulmonar resultante provoca infiltrado pulmonar difuso, redução da complacência respiratória, aumento do shunt intrapulmonar e dispneia (Raghavendran et al., 2011; Ware e Matthay, 2005).

As proteínas extravasadas, associadas ao infiltrado inflamatório e à lesão dos pneumócitos do tipo II, comprometem a integridade do surfactante pulmonar. A deficiência do surfactante favorece o colabamento alveolar (atelectasia), prejudica a mecânica respiratória e compromete as trocas gasosas, levando à insuficiência respiratória grave (Swenson e Swenson, 2021). Embora a SDRA seja primariamente uma condição respiratória, a inflamação sistêmica, o edema, a disfunção do surfactante e a hipoxemia profunda podem contribuir para lesões em múltiplos órgãos, culminando em falência múltipla de órgãos e, frequentemente, na morte do paciente (McGonagle et al., 2020; Ragab et al., 2020)

Do ponto de vista patológico, a SDRA evolui em fases. A primeira delas é a fase exsudativa ou inflamatória aguda, caracterizada pela intensa infiltração de neutrófilos e macrófagos nos alvéolos e pelo início do edema pulmonar, devido ao extravasamento de fluido rico em proteínas. Essa fase geralmente dura cerca de sete dias. Pacientes que apresentam melhora clínica durante esse período têm maior

probabilidade de resolução completa da doença, pois a resposta inflamatória ainda é potencialmente reversível nessa etapa (Matthay et al., 2012).

A segunda fase da SDRA é denominada fase fibroproliferativa. Ela se caracteriza pela organização do exsudato inflamatório e pelo início da fibroelastogênese, com intensa produção de matriz extracelular no interior dos alvéolos, que passam a ser preenchidos por tecido conjuntivo. Nessa etapa, observa-se uma diminuição do edema pulmonar, ao mesmo tempo em que as paredes alveolares sofrem proliferação de pneumócitos do tipo II, resultando em um processo hiperplásico. Além disso, há acúmulo de proteínas como fibronectina e colágeno no parênquima pulmonar (Matthay et al., 2012; Matthay et al., 2019).

A última fase da SDRA é a fase fibrótica, marcada pela deposição anormal e excessiva de proteínas da matriz extracelular, com graus variáveis de fibrose intersticial e presença significativa de colágeno. Essa condição contribui para a hipoxemia progressiva, disfunção de múltiplos órgãos e está associada a uma taxa de mortalidade que pode atingir até 80% dos pacientes (Matthay et al., 2019).

Infecções por patógenos também podem desencadear lesão pulmonar e contribuir para o desenvolvimento da SDRA. Moléculas endógenas e produtos microbianos interagem com receptores do tipo toll-like (TLRs) presentes nos pulmões, ativando o sistema imunológico inato. Embora esse mecanismo tenha como objetivo eliminar o patógeno, ele também pode agravar a lesão tecidual ao promover uma resposta inflamatória exacerbada (Hupper et al., 2019; Imai et al., 2008).

Um dos eventos marcantes nesse processo é a tempestade de citocinas, caracterizada por um aumento acentuado na liberação desses mediadores inflamatórios (Zhou e Lu, 2024). Citocinas como interleucina 1 beta (IL-1β), interleucina 6 (IL-6) e fator de necrose tumoral (TNF), produzidas principalmente por macrófagos e linfócitos ativados, desempenham papel central na sinalização inflamatória, interagindo com receptores de superfície celular e amplificando a resposta imune (De Virgiliis e Giovanni, 2020; Yang et al., 2021). Estudos mostram que concentrações elevadas dessas citocinas estão associadas a desfechos fatais em pacientes com SDRA (Wang et al., 2021).

Por outro lado, citocinas com perfil anti-inflamatório, como interleucinas 4 e 10 (IL-4 e IL-10), também estão presentes na SDRA e podem modular negativamente a resposta inflamatória (Jarczak e Nierhaus, 2022).

Além das citocinas, outros mediadores inflamatórios, como bradicinina, histamina e serotonina, também desempenham papéis relevantes na inflamação pulmonar aguda e no desconforto respiratório (Roche e Roche, 2020; Raban et al., 2021).

Portanto, o descontrole da resposta inflamatória, caracterizado pelo recrutamento excessivo de neutrófilos, estresse oxidativo e liberação contínua de mediadores inflamatórios, gera um desequilíbrio na homeostase pulmonar. Essa cascata inflamatória culmina na ruptura das barreiras endotelial e epitelial, provocando edema alveolar, prejuízo da mecânica pulmonar e comprometimento das trocas gasosas, características fundamentais da SDRA (Hussain et al., 2020). A Figura 1 ilustra os principais mecanismos fisiopatológicos envolvidos na SDRA, incluindo a ativação de células inflamatórias, lesão do endotélio alveolar e disfunção da barreira alvéolo-capilar.

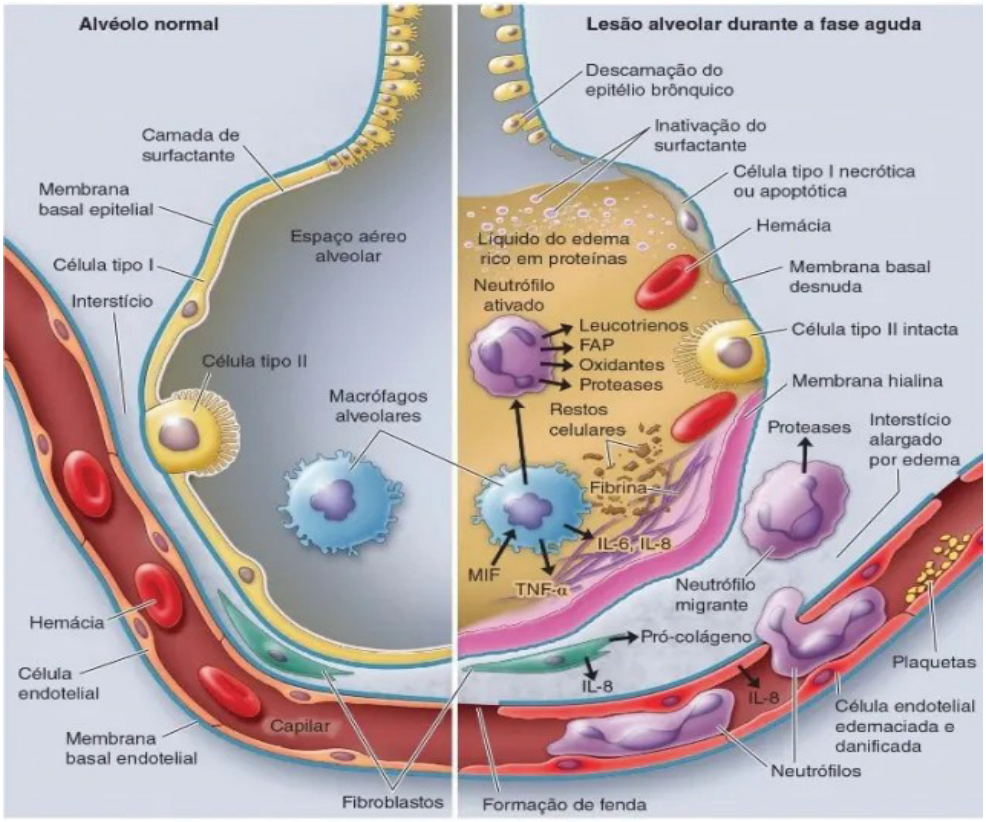


Figura 1. Fisiopatologia da SDRA. Destacando a ruptura da barreira alvéolo-capilar, o acúmulo de edema e a infiltração de células inflamatórias que comprometem a troca gasosa e favorecem a progressão da lesão pulmonar. Fonte: Hussain et al., 2020.

1.4 MODELO EXPERIMENTAL DE LPA INDUZIDA POR LPS

Diversos modelos experimentais têm sido utilizados para estudar a SDRA, sendo a maioria classificada como modelos de LPA, já que a SDRA é uma forma grave dessa condição. Contudo, nenhum modelo reproduz integralmente a complexidade da doença humana. O modelo ideal deve mimetizar características fisiopatológicas humanas, como a inflamação desregulada e a ruptura da barreira alvéolo-capilar (D'Alessio, 2018; Rocco e Nieman, 2016).

A escolha do modelo varia conforme fatores como custo, necessidade de amostragem de sangue, número de animais e disponibilidade de reagentes. Entre os diversos métodos de indução, destaca-se o uso de lipopolissacarídeo (LPS), uma endotoxina derivada de bactérias gram-negativas como *Escherichia coli*, que ativa a resposta imune por meio de TLR-4, desencadeando inflamação e apoptose endotelial (Ballard-Croft et al., 2012; Chen et al., 2010).

Os modelos experimentais em animais por LPS envolvem estudos com coelhos, ratos, camundongos, ovelhas, gatos, porcos e entre outros. O LPS pode ser administrado por diferentes vias (intraperitoneal, intravenosa, intratraqueal ou nasal), simulando condições como sepse ou pneumonia, principais causas de SDRA. As vias locais (nasal e intratraqueal) são preferidas por induzirem lesão pulmonar mais específica. Em geral, os primeiros sinais de dano aparecem nas primeiras 6 a 24 horas após a exposição, com acúmulo de neutrófilos, edema alveolar, espessamento da parede alveolar e necrose celular (Matute-Bello et al., 2011).

Achados laboratoriais incluem aumento de neutrófilos, proteínas totais, citocinas e atividade de mieloperoxidase no lavado broncoalveolar (LBA), além de inflamação, hemorragia, atelectasia e formação de membranas hialinas nos tecidos pulmonares (Ballard-Croft et al., 2012).

Dentre as considerações relatadas, este modelo fornece informações importantes sobre o mecanismo inflamatório causado pela administração de endotoxinas, sendo apropriado para estudar a patogênese da LPA/SDRA. Sendo, portanto, um modelo aplicável, com alta reprodutibilidade, com elevada relevância na clínica (Ballard-Croft et al., 2012) e o modelo a ser empregado nesse estudo.

1.5 RECEPTORES TRP NAS VIAS AÉREAS

No trato respiratório, estão presentes receptores capazes de sensibilizar as vias aéreas e desencadear respostas a diversos estímulos (Müller et al., 2022). Dentre esses receptores, destacam-se os canais de potencial transitório (TRP), uma grande família de proteínas de canais iônicos, dividida em seis subfamílias com base na homologia de sequência de aminoácidos: Canônico (TRPC), Vanilóide (TRPV), Melastatina (TRPM), Policistina (TRPP), Mucolipina (TRPML) e Anquirina (TRPA) (Achanta e Jordt, 2020; Gu e Lee, 2021).

A nomenclatura desses canais tem origem na descoberta feita por Cosens e Manning em estudos com *Drosophila*, nos quais se observou uma resposta transitória à exposição à luz intensa (Cosens e Manning, 1969; Grace et al., 2014). Estruturalmente, os canais TRP são formados por seis domínios transmembrana, com o poro do canal localizado entre os domínios cinco e seis. Além disso, apresentam terminais C e N intracelulares, com diferentes graus de repetições de anquirina (Grace et al., 2014; Müller et al., 2022).

A ativação desses canais nos nervos sensoriais do trato respiratório permite o influxo de cátions, levando à despolarização da membrana neuronal. Esse processo pode resultar tanto na ativação quanto na inativação de canais iônicos dependentes de voltagem, modulando assim a excitabilidade celular. Os canais TRP estão envolvidos em respostas fisiológicas e participam da regulação positiva durante processos inflamatórios nas vias aéreas, contribuindo para manifestações como hipersensibilidade, tosse e broncoespasmo, além de estarem relacionados a diversas doenças respiratórias (Belvisi e Birrell, 2017; Gu e Lee, 2021).

No contexto das vias aéreas, a expressão dos TRP se restringe principalmente aos receptores TRPV1 (vanilóide tipo 1), TRPA1 (anquirina tipo 1) e TRPV4 (vanilóide tipo 4). Esses receptores estão envolvidos em processos fisiológicos e patológicos associados a doenças inflamatórias respiratórias, como asma, fibrose cística, câncer, doença pulmonar obstrutiva crônica (DPOC) e SDRA (Koivisto et al., 2022; Müller et al., 2022; Wallace, 2017).

1.5.1 RECEPTOR TRPV1

O receptor TRPV1 é um canal de cátion polimodal que pode ser ativado por diversos estímulos (Luu et al., 2021). Ele responde diretamente a agentes como capsaicina, resiniferatoxina, baixo pH extracelular, anandamida, temperaturas elevadas (> 42 °C) e derivados do ácido araquidônico, entre outros. Além disso, pode ser ativado indiretamente por mediadores endógenos como bradicinina e prostaglandina E_2 (PGE_2) (Belvisi e Birrell, 2017) (Figura 2).

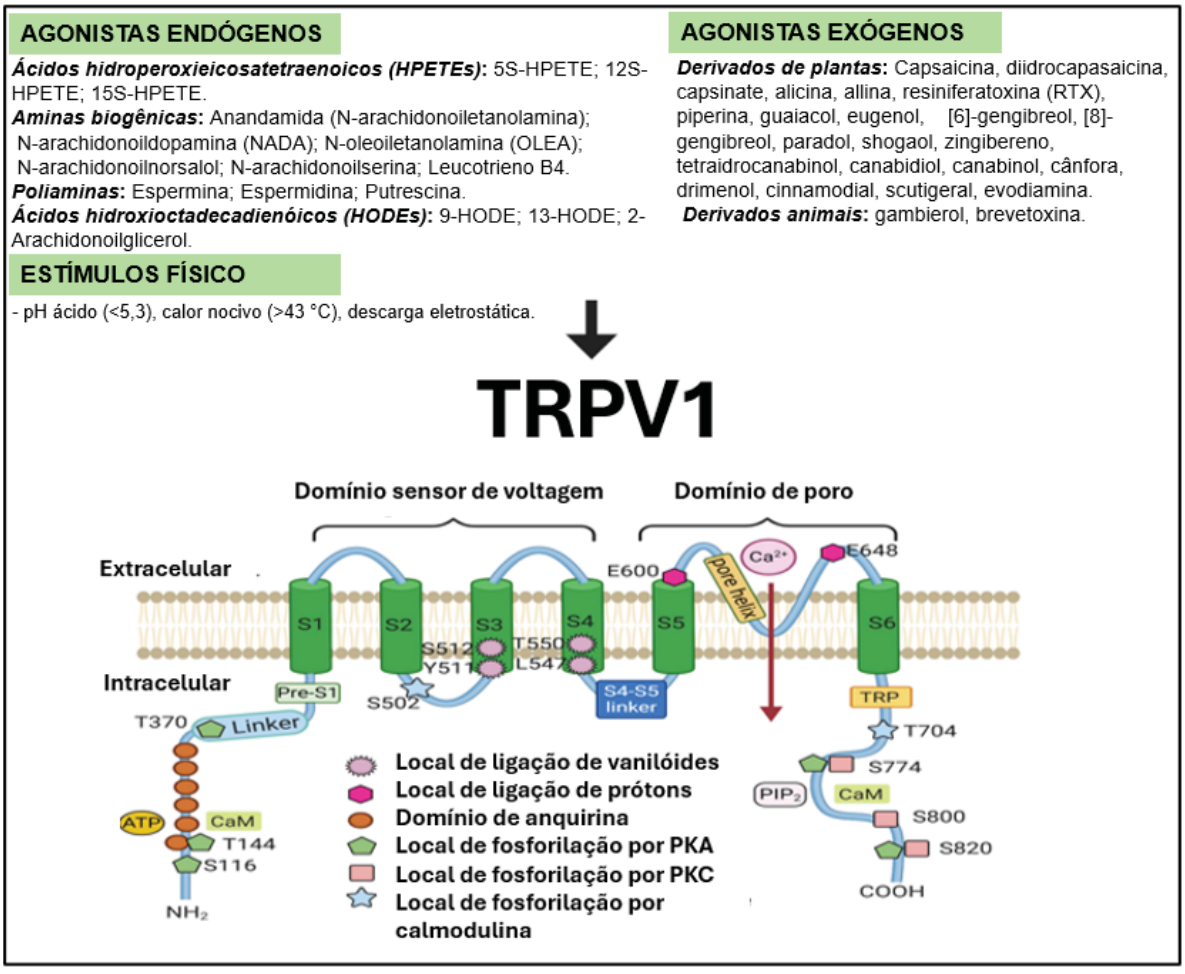


Figura 2. Representação esquemática dos ativadores e da estrutura do canal iônico TRPV1. Fonte: Achanta e Jordt, 2020, adaptado pelo autor.

No trato respiratório, o TRPV1 é expresso na musculatura lisa pulmonar, no epitélio da traqueia, brônquios e laringe, bem como em células endoteliais vasculares e células dendríticas do pulmão (Müller et al., 2022). Além disso, está presente nas

fibras aferentes sensoriais do tipo C que inervam todo o trato respiratório, sendo que algumas dessas fibras também expressam os canais TRPA1 e TRPV4.

A ativação do TRPV1 promove o aumento do cálcio intracelular e da excitabilidade celular, desencadeando potenciais de ação que levam à liberação de mediadores como taquicininas e o peptídeo relacionado ao gene da calcitonina (CGRP) (Lee e Gu, 2009; De Oliveira et al., 2016). Como consequência, pode ocorrer uma resposta sensorial exacerbada nas vias aéreas, caracterizada por irritação, dispneia, tosse, broncoconstrição, extravasamento de plasma e quimiotaxia de células inflamatórias, em decorrência da liberação de neuropeptídeos (Lee e Gu, 2009).

Estudos demonstram a interação entre neurônios positivos para TRPV1 e células do sistema imune, sendo essa comunicação fundamental na mediação da inflamação das vias aéreas após exposição a alérgenos inalados ou infecções virais (Nahama et al., 2020; Talbot et al., 2012). Além de estar envolvido na resposta à tosse, com expressão aumentada em pacientes com tosse crônica, o TRPV1 participa das alterações na reatividade das vias aéreas induzidas por agentes irritantes (Nahama et al., 2020).

Os canais TRPV1 são os receptores da família TRP mais amplamente estudados no contexto do sistema respiratório. Sua relevância decorre da abundante expressão no trato respiratório e do envolvimento em processos fisiológicos e patológicos das vias aéreas (Liu et al., 2022; Müller et al., 2022). Por esse motivo, esses canais têm sido extensivamente investigados em modelos animais, especialmente em estudos relacionados à tosse, asma e DPOC (Liu et al., 2022; Müller et al., 2022).

O papel do TRPV1 no desencadeamento da tosse crônica já está bem estabelecido. Diversos estudos demonstraram que moléculas capazes de sensibilizar esse canal, como a capsaicina, aumentam sua expressão e, consequentemente, promovem o surgimento da tosse. Em contrapartida, o bloqueio do TRPV1 por antagonistas tem se mostrado eficaz na atenuação desse reflexo (Zhang et al., 2018). Além disso, evidências indicam que fármacos como sinvastatina, captopril e metabólitos do paracetamol também podem modular a atividade do TRPV1, contribuindo para o aparecimento de alterações nas vias aéreas como broncoconstrição e tosse (Amorim et al., 2021; Eberhardt et al., 2017; De Oliveira et al., 2016).

Com relação à asma e à DPOC, o TRPV1 também tem demonstrado importante participação. Estudos com modelos animais revelaram que o bloqueio deste receptor pode reduzir a inflamação e mitigar o remodelamento associado à asma crônica (Choi et al., 2018). Também, observou-se uma expressão significativamente aumentada de TRPV1 nas vias aéreas de pacientes asmáticos e com DPOC, em comparação a indivíduos saudáveis (Baxter et al., 2014).

A ativação do TRPV1 aumenta a liberação de várias moléculas pró-inflamatória, incluindo a substância P (Jia e Lee, 2007) e citocinas como a IL-6 (Malagarie-Cazenave et al., 2011). Interessantemente, Cabral e Giusti-Paiva, 2016, demonstraram que o pré-tratamento com um antagonista do receptor TRPV1 preveniu tanto o aumento da lesão quanto da resistência pulmonar em modelo de lesão aguda pulmonar induzida por LPS em camundongos (Cabral e Giustini-Paiva, 2016). Esses achados reforçam o papel relevante desse canal na hipersensibilidade das vias aéreas e no desenvolvimento de diversas doenças respiratórias.

1.5.2 RECEPTOR TRPA1

Com relação ao receptor TRPA1, este é amplamente expresso em neurônios sensoriais e pode ser ativado por temperaturas frias (abaixo de 17 °C), espécies reativas de oxigênio (ERO) e 4-hidroxinonenal (4-HNE). Além disso, assim como ocorre com o receptor TRPV1, mediadores inflamatórios, como fatores de crescimento, bradicinina, proteases e as proteínas quinase A e C (PKA e PKC, respectivamente), podem sensibilizar o canal, reduzindo seu limiar de ativação (Belvisi e Birrell, 2017). Irritantes exógenos, como o alil isotiocianato (presente na mostarda), o cinamaldeído (presente na canela), bem como substâncias como a acroleína e o crotonaldeído, encontrados na fumaça do cigarro, também são capazes de ativar o TRPA1 (Moore et al., 2017) (Figura 3).

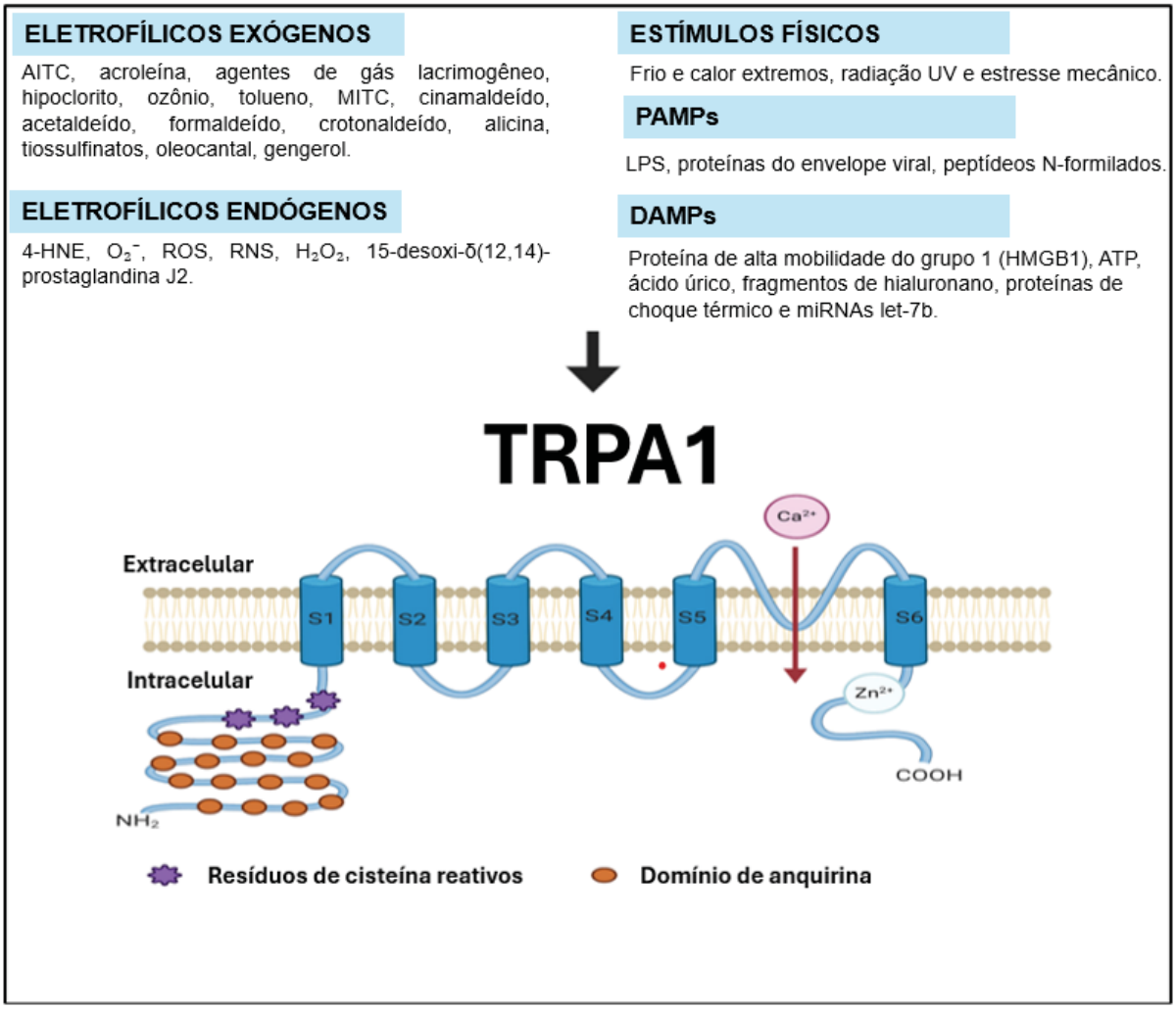


Figura 3. Representação esquemática dos ativadores e da estrutura do canal iônico TRPA1. Fonte: ACHANTA & JORDT, 2020, adaptado pelo autor.

O TRPA1 é expresso em diferentes tipos celulares do pulmão, incluindo fibroblastos pulmonares e neurônios sensoriais, como as fibras C, que inervam a maioria dos tecidos do trato respiratório, abrangendo traqueia, brônquios, bronquíolos terminais e respiratórios, ductos alveolares e alvéolos (Donavan e Hansbro, 2019; Moore et al., 2017).

Após sua ativação, o TRPA1 permite o influxo de cálcio para o interior da célula, promovendo a dessensibilização e a geração de potenciais de ação ao longo da fibra sensorial. Esse processo, assim como o observado para o TRPV1, desencadeia inflamação neurogênica por meio da liberação de neuropeptídeos pró-inflamatórios. Nas vias aéreas, o TRPA1 está associado à indução de tosse, broncoconstrição e hiper-reatividade brônquica, tanto em humanos quanto em modelos animais. Sua

ativação tem sido observada em pacientes com asma e DPOC, especialmente em decorrência da liberação de mediadores inflamatórios (Bonvini e Belvisi, 2017; Jentsch et al., 2020; Koivisto et al., 2022).

Estudos recentes demonstram que a inibição farmacológica dos receptores TRPA1 é capaz de reduzir a inflamação das vias aéreas e a hiper-reatividade brônquica, tanto in vitro quanto in vivo. Assim, o canal TRPA1 tem despertado grande interesse como alvo terapêutico para intervenções farmacológicas no tratamento de doenças respiratórias (Jha et al., 2015; Koivisto et al., 2022).

O receptor TRPA1 também pode estar envolvido em casos de lesão pulmonar. Balestrini et al., 2021, demonstrou que um o bloqueio do canal TRPA1 por seu antagonista conhecido como GDC-0334 inibiu respostas das vias aéreas como hiper-reatividade, tosse e até mesmo formação de edema pulmonar (Balestrini et al., 2021).

1.5.3 RECEPTOR TRPV4

O TRPV4 é um canal catiônico não seletivo com permeabilidade ao cálcio (Ca^{2+}) e ao magnésio (Mg^{2+}) e que, assim como os canais TRPV1 e TRPA1, participam de doenças inflamatórias do pulmão, como asma, DPOC e fibrose cística (Bonvini et al., 2016; Scheraga et al., 2017). O receptor TRPV4 pode ser ativado por diferentes estímulos físicos e químicos, incluindo temperaturas entre 27 e 35°C, força mecânica e substâncias endógenas como ácidos epoxieicosatrienóicos, anandamida e estímulos hipotônicos (Grace et al., 2017). As variações na tonicidade do fluido extracelular podem alteram a tensão na membrana celular, causando deformação mecânica da membrana e dessa forma, resultam na ativação do TRPV4 (White et al., 2016) (Figura 4).

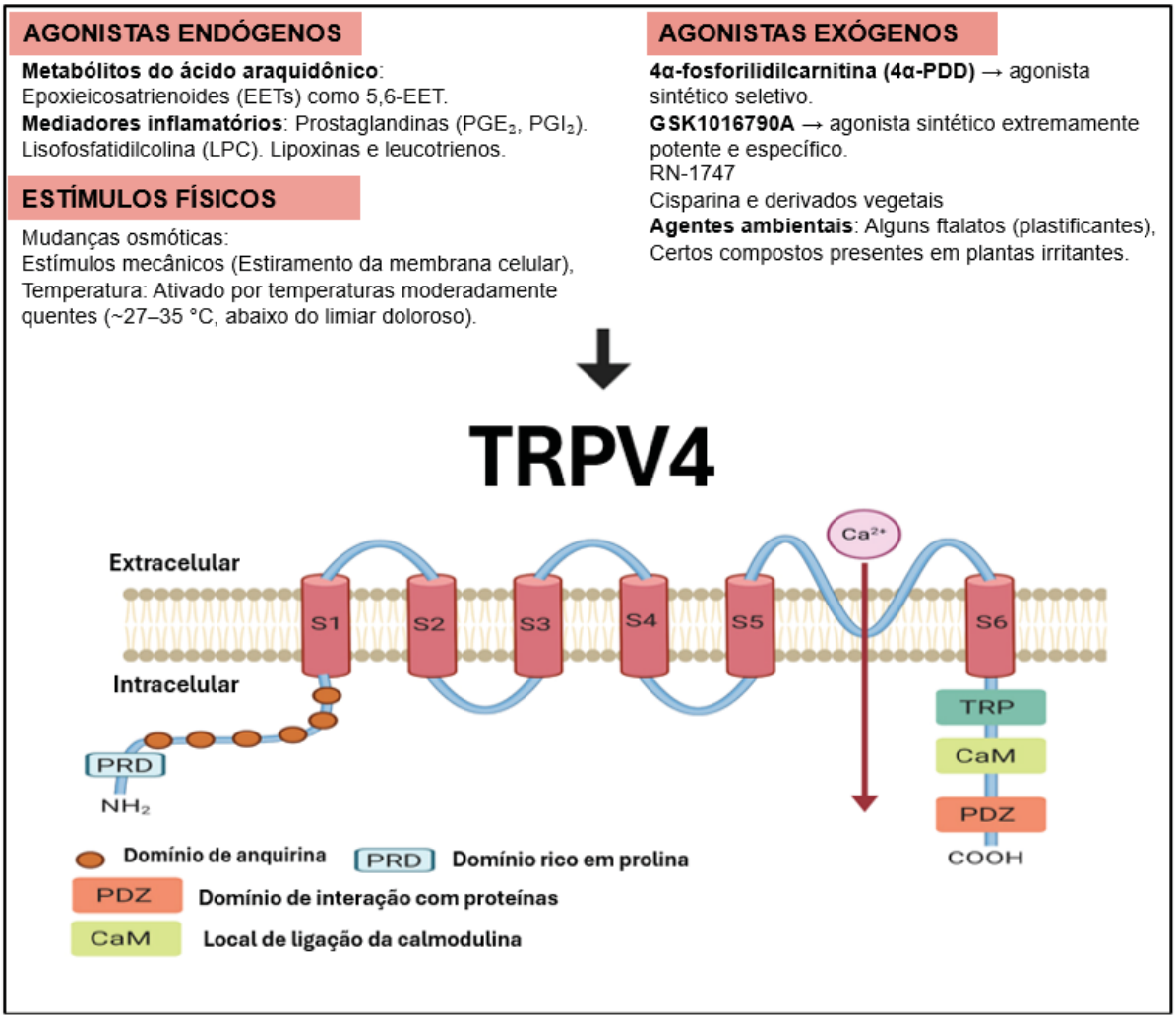


Figura 4. Representação esquemática dos ativadores e da estrutura do canal iônico TRPV4. Fonte: ACHANTA & JORDT, 2020, adaptado pelo autor.

O canal TRPV4 também é amplamente distribuído nas células epiteliais dos brônquios humanos, células estruturais como musculatura lisa das vias aéreas, fibroblastos e vasos pulmonares, células inflamatórias como macrófagos e neurônios sensoriais (Belvisi e Birrell, 2017). Além disso, esse canal é localizado com TRPV1 em neurônios sensoriais que expressam neuropeptídeos (Benemei et al., 2015).

Alguns estudos demonstraram que a ativação do TRPV4 causa tosse em modelo animal (Bonvini et al., 2016; Bonvini e Belvisi, 2017). Além disso, McAlexander e outros pesquisadores demonstraram que ativação do TRPV4 causa constrição de brônquios isolados das vias aéreas humanas (McAlexander et al., 2014). Outros achados demonstram a participação do TRPV4 na ruptura da barreira alveolar e sugerem sua participação na patogênese da LPA (Luo et al., 2025).

O canal TRPV4 está envolvido no desenvolvimento de edema pulmonar e associado à inflamação e lesão tecidual (Simonsen et al.,2017). Atuando na proteção da barreira alvéolo capilar, o bloqueio desse receptor apresenta uma importante contribuição nas doenças respiratórias. Averígua-se que a ativação desse canal participa na disrrupção da permeabilidade capilar pulmonar via liberação de proteases, citocinas e ERO favorecendo o aparecimento de edema pulmonar (Alvarez et al., 2006; Koivisto et al., 2022; Thorneloe et al., 2012).

Atualmente, o canal TRPV4 tem sido apontado como um importante modulador da permeabilidade da barreira alvéolo-capilar. Estudos demonstram que sua ativação contribui para o extravasamento plasmático, edema pulmonar e agravamento da lesão pulmonar (Weber et al., 2020). Em modelos experimentais, a inibição do TRPV4 mostrou-se eficaz em reduzir a lesão pulmonar induzida por ventiladores mecânicos e ácidos em roedores (Hamanaka et al., 2010; Balakrishna et al., 2014). Clinicamente, o bloqueio do TRPV4 tem sido investigado em pacientes com COVID-19 nos estágios iniciais da infecção, antes da progressão para SDRA, mostrando potencial para proteger a barreira alvéolo-capilar e prevenir complicações como edema pulmonar, fibrose ou outras sequelas tardias (Koivisto et al., 2022).

1.6 RECEPTORES DE BRADICININA NAS VIAS AÉREAS: B_1 E B_2 NA INFLAMAÇÃO PULMONAR

A bradicinina (BK) é um peptídeo vasoativo com importante papel na resposta inflamatória, promovendo vasodilatação, aumento da permeabilidade vascular e recrutamento de células inflamatórias. Também atua sobre a musculatura lisa, podendo causar broncoconstrição e contrações uterinas e intestinais, além de estimular a liberação de prostanoides, citocinas e outros mediadores inflamatórios (Ricciardolo et al., 2018).

Sua produção ocorre por meio do sistema calicreína-cininas, que envolve o fator XII, a pré-calicreína e o cininogênio de alto peso molecular. A ativação sequencial dessas proteínas leva à liberação da bradicinina, especialmente em situações de lesão ou ativação endotelial. Além disso, é rapidamente degradada por enzimas,

principalmente pela enzima conversora de angiotensina, que está abundantemente expressa no endotélio pulmonar (Yilmaz, 2019).

Devido às suas propriedades vasoativas e pró-inflamatórias, a bradicinina tem sido associada a diversas doenças inflamatórias e respiratórias, nas quais contribui para o aumento da permeabilidade vascular e edema pulmonar. Seus efeitos são mediados principalmente por dois receptores acoplados à proteína G: o receptor B_1 (B_1) e o receptor B_2 (B_2) (Campanholle et al., 2010; Wang, 2015).

O receptor B_1 de bradicinina é induzível, ou seja, sua expressão aumenta em situações de lesão tecidual, infecção ou inflamação crônica. A ativação do receptor B_1 ocorre principalmente por metabólitos da bradicinina (como a des-Arg9-BK), e está relacionada ao recrutamento de leucócitos, ao aumento da liberação de citocinas pró-inflamatórias e à manutenção da resposta inflamatória tardia (Nasseri et al., 2015; Székacs et al., 2021).

Estudos experimentais reforçam o papel desses receptores na lesão pulmonar. Nasseri et al. (2015) demonstraram que a administração sistêmica de BI113823, um antagonista não peptídico seletivo para o receptor B_1, conferiu proteção contra a LPA induzida por LPS em modelo murino (Nasseri et al., 2015). De forma semelhante, Campanholle et al. (2010) observaram que o bloqueio do receptor B_1 preveniu a LPA induzida por LPS inalatório (Campanholle et al., 2010).

Por outro lado, o receptor B_2 de bradicinina é constitutivamente expresso em tecidos saudáveis e é ativado pela bradicinina intacta. Sua ativação promove vasodilatação, aumento da permeabilidade vascular, contração do músculo liso brônquico e produção de muco, contribuindo para os sintomas inflamatórios nas vias aéreas, como tosse, broncoconstrição e edema (Nasseri et al., 2015; Székacs et al., 2021). Além disso, a estimulação do receptor B_2 pode induzir a liberação de mediadores pró-inflamatórios como prostaglandinas e NO, além de interagir com canais iônicos, amplificando o estímulo sensorial e a resposta inflamatória (Al-Shamlan e El-Hashim, 2019).

Em modelos murinos de inflamação pulmonar induzida por LPS, tanto o receptor B_1 quanto o B_2 demonstraram estar implicados na exacerbação do dano tecidual, edema e inflamação neutrofílica (Al-Shamlan e El-Hashim, 2019). Dessa forma, os receptores B_1 e B_2 desempenham papéis distintos, porém complementares, na modulação da inflamação pulmonar. O receptor B_2, constitutivamente expresso, medeia as respostas iniciais e agudas por meio da ativação das vias de fosfolipase C

(PLC)–PKC– proteína quinase ativada por mitógeno (MAPK), resultando em aumento do Ca^{2+} intracelular, liberação prostaglandinas, e aumento da permeabilidade vascular e do recrutamento de neutrófilos. Em contraste, o receptor B_1 é induzido em resposta a mediadores pró-inflamatórios, sendo ativado por metabólitos da bradicinina e sustentando a inflamação crônica, com consequente produção de ERO e manutenção do dano tecidual, tornando ambos potenciais alvos para intervenções farmacológicas em doenças respiratórias inflamatórias (Campanholle et al., 2010).

Embora o receptor B_1 esteja mais diretamente associado a processos inflamatórios persistentes, o receptor B_2 também contribui significativamente para os eventos iniciais da inflamação aguda. Assim, considerando que a resposta inflamatória exacerbada é um dos principais determinantes da SDRA, a sinalização mediada por bradicinina e seus receptores pode representar um importante mecanismo na patogênese da síndrome, além de uma via terapêutica promissora (Campanholle et al., 2010).

1.7 ENVOLVIMENTO DOS CANAIS TRPV1, TRPA1, TRPV4 E DOS RECEPTORES DE BRADICININA NA SDRA

Diversos estudos já estabeleceram que a bradicinina pode induzir a sensibilização dos canais TRP e alterar seus níveis de expressão através dos receptores B_1 e B_2, amplificando sua reatividade frente a estímulos posteriores (Al-Shamlan e El-Hashim; Cernit et al., 2020).

Nesse sentido, o receptor B_1, cuja expressão é induzida em condições inflamatórias, tem sido associado à modulação de canais TRP. Em modelos de inflamação e dor, a estimulação do receptor B_1 induz a expressão e ativação sustentada de TRPV1 (Cernit et al., 2020), TRPA1 (Meotti et al., 2017) e TRPV4 (Fialho et al., 2024) intensificando a resposta inflamatória e o recrutamento celular.

O receptor B_2, amplamente expresso em tecidos saudáveis, também está associado à ativação de canais TRP em modelos inflamatórios (Fialho et al., 2024). No contexto pulmonar, já foi caracterizada uma importante interação funcional entre receptores B_2 e receptores TRPV1, TRPA1 e TRPV4.

Coletivamente, dados reforçam que TRPA1 e TRPV4 participam do extravasamento plasmático e da broncoconstrição, mediado pela ativação do receptor B_2 nas vias aéreas de ratos tratados com captopril (Jentsch et al., 2020). Em relação ao receptor TRPV1, Amorim et al., (2021) demonstraram que o tratamento com sinvastatina parece promover a liberação de bradicinina, que, por meio dos receptores B_2, libera NO, que pode então ativar o TRPV1 para promover extravasamento plasmático e broncoconstrição nas vias aéreas de ratos. Al-Shamlan e El-Hashim (2019) evidenciaram que a administração central de bradicinina aumenta a responsividade do reflexo da tosse e intensifica a obstrução das vias aéreas por meio do receptor B_2 e envolvimento dos canais TRPV1 e TRPA1, por meio de metabólitos das enzimas cicloxigenase **(**COX) e 12-lipoxigenase (12-LOX).

Estudos demonstram que a ativação de receptores B_1 e B_2 por bradicinina promove a fosforilação e sensibilização dos canais TRP via sinalização intracelular envolvendo PLC, PKC e PKA (Al-Shamlan e El-Hashim, 2019; Amorim et al. 2021; De Oliveira et al. 2016). Essa ativação indireta dos receptores TRP promove o influxo de cálcio intracelular e ativação de vias como PKC, MAPK e fator nuclear kappa B (NF-κB), potencializando a excitabilidade neuronal, produção de citocinas, recrutamento de células inflamatórias e liberação de neuropeptídeos como substância P e CGRP, resultando em broncoconstrição e aumento da permeabilidade vascular – eventos típicos de inflamação pulmonar (Al-Shamlan e El-Hashim, 2019; Amorim et al. 2021; De Oliveira et al. 2016).

Esses dados sustentam a hipótese de que os receptores de bradicinina modulam canais TRP, contribuindo para a fisiopatologia de doenças pulmonares. O bloqueio seletivo de receptores B_1/B_2 ou dos canais TRPV1/TRPA1/TRPV4 tem se mostrado eficaz em reduzir a inflamação e o dano tecidual em modelos experimentais, destacando essa interação funcional como um alvo terapêutico promissor (Amorim et al., 2021; Campanholle et al., 2010).

Durante a SDRA, a lesão pulmonar desencadeia a liberação de citocinas, bradicinina, espécies reativas de oxigênio e alterações mecânicas na barreira alveolo-capilar, que em conjunto promovem a ativação e sensibilização dos receptores TRPs (TRPV1, TRPA1, TRPV4) e dos receptores B_1 e B_2 de bradicinina, amplificando a inflamação e a disfunção pulmonar (Campanholle et al., 2010; Koivisto et al., 2022).

Embora os receptores TRPV1, TRPA1 e TRPV4, bem como os receptores de bradicinina B_1 e B_2, estejam implicados na SDRA, ainda não foi esclarecido se a

interação funcional entre esses receptores poderia amplificar a resposta inflamatória e agravar a lesão pulmonar. No contexto de modelos experimentais de LPA, investigar a participação conjunta desses receptores pode fornecer novos insights sobre os mecanismos fisiopatológicos envolvidos e identificar potenciais alvos terapêuticos.

Nesse sentido, hipotetizamos que a ativação dos receptores de bradicinina B_1/B_2 potencializa a atividade dos canais TRP (TRPV1, TRPA1 e TRPV4), contribuindo para a fisiopatologia da SDRA. Para testar essa hipótese, utilizamos o modelo experimental de LPA induzida por LPS, avaliando o papel desses receptores na modulação da lesão pulmonar.

2. PLANO DA TESE

2.1 JUSTIFICATIVA

A SDRA é uma doença pulmonar que apresenta alta incidência e acomete todas as faixas etárias, apresentando alta morbidade e mortalidade no mundo.

O processo inflamatório característico da doença poderia sensibilizar canais TRP como TRPV1, TRPA1 e TRPV4 presentes nas vias aéreas, uma vez que mediadores inflamatórios envolvidos na SDRA, como bradicinina e seus receptores podem modular esses canais TRP. Uma vez ativados esses receptores poderiam promover a liberação de citocinas, entre outros, exacerbando o processo inflamatório na SDRA. Porém até o momento não existem estudos sugerindo uma provável relação entres canais TRP, receptores de bradicinina e SDRA.

Os resultados do presente estudo, em modelos de animais com LPA irão fornecer evidências sobre a participação dos receptores TRPV1, TRPA1 e TRPV4 na SDRA contribuindo assim para um melhor entendimento dessa doença. Embora o modelo utilizado não reproduza integralmente todas as características da SDRA, os achados podem oferecer informações importantes que contribuam para a compreensão dos mecanismos envolvidos nessa doença e, potencialmente, subsidiem o desenvolvimento de estratégias terapêuticas futuras. Vale também ressaltar que este projeto oferece um leque de possibilidades de estudo, que poderão ser aprofundadas a partir dos resultados obtidos e das propostas desenvolvidas ao longo desta pesquisa.

2.2 OBJETIVO GERAL

Investigar a participação dos receptores TRPV1, TRPA1 e TRPV4, bem como dos receptores de bradicinina B_1 e B_2, e uma possível interação funcional entre eles, na fisiopatologia da LPA induzida por LPS em camundongos.

2.3 OBJETIVOS ESPECÍFICOS

1) Avaliar os efeitos dose-dependentes e temporais do LPS administrado por via intratraqueal na indução de LPA em camundongos, por meio de análise histológica, perda de peso corporal e quantificação de proteínas no LBA.

2) Analisar se a inibição farmacológica dos receptores TRPV1, TRPA1 e TRPV4 e receptores B_1 e B_2 de bradicinina atenuam o edema pulmonar e a perda de peso corporal em modelo murino de LPA induzida por LPS.

3) Verificar se o bloqueio dos receptores TRPV1, TRPA1 e TRPV4 e dos receptores B_1 e B_2 de bradicinina reduzem o infiltrado celular nas vias aéreas em camundongos submetidos à LPA induzida por LPS.

4) Avaliar alterações histológicas nos pulmões de camundongos com LPA induzida por LPS, bem como os efeitos do bloqueio farmacológico dos receptores TRPV1, TRPA1 e TRPV4 e dos receptores B_1 e B_2 de bradicinina na reversão dessas alterações.

5) Averiguar a deposição de colágeno nos pulmões de camundongos com LPA induzida por LPS, bem como os efeitos do bloqueio farmacológico dos canais TRPV1, TRPA1 e TRPV4 e dos receptores B_1 e B_2 de bradicinina na modulação desse processo.

6) Quantificar os níveis de citocinas e proteínas totais no LBA e investigar se a modulação dos receptores TRPV1, TRPA1 e TRPV4 e receptores B_1 e B_2 de bradicinina influenciam o perfil inflamatório em camundongos com LPA induzida por LPS.

7) Investigar, por meio de imunohistoquímica, alterações na expressão dos receptores TRPV1, TRPA1 e TRPV4 nos pulmões de camundongos com LPA induzida por LPS.

8) Investigar os efeitos do bloqueio dos receptores TRPV1, TRPA1 e TRPV4 B_1 e B_2 de bradicinina, por meio de seus antagonistas, sobre a expressão do canal TRPV1, TRPA1 e TRPV4 nos pulmões de camundongos com LPA induzida por LPS.

3. ARTIGOS CIENTÍFICOS

3.1 MANUSCRITO ORIGINAL 1

Publicado na revista *Pulmonary Pharmacology and Therapeutics*

FUNCTIONAL INTERPLAY BETWEEN BRADYKININ RECEPTORS AND TRANSIENT RECEPTOR POTENTIAL VANILLOID-1 IN LIPOPOLYSACCHARIDE-INDUCED ACUTE LUNG INJURY IN MICE

Pulmonary Pharmacology & Therapeutics 90 (2025) 102384

Contents lists available at ScienceDirect

Pulmonary Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/ypupt

Functional interplay between bradykinin receptors and transient receptor potential vanilloid-1 in lipopolysaccharide-induced acute lung injury in mice

Mayara Alves Amorim [a], Vitor Hélio Souza Oliveira [a], João B. Calixto [b], Eunice André [a,*]

[a] Department of Pharmacology, Federal University of Paraná, Curitiba, Brazil
[b] Centro de Inovação e Ensaios Pré Clínicos CIEnP, Florianópolis, SC, Brazil

ARTICLE INFO

Keywords:
LPS-induced lung injury
Acute respiratory distress syndrome
Cytokines
Bradykinin receptors
TRPV1

ABSTRACT

In this study, we investigated the functional interplay between bradykinin receptors and the transient receptor potential vanilloid-1 (TRPV1) channel in a mouse model of acute lung injury (ALI) induced by lipopolysaccharide (LPS). Lung and bronchoalveolar lavages were collected at 6 and 24 h after the induction of ALI and evaluated for changes in body weight, inflammatory marker levels, lung injury, and TRPV1 expression. Pretreatments with a TRPV1 antagonist (capsazepine) or B_1 and B_2 receptor antagonists, i.e., DALBK and HOE 140, respectively, were evaluated in this ALI mouse model. The histological score revealed higher levels of lung injury in mice treated with LPS (5 and 10 mg/kg), assessed at both 6 and 24 h, compared to the vehicle-treated group. A loss of body weight was observed within 24 h of ALI induction. Furthermore, collagen deposition, pulmonary oedema, leukocyte influx, and increased cytokine levels were also observed following LPS administration. Pretreatment with capsazepine, DALBK, or HOE 140 not only reversed all inflammatory parameters but also prevented the increased expression of TRPV1 observed in the lungs of mice subjected LPS-induced ALI. Our data suggest that, following LPS-induced ALI, bradykinin activates both B_1 and B_2 receptors associated with the subsequent activation of TRPV1. These findings suggest that bradykinin can activate both B_1 and B_2 receptors, which may contribute functionally to TRPV1 upregulation and activation during LPS-induced ALI. This novel pathway appears to sustain inflammation, offering a new therapeutic target for ALI and ARDS.

1. Introduction

Acute respiratory distress syndrome (ARDS) is a manifestation of acute lung injury (ALI) resulting from severe pulmonary lesions leading to alveolar damage and collapse [1]. Although ALI and ARDS originate from different injuries and conditions, their similar clinical features suggest they must be considered a single entity [2]. Both these diseases are potentially fatal in critically ill patients. ARDS is characterised by noncardiogenic pulmonary oedema, severe hypoxemia, hypercapnia, diffuse chest radiography infiltrates, and a notable decrease in pulmonary compliance leading to alveolar collapse [1,3,4]. It can affect people of any age, regardless of the underlying cause; thus far, there is no effective therapy available, leading to high morbidity and mortality in 46 %–60 % of patients [1,5].

In this context, a comprehensive elucidation of the pathophysiology of ARDS will facilitate the development of new therapies. It has been demonstrated that the transient receptor potential vanilloid-1 (TRPV1) channel is involved in various physiological and pathological functions and, therefore, it has emerged as an attractive target for treating respiratory diseases [6]. TRPV1 channels are particularly activated in response to various exogenous and endogenous noxious stimuli, such as capsaicin, low pH (<6.5), harmful temperatures (>43 °C) and increased levels of several inflammatory mediators (for review see: [7]). When activated, TRPV1 channels contribute to lung inflammation aggravation, resulting in pulmonary oedema, breathing difficulties, and tissue damage (for review see: [8,9]). The role of TRPV1 has been widely investigated in the pathogenesis of various airway diseases, such as asthma, chronic obstructive pulmonary disease (COPD), cystic fibrosis, and chronic cough [7,10,11]. In addition, TRPV1 has been implicated in ALI, and preclinical studies have demonstrated that blocking TRPV1 using selective antagonists can prevent pulmonary injury in mice [12,13].

* Corresponding author. Department of Pharmacology, Federal University of Paraná, Curitiba, PR, 81531-980, Brazil.
E-mail address: eunice.andre@ufpr.br (E. André).

https://doi.org/10.1016/j.pupt.2025.102384
Received 12 May 2025; Received in revised form 30 July 2025; Accepted 9 August 2025
Available online 10 August 2025
1094-5539/

In addition, studies have demonstrated that kinins, acting on their B_1 and B_2 receptors, are involved in the pathological events of the airways [14–16]. B_1 receptors exhibit relatively low expression under normal physiological conditions but are highly inducible following inflammatory conditions, whereas B_2 receptors are normally constitutively expressed [14,17–19]. In addition, previous studies have demonstrated an interaction between bradykinin receptors and TRPV1, and this interaction appears to contribute to an increase in the sensitivity of TRPV1, making it relatively more responsive to subsequent stimuli [20–22]. This interaction between bradykinin receptors and the TRPV1 channel has been reported in lung tissue, contributing to airway changes such as bronchoconstriction, plasma leakage, and coughing [21,23,24].

However, its role in ARDS remains unknown. Based on the hypothesis that bradykinin receptors trigger TRPV1 activation in ARDS, we aimed to investigate whether a functional association between bradykinin receptors and TRPV1 contributes to the inflammatory responses in an ALI model induced by intratracheal lipopolysaccharide (LPS) administration.

2. Material and methods

2.1. Animals and ethics statement

C57BL/6 male and female mice, aged 7–8 weeks, weighing 20–25 g, were used in this study. The animals were obtained from the Bioterium Complex of the Biological Sciences Sector at the Federal University of Paraná Brazil. The mice were housed in the groups of four animals per cage, filled with shaving material and maintained under controlled temperature conditions (22 ± 2 °C) and illumination (light/dark cycle of 12 h), with food and water *ad libitum*. The experimental procedures were approved by the local Ethics Committee of Animal Experimentation of the Federal University of Paraná (Protocol Nos. 1432/2021 and 1602/2024) and were performed following the ARRIVE guidelines, as previously outlined by Sert et al. [25]. The animals were acclimatised to the laboratory conditions for at least an hour before the experiments. Since no significant difference was observed between male and female mice, both sexes were included together in the analyses to increase statistical power and reduce variations. Subsequently, the animals were randomly (random.org) assigned to groups, with a minimum of six animals in each experimental group, a prerequisite to obtain robust and consistent data and perform statistical analyses. The animals were initially allocated to the negative control (vehicle) and induction (LPS) groups. Following intratracheal instillation of LPS or saline, the LPS-exposed animals were randomly reassigned to experimental groups, receiving either one of the tested antagonists or the corresponding vehicle, ensuring treatment blinding. The treatment blinding was performed in a manner where the experimenter had no prior knowledge of the assigned treatments, minimizing potential bias. All raw data were included in the calculation of means, statistical analyses, or result analyses, and no data were excluded.

2.2. Experimental model of ALI in mice

In this study, ALI was induced by the administration of LPS from *Escherichia coli* (endotoxin activity: not less than 500,000 endotoxin units per mg of LPS; O111:B4, product number LPS25; Sigma Aldrich) at a dose of (2, 5, or 10 mg/kg in 50 μL intratracheally (i.t.) in both male and female C57BL/6 mice, whereas the mice in the control group were administered with vehicle (50 μL saline). In this study, ALI was induced in C57BL/6 mice using intratracheal LPS at doses of 2, 5, or 10 mg/kg. These doses were chosen based on variability reported in the literature [26–30]. To define the most appropriate conditions for our model, we tested all three doses at two different time points: 6 and 24 h after LPS administration. This approach allowed us to evaluate the progression of the inflammatory response and select the dose that best suited our experimental goals.

For this procedure, the mice were weighed and then anaesthetised intraperitoneally (i.p.) with ketamine/xylazine (100 and 15 mg/kg, respectively) before being placed in the supine position. Under aseptic conditions, a 5-mm vertical incision was carefully made on the skin of the neck. The trachea was then identified, and either LPS or its vehicle (50 μL saline) was carefully injected into the trachea using a sterile 30-gauge needle. The skin was sutured, and after 6 or 24 h, the animals were weighed again and euthanised with a dose of 50 mg/kg sodium thiopental (i.p.) following the application of lidocaine (10 mg/mL, i.p.). Subsequently, transcardial perfusion with 0.9 % saline solution was performed, and the lungs were removed for histological and immunohistochemical analyses. In addition, bronchoalveolar lavage fluid (BALF) was collected for the subsequent analyses of cellular infiltrates and protein and cytokine levels.

2.3. Effects of the treatments of animals with the antagonists of TRPV1 or B_1 and B_2 receptors

To evaluate the potential functional association of TRPV1 and B_1 and B_2 bradykinin receptors in LPS-induced ALI in mice, animals were first weighed and then pretreated with capsazepine (10 μmol/kg, i.p.) [31], a TRPV1 channel antagonist. Another group of mice received Des-Arg9-Leu8-bradykinin (DALBK; 50 nmol/kg, i.p.) or icatibant (HOE 140; 10 nmol/kg, i.p.), B_1 and B_2 receptor antagonists, respectively. The doses of the antagonists capsazepine, DALBK, and HOE 140 were chosen as described previously [32,33].

Approximately 15 min after pretreatment with these antagonists (capsazepine, DALBK, or HOE 140) or their respective vehicles (vehicle for capsazepine: 0.9 % NaCl composed of 10 % DMSO and 5 % Tween 80; vehicle for DALBK and HOE 140: 0.9 % NaCl), the animals were exposed to LPS (5 mg/kg, i.t.) to induce ALI. Nearly 6 or 24 h after LPS administration, the mice were reweighed and euthanised with a sodium thiopental overdose of 100 mg/kg (i.p.), following lidocaine administration (10 mg/mL, i.p.). The lungs were removed for histological and immunohistochemical analyses, and BALF was collected for subsequent analyses.

2.4. Histological analyses

The lung tissues of mice were collected and fixed in 4 % paraformaldehyde, embedded in paraffin, sliced into 4 μm sections, and subjected to haematoxylin and eosin (H&E) staining to evaluate histological changes or to Masson's trichrome staining to determine collagen content in the lung tissue.

Lung histology was evaluated by a pathologist using optical microscopy, classifying pathological changes on a scale of 0–3: normal (0), mild (1), moderate (2), and severe (3) [34]. The pathologist was blinded and had no prior knowledge of the treatment assignments. The parameters evaluated were alveolar congestion, inflammatory cell infiltration, oedema, and alveolar haemorrhage [34]. The values were expressed as the sum of scores obtained after histopathological analysis.

To evaluate collagen content, lung tissues were subjected to Masson's trichrome staining, and the stained slices were observed under an optical microscope. The percentage of collagen was defined as the total collagen amount/total area of the tissue in the image and analyzed using ImageJ 1.4d [35,36].

2.5. Quantification of total protein content in BALF

After the euthanasia of mice, the trachea was exposed, and a cannula was inserted via the cervical mid-incision. The airways were washed thrice with 1 mL of phosphate-buffered saline (PBS), and the aspirated content was collected in silicone tubes on ice. Subsequently, the entire exudate was centrifuged at $290 \times g$ for 8 min at 4 °C, and the supernatant obtained was used to measure protein levels using Bradford reagent. Protein concentration was determined by interpolating data to a

standard curve (0.1–1.0 mg/mL) of bovine serum albumin (BSA) solution. The absorbance of the samples was recorded at 595 nm using a spectrophotometer.

2.6. Measuring the wet/dry weight ratio

Mice were sacrificed 6 and 24 h after LPS administration, and the wet/dry weight (W/D) ratio of the lungs was estimated to evaluate pulmonary oedema. The wet weight was measured immediately after removing the lungs, and the dry weight was obtained by drying the lungs for 72 h at 60 °C in an oven. The W/D ratio was estimated, with the following calculation: weight of the wet lung (mg) – weight of the dry lung (mg)/body weight (g) [34,37]. Values are expressed as lung weight (mg)/g body weight.

2.7. Analysis of leukocyte influx in BALF

Leukocyte influx into the lungs was evaluated in the BALF of mice subjected LPS-induced ALI. After the collection of BALF, the entire exudate was centrifuged at 290×*g* for 8 min at 4 °C, and the sediment cell pellet was resuspended in 100 μL of PBS. The total number of cells was determined using a Neubauer chamber (American Optical, Southbridge, MA, USA), after the addition of Türk solution (0.5 mg/mL in PBS) under the optical microscope (at 400X magnification). The results are expressed as the total number of cells ($\times 10^6$) [38].

2.8. Immunohistochemical analyses

Immunohistochemistry was performed to evaluate TRPV1 channel expression in the lungs of mice. The lung tissues were fixed in 4 % paraformaldehyde, embedded in paraffin, and sliced into 5-μm thick sections. Subsequently, the sections were blocked and incubated overnight at 4 °C with a primary antibody against TRPV1 (1:100). After washing 4 times with PBS, the sections were incubated with a goat anti-rabbit secondary antibody (1:100) for 1 h at room temperature in a humid chamber and then washed 4 times with 1 % BSA in PBS. Positive signals were detected using the 3,3′-diaminobenzidine substrate kit (BD Pharmingen, San Jose, CA, USA). The stained tissue sections were counterstained with H&E. Immunoreactivity for TRPV1 channel was visualised under an Olympus System Microscope using the imaging software CellF (2008; Olympus Soft Imaging Solutions GmbH). The images were analyzed using ImageJ 1.4d, and the expression levels of TRPV1 were determined as the average of the grey values normalised based on nuclei number from two to four images per lung [39,40].

2.9. Determination of cytokine levels in BALF

The quantification of the levels of interleukin (IL)-1β, IL-6, IL-10, IL-17, IL-23, tumour necrosis factor-alpha (TNF-α), and interferon-gamma (IFN-γ) levels in BALF was performed at both time points of 6 and 24 h after LPS-induced ALI using enzyme-linked immunosorbent assay kits, and all procedures were performed following the manufacturer's instructions.

2.10. Drugs and reagents

The following drugs and reagents were used: LPS from *Escherichia coli* (O111:B4, product number: LPS25, not less than 500,000 Endotoxin Units per mg of LPS), BSA, Bradford reagent, DALBK acetate salt, HOE140, goat anti-rabbit secondary antibody, all from Sigma Chemical Co., St. Louis, USA. Capsazepine and rabbit anti-TRPV1 antibody polyclonal were purchased from Alomone Labs, Jerusalem, Israel. DAB Substrate Kit was purchased from BD Pharmingen, San Jose, CA, USA. LPS, DALBK acetate salt and HOE140 were dissolved in 0.9 % of NaCl. Capsazepine solutions were made using 0.9 % NaCl composed of 10 % DMSO and 5 % Tween 80. The solutions were diluted on the day of the experiment just prior to use. The cytokines IL-1, IL-6, IL-17, IL-23, TNF, IFN-γ and IL-10 were obtained from Peprotech Brazil-Funpec, while IL-17 and IL-23 were purchased from Biolegend company, UK.

2.11. Statistical analysis

The reported values were expressed as mean ± standard error of mean (SEM) of a minimum of six animals per group and all experiments were repeated at least three times. Statistical comparisons of data were performed by one-way analysis of variance (one-way ANOVA) followed by Newman Keuls (GraphPad Prism software version 8). The body weight data were analyzed using two-way ANOVA followed by Tukey's post-hoc considering the independent variables 'treatment' (vehicle vs. LPS) and 'time' (0, 6 or 24 h). In all experiments, values of $P \leq 0.05$ were considered statistically significant and all statistical analyses were conducted using GraphPad Prism 8.0 (San Diego, CA, USA).

3. Results

3.1. Pathological alterations in the lung tissues of mice with LPS-induced ALI

The administration of LPS at a dose of 2 mg/kg (i.t.) caused no significant histological changes in the lung tissues of treated mice (Fig. 1A–C). However, the lung tissues of the animals treated with LPS at the doses of 5 and 10 mg/kg (i.t.) exhibited significant pulmonary lesions characterised by histological changes, including alveolar congestion, inflammatory cell infiltration, oedema, and alveolar haemorrhage (Fig. 1A–C). Histological assessment using injury scores demonstrated that the extent of lung injury in the LPS-treated group was relatively more severe compared with that in the vehicle-treated group. The histological average score (Fig. 1B and C) of mice treated with 5 mg/kg dose of LPS at both time points of 6 and 24 h after administration were 7.66 ± 0.21 and 6.66 ± 0.21, respectively, which were significantly higher than those in mice in the vehicle-treated group (2.50 ± 0.22 and 2.33 ± 0.21, respectively). Similarly, at the 10 mg/kg dose of LPS, the histological score (Fig. 1B and C) at both analysis time points (6 and 24 h following administration) were significantly higher (6.50 ± 0.22 and 6.33 ± 0.21, respectively) than those in the vehicle-treated group (2.50 ± 0.22 and 2.33 ± 0.21, respectively).

In addition, ALI induced by LPS (5 mg/kg) did not result in a loss of body weight at 6 h after the induction of ALI; however, a significant loss of body weight (7.89 % ± 1.09 %) was evident 24 h after the induction of ALI (Fig. 1D and E).

The data presented in Fig. 1F demonstrate that protein levels in supernatant samples from BALF were also significantly higher in mice subjected ALI induced by LPS (5 mg/kg) at both analysis time points of 6 and 24 h after LPS administration (658.1 ± 67.99 μg/mL, and 638.6 ± 44.82 μg/mL, respectively) than those in mice in the vehicle-treated group (312.6 ± 53.45 and 312.9 ± 47.44 μg/mL, respectively).

3.2. TRPV1 channel mediates histological alterations in the lung tissues of mice with LPS-induced ALI

The histological changes observed at the dose of 5 mg/kg of LPS, at both analysis time points, were prevented by pretreatment with capsazepine (histological average score: 3.33 ± 0.21 and 2.50 ± 0.22, respectively) compared with that in the vehicle-treated group (histological average score: 7.00 ± 0.63 and 6.66 ± 0.21, respectively) (Fig. 2A–C).

Additionally, there was a significant increase in collagen accumulation in the lungs of mice subjected ALI induced by LPS (5 mg/kg, i.t.) at both 6- and 24-h analysis time points (41.72 % ± 4.85 % and 42.31 % ± 4.16 % of the total area, respectively), compared with that in the vehicle-treated group (25.16 % ± 2.89 % and 21.74 % ± 1.37 % of the total area, respectively) (Fig. 2B–D). Moreover, pretreatment with

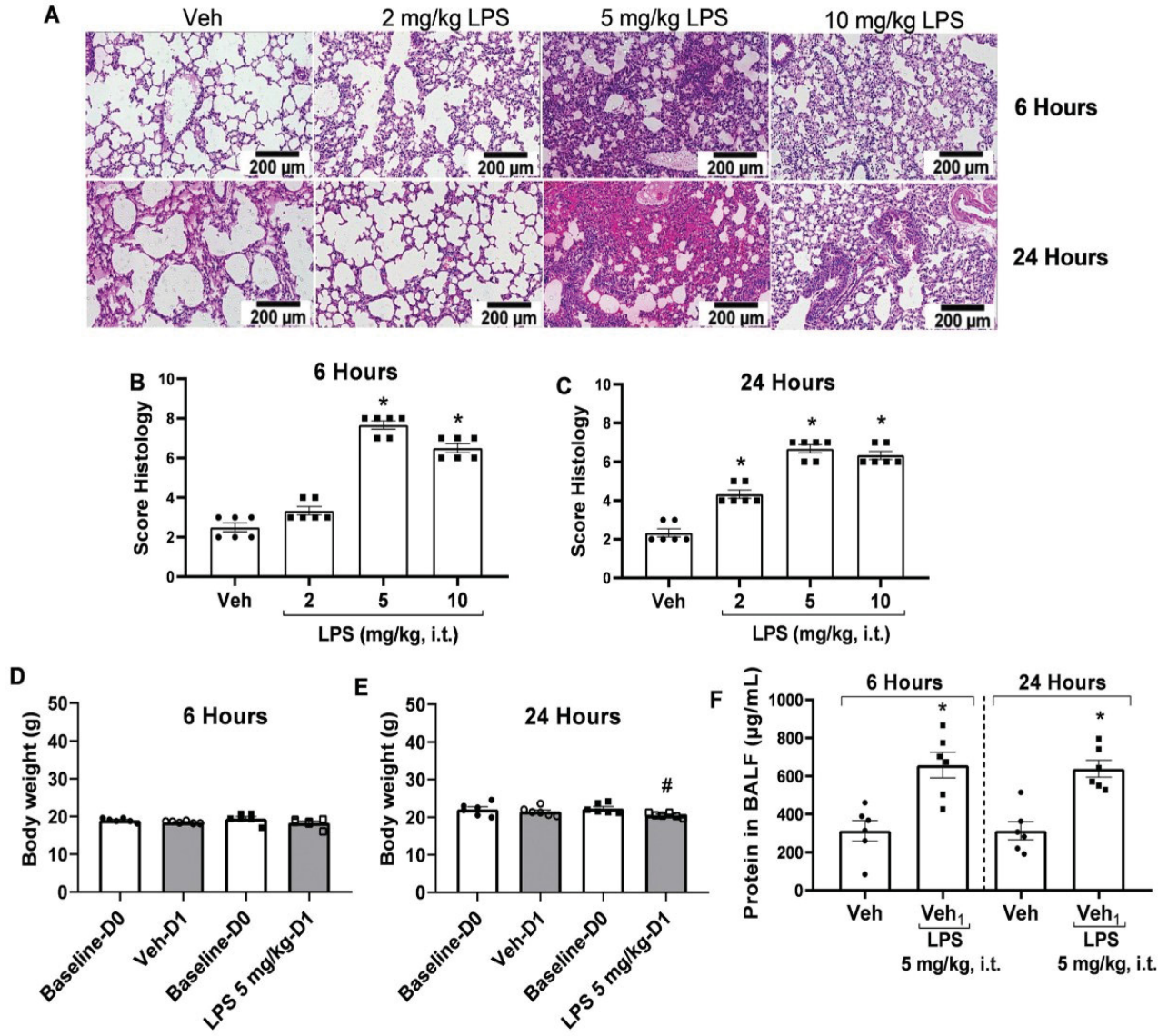


Fig. 1. Lung injury assessed at 6 and 24 h after administration intratracheal lipopolysaccharide (LPS). (A) Representative light microphotograph of the lungs of mice treated with 2, 5 or 10 mg/kg intratracheal doses of LPS. (B, C) Average histological scores of pulmonary lesions observed at 6 and 24 h after treatment subjected LPS (2, 5 or 10 mg/kg). (D,E) Alteration in body weight at 6 and 24 h after treatment with LPS (5 mg/kg). (F) Quantification of total protein content in bronchoalveolar lavage fluid (BALF) at 6 and 24 h after treatment with LPS (5 mg/kg). Baseline-D0 represents the body weight before the administration of vehicle or LPS, respectively. Veh D1 or LPS D1 represents the body weight at 6 and 24 h after the administration of vehicle or LPS, respectively. Each point represents the mean (standard error of the mean, SEM) of six mice. $^{*}P \leq 0.05$ compared with vehicle-treated group (Veh), (one-way ANOVA followed by Student–Newman–Keuls test). $^{\#}P \leq 0.05$ compared with LPS D0 group, (two-way ANOVA applied to body weight changes, followed by Tukey's test for post-hoc analysis).

capsazepine restored the increased accumulation of lung collagen to its normal levels in LPS-treated mice at both analysis time points (19.16 % ± 2.84 % and 23.23 % ± 3.96 % of the total area, respectively) compared with that in mice in the respective vehicle-treated group (41.72 % ± 4.85 % and 42.31 ± 4.16 % of the total area, respectively) (Fig. 2B–D).

3.3. TRPV1 modulates plasma protein levels, leukocyte influx, lung weight, and body weight in mice with LPS-induced ALI

In BALF, the albumin content was quantified to assess the integrity of the alveolar-capillary barrier. The increase in endothelial permeability indicated by elevated protein content in BALF at both analysis time points of 6 and 24 h post-LPS administration (626.1 ± 52.83 and 660.6 ± 42.05 µg/mL, respectively) was inhibited by pretreatment with capsazepine (332.6 ± 48.43 µg/mL and 359.1 ± 33.48 µg/mL, respectively) (Fig. 3A). In addition, mice subjected ALI induced by LPS (5 mg/kg, i.t.) exhibited pulmonary oedema, characterised by an increase in lung weight at both analysis time points of 6 and 24 h (9.00 ± 0.66 and 7.40 ± 0.44 mg/g, respectively) after the treatment compared with that in mice in the vehicle-treated group (5.21 ± 0.10 and 5.00 ± 0.55 mg/g, respectively) (Fig. 3B). The increase in lung weight observed at 6 and 24 h after LPS administration was prevented by pretreatment with capsazepine (5.61 ± 0.46 and 5.29 ± 0.47 mg/g, respectively) (Fig. 3B).

In addition, an increased influx of leukocytes in BALF was observed at 6 and 24 h (LPS: 38.97 ± 3.81 × 10^6 and 34.10 ± 1.63 × 10^6 cells, respectively) after LPS administration compared with that in the vehicle-treated group (Veh-LPS: 20.57 ± 1.09 × 10^6 and 18.99 ± 1.07 × 10^6 cells, respectively) (Fig. 3C). However, pretreatment with capsazepine prevented the increase in the influx of leukocytes at both analysis time points following LPS administration (23.14 ± 2.33 × 10^6 and 22.18 ± 1.39 × 10^6 cells, respectively) (Fig. 3C). Furthermore, the

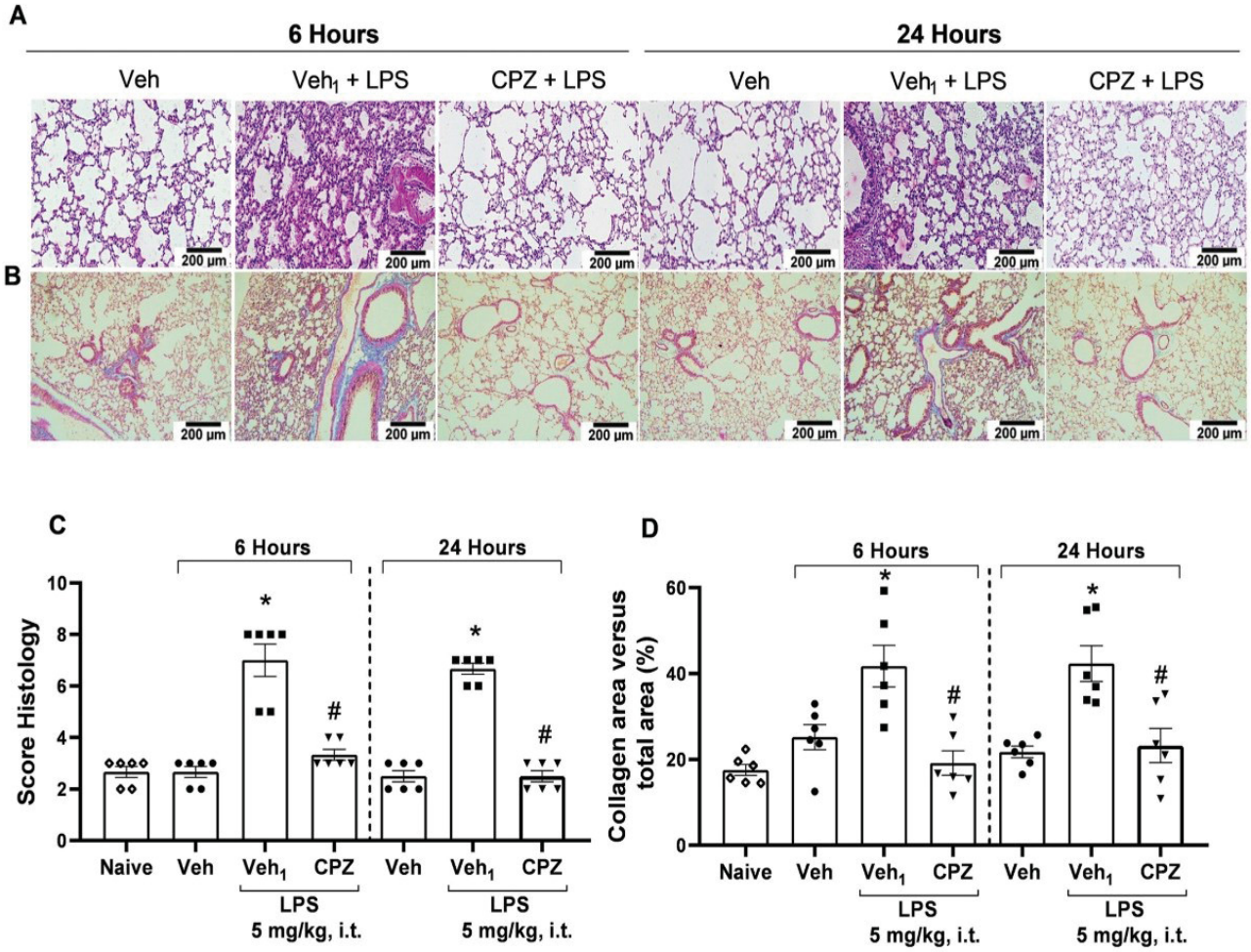


Fig. 2. Effects of TRPV1 channel antagonist (capsazepine) on histological changes and collagen deposition at 6 and 24 h after the intratracheal administration of lipopolysaccharide (LPS) in mice. (A) Representative light microphotograph of histological alterations and (B) collagen deposition in the lungs of mice pretreated with capsazepine or its vehicle and collected at 6 and 24 h after treatment with LPS or vehicle, respectively. (C) Histology injury score and (D) quantification of the collagen-stained areas of the lung tissues of mice. Each point represents the mean (standard error of the mean, SEM) of five to six mice. $^{*}P \leq 0.05$ compared with the vehicle-treated group (Veh), $^{\#}P \leq 0.05$ compared with the vehicle of antagonists + LPS-treated group (Veh1) (one-way analysis of variance followed by Student–Newman–Keuls test).

data revealed that the pharmacological blockade of TRPV1 channels with capsazepine prevented the loss of body weight in mice observed 24 h after the administration of LPS (Fig. 3D).

3.4. *TRPV1 is upregulated in the lungs of mice with LPS-induced ALI*

The administration of LPS (5 mg/kg, i.t.) resulted in an increased expression of TRPV1 channel at 6 and 24 h analysis time points (relative expression: 8.58 ± 0.97 and 6.45 ± 0.71 mg/g, respectively) after the treatment compared with that in the vehicle-treated group (relative expression: 4.46 ± 0.79 and 3.10 ± 0.25 mg/g, respectively) (Fig. 3E and F). Pretreatment with capsazepine (30 µmol/kg, i.p.) abolished the increase in TRPV1 expression in the lungs of mice subjected LPS-induced ALI at both analysis time points (6 and 24 h) post-LPS administration (Fig. 3E and F). Pretreatment with capsazepine restored the relative expression of TRPV1 in mice at 6 and 24 h post-LPS administration (3.06 ± 0.45 and 3.1 ± 0.51 mg/g, respectively) achieving values similar to those observed in the group treated only with the vehicle of capsazepine and LPS (4.20 ± 0.86 and 3.1 ± 0.25 mg/g, respectively).

3.5. *B_1 and B_2 receptors contribute to histopathological changes in the lung tissues of mice with LPS-induced ALI*

The pretreatment of mice with the B_1 antagonist DALBK (50 nmol/kg, i.p.) 15 min before LPS administration (5 mg/kg, i.t.) completely prevented the histological alterations at both analysis time points of 6 and 24 h (histological score: 2.83 ± 0.30 and 3.16 ± 0.47, respectively) compared with that in mice in the respective vehicle-treated group (histological score: 6.83 ± 0.60 and 6.50 ± 0.42, respectively) (Fig. 4A and B). Moreover, these effects were also observed in mice pretreated with the B_2 antagonist HOE 140 (10 nmol/kg, i.p.). HOE 140 pretreatment completely prevented the histological alterations at both 6 and 24 h after the induction of ALI by LPS (histological score: 3.33 ± 0.21 and 4.00 ± 0.36, respectively) compared with that in mice in the respective vehicle-treated group (histological score: 5.83 ± 0.30 and 6.00 ± 0.36, respectively) (Fig. 4A–C). In addition, the increase in collagen deposition (24.52 % ± 1.49 % and 23.07 % ± 1.34 % of the total area, respectively) in the airways of mice treated with LPS at 6 and 24 h following LPS administration was prevented by the pretreatment of mice with DALBK (15.06 % ± 2.52 % and 12.67 % ± 1.53 % of the total area, respectively) (Fig. 5A and B). Moreover, pretreatment with HOE 140 inhibited the increase in collagen deposition in the airways of mice at both 6 and 24 h after the induction of ALI (8.60 % ± 1.48 % and 9.33 %

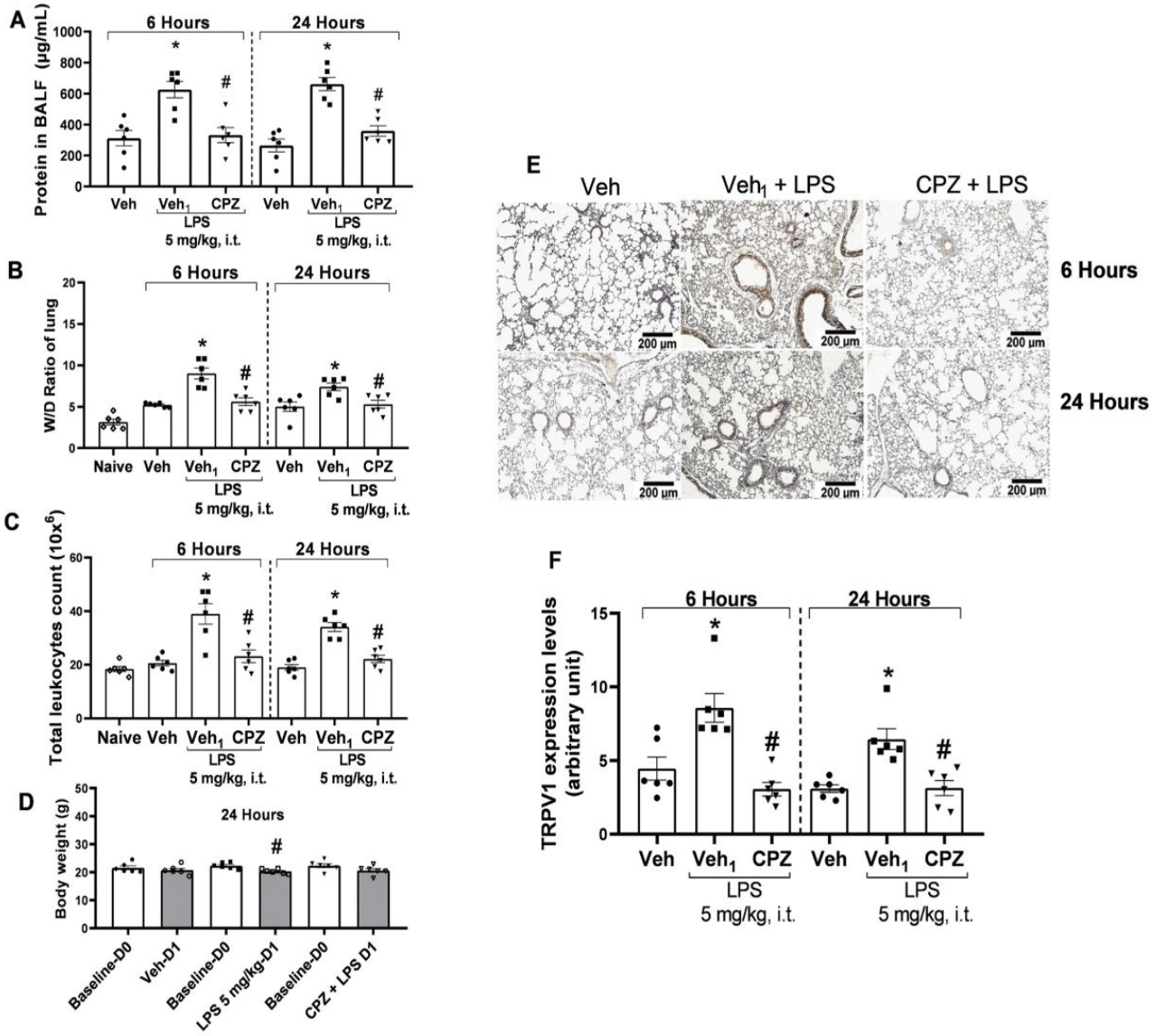


Fig. 3. Effects of TRPV1 channel antagonist (capsazepine) on protein content, lung weight, leukocyte count, body weight, and TRPV1 channel protein expression at 6 and 24 h after the intratracheal administration of LPS in mice. (A) Quantification of total protein content in bronchoalveolar lavage fluid (BALF). (B) Wet/Dry (W/D) weight ratio of lung. (C) Leukocyte infiltration in BALF. (D) Alteration in body weight at 24 h after treatment with LPS. (E, F) Immunoreactivity of TRPV1 in the lungs of mice pretreated with capsazepine (CPZ) and collected at 6 and 24 h after LPS administration. The W/D ratio was estimated using the following formula: weight of the wet lung (mg) – weight of the dry lung (mg)/body weight (g). Each point represents the mean (standard error of the mean, SEM) of six mice. $^{*}P \leq 0.05$ compared with the vehicle-treated group (Veh), $^{\#}P \leq 0.05$ compared with the vehicle of antagonists + LPS-treated group (Veh1) (one-way ANOVA followed by Student–Newman–Keuls test) or compared with the LPS baseline-D0 group, (two-way ANOVA applied to body weight changes, followed by Tukey's test for post-hoc analysis).

± 1.67 % of the total area, respectively) compared with that in mice in the respective vehicle-treated group (17.79 % ± 1.27 % and 18.45 % ± 1.32 % of the total area, respectively) (Fig. 5A–C).

3.6. B_1 and B_2 receptors contribute to changes in body weight and the levels of inflammatory markers in mice with LPS-induced ALI

Increased endothelial permeability, as indicated by the significantly increased protein content in BALF following intratracheal LPS treatment, was prevented by pretreatment with DALBK or HOE 140 (Fig. 6A). In addition, pretreatment with DALBK or HOE 140 prevented the increase in lung weight at 6 h (DALBK + LPS: 5.68 ± 0.86 mg/g: HOE 140 + LPS: 4.91 ± 0.76 mg/g) and 24 h (DALBK + LPS: 5.95 ± 0.49 mg/g; HOE 140 + LPS: 6.73 ± 0.40 mg/g) after LPS administration compared with that in their respective vehicle-treated groups (Veh-DALBK + LPS: 14.76 ± 1.39 and 10.63 ± 1.20 mg/g, respectively; Veh-HOE 140 + LPS: 12.34 ± 1.84 and 11.47 ± 1.63 mg/g, respectively) (Fig. 6B and C). Furthermore, both kinin antagonists prevented the influx of leukocytes in the BALF of mice subjected LPS-induced ALI at both analysis time points of 6 h following LPS administration (DALBK + LPS: [21.02 ± 1.71] × 10^6 cells; HOE 140 + LPS: [18.23 ± 1.99] × 10^6 cells) and 24 h following LPS administration (DALBK + LPS: 18.43 ± 2.07 × 10^6 cells; HOE 140 + LPS: 17.28 ± 2.20 × 10^6 cells) compared with that in their respective vehicle-treated groups (Veh-DALBK + LPS: 34.37 ± 4.85 × 10^6 cells and 31.58 ± 2.45 × 10^6 cells, respectively; Veh-HOE 140 + LPS: 36.53 ± 3.70 × 10^6 cells and 30.23 ± 1.79 × 10^6 cells, respectively) (Fig. 6D and E). In addition, the loss of body weight observed in mice subjected LPS-induced ALI at 24 h following LPS administration was prevented by pretreatment with DALBK or HOE 140 (Fig. 6F).

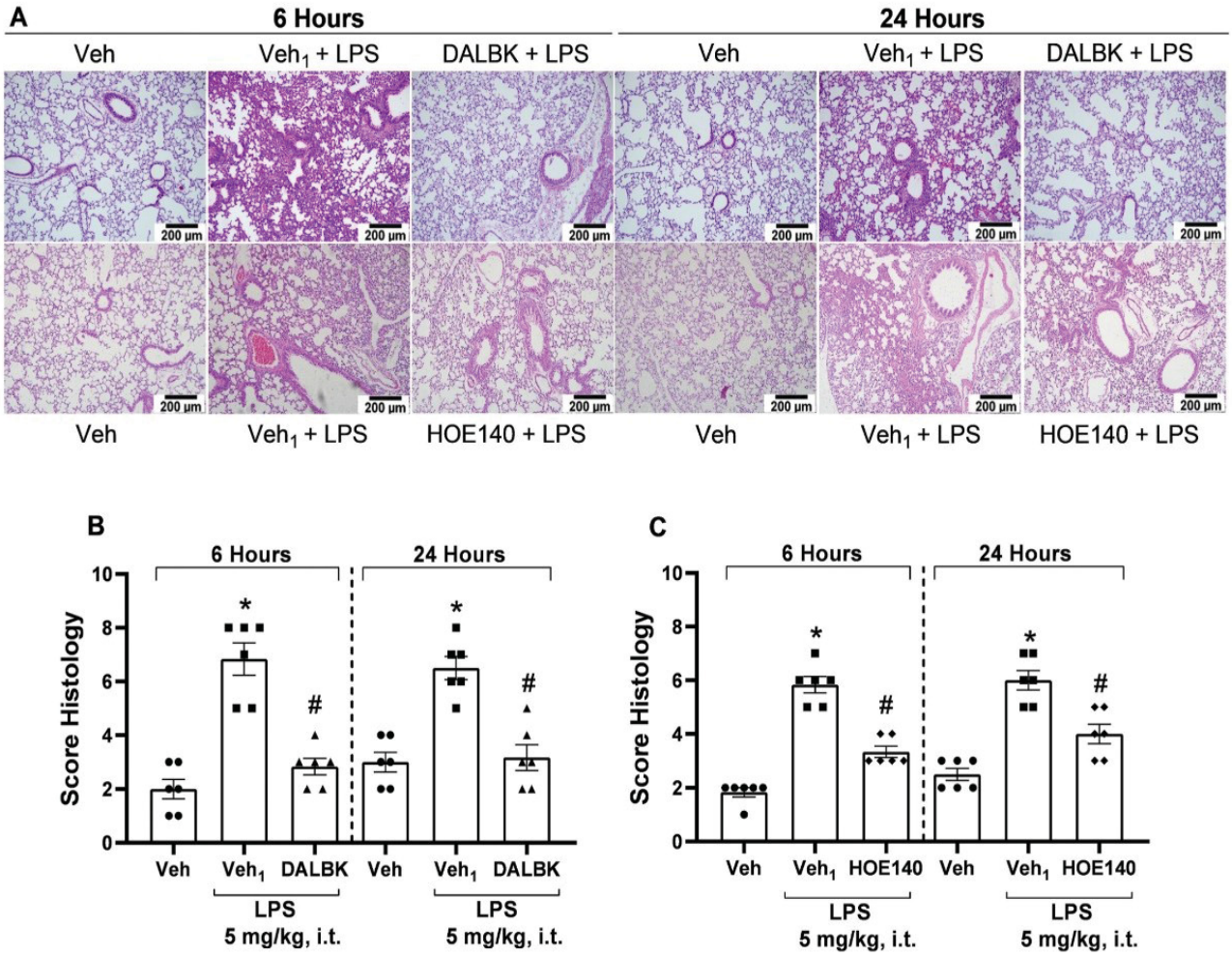


Fig. 4. Effects of B_1 (DALBK) and B_2 (HOE 140) receptor antagonists on histological changes at 6 and 24 h after the intratracheal administration of lipopolysaccharide (LPS) in mice. (A) Representative light microphotograph of histological alterations and (B, C) histological average scores in the lung tissue of mice pretreated with DALBK or HOE 140 at 6 and 24 h after LPS administration. Each point represents the mean (standard error of the mean, SEM) of six mice. $^{*}P \leq 0.05$ compared with the vehicle treated group (Veh), $^{\#}P \leq 0.05$ compared with the vehicle of antagonists + LPS-treated group (Veh1) (one-way ANOVA followed by Student–Newman–Keuls test).

3.7. B_1 and B_2 receptors are involved in upregulating the expression of TRPV1 channel in the lungs of mice with LPS-induced ALI

The results depicted in Fig. 7A–C demonstrate the significant upregulation of TRPV1 at 6 and 24 h after treatment with LPS. However, pretreatment with DALBK (50 nmol/kg, i.p.) completely abolished this upregulation at both analysis time points of 6 and 24 h following LPS administration (arbitrary unit: 3.28 ± 0.38 and 3.39 ± 0.35, respectively) compared with that in the respective vehicle-treated group (arbitrary unit: 8.70 ± 0.51 and 6.79 ± 0.61, respectively). The TRPV1 expression levels after treatment with DALBK were similar to those observed in the group treated only with the vehicle of DALBK and LPS at both time points of 6 and 24 h after administration (arbitrary unit: 3.42 ± 0.30 and 3.50 ± 0.32, respectively) (Fig. 7A and B).

Similar results were obtained following the pretreatment of animals with HOE 140. Thus, pretreatment with B_2 antagonist HOE 140 also prevented the upregulation of TRPV1 at 6 (arbitrary unit: 3.67 ± 0.41) and 24 h (arbitrary unit: 2.90 ± 0.57) after LPS-induced ALI compared with that in the respective vehicle-treated group (arbitrary unit: 6.71 ± 0.69 and 6.18 ± 0.24, respectively) (Fig. 7A–C).

3.8. B_1 and B_2 receptors and TRPV1 contribute to cytokine release within the bronchoalveolar space in mice with LPS-induced ALI

Fig. 8 shows the increased levels of inflammatory cytokines (IL-1β, IL-6, IL-10, IL-17, IL-23, TNF-α, and IFN-γ) and anti-inflammatory IL-10 in the bronchoalveolar space in mice subjected LPS-induced ALI. The pretreatment of mice with capsazepine, DALBK, or HOE 140, effectively prevented the increase in cytokine levels in the BALF of mice subjected LPS-induced ALI (Fig. 8). Although not statistically significant, a decrease in the levels of the anti-inflammatory cytokine IL-10 was also observed in mice pretreated with capsazepine, DALBK, or HOE 140 before LPS administration for ALI induction.

4. Discussion

ARDS is a common cause of respiratory failure observed in critically ill patients. Although the characteristics of ARDS are already defined in humans, a lack of consensus regarding the main characteristics of this disease exists in animal models, making preclinical studies difficult. Although the animal model of ALI does not fully replicate all features of ARDS, it is widely accepted as an experimental model for this disease [41,42].

According to the American Thoracic Society, animal models of ALI

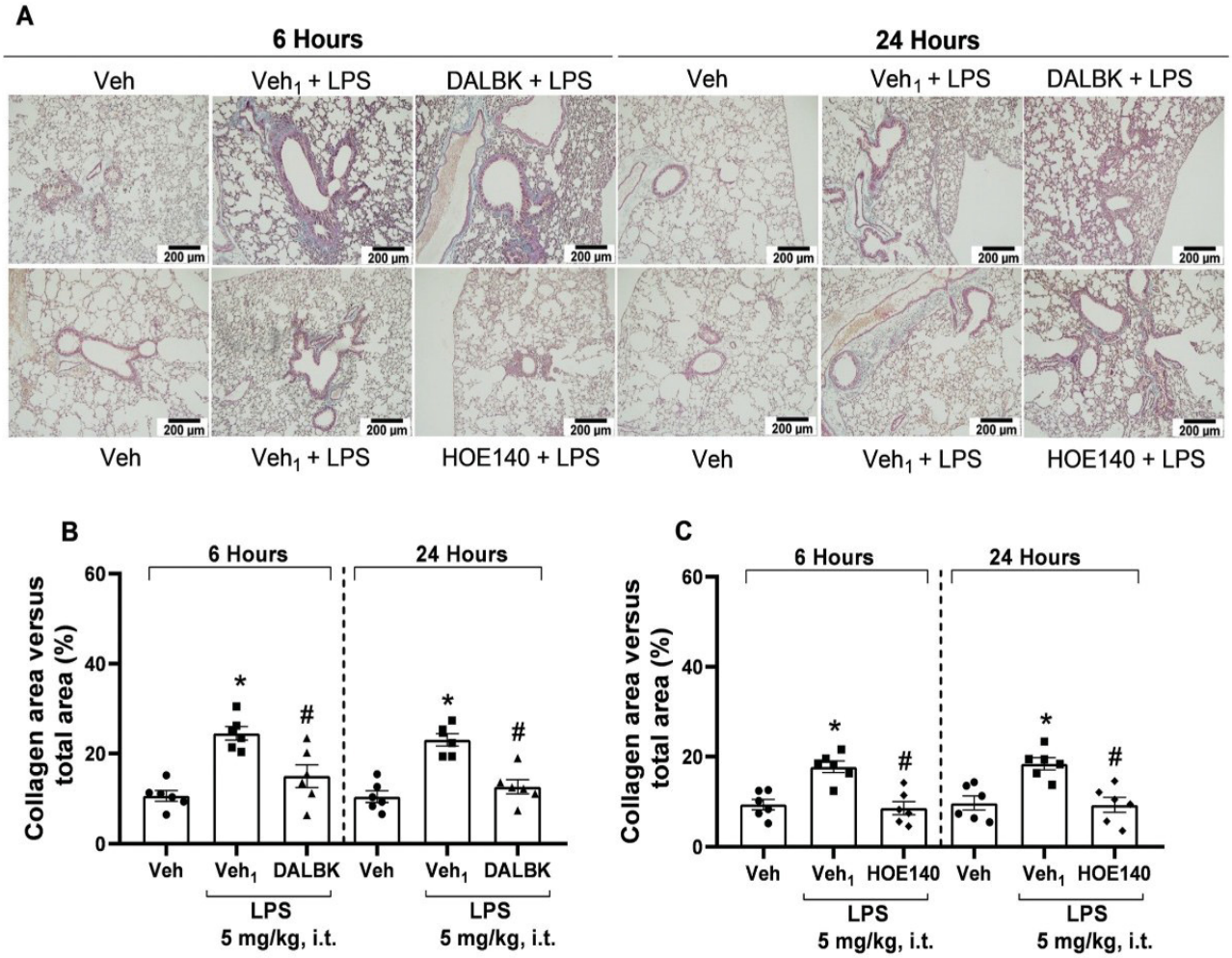


Fig. 5. Effects of B_1 (DALBK) and B_2 (HOE 140) receptor antagonists on collagen deposition at 6 and 24 h after the intratracheal administration of lipopolysaccharide (LPS) in mice. (A) Representative light microphotograph of collagen deposition in the lungs of mice pretreated with DALBK, HOE 140, or their vehicles and collected at 6 and 24 h after treatment with LPS. (B, C) Quantification of collagen deposition is presented as the percentage (%) of collagen area versus the total stained area. Each point represents the mean (standard error of the mean, SEM) of six mice. $^{*}P \leq 0.05$ compared with the vehicle-treated group (Veh), $^{\#}P \leq 0.05$ compared with the vehicle of antagonists + LPS-treated group (Veh1) (one-way ANOVA followed by Student–Newman–Keuls test).

must present, in addition to a rapid onset within 24 h, at least three of the four main characteristics: histological evidence of tissue injury, disruption of the alveolar-capillary barrier, presence of an inflammatory response, and signs of physiological dysfunction [41,42]. In our experimental model based on using LPS for ALI induction, we observed a rapid onset of ALI (within 6 and 24 h following LPS administration), an effect that was characterised by histopathological changes, such as increased lung injury score and collagen deposition. Lung injury was evident with 5 and 10 mg/kg LPS at early time points, whereas the 2 mg/kg dose induced detectable changes only after 24 h. This delay may reflect differences in mouse strains and experimental protocols, as most studies using 2 mg/kg assess outcomes only at 24 h after LPS administration [27–29]. Although the response to 10 mg/kg seemed less strong than to 5 mg/kg, this difference was not statistically significant. This reduction might be due to compensatory mechanisms like endotoxin tolerance, reported in mouse models with high LPS doses [43].

In our study, we observed significant changes in the alveolar-capillary barrier, with marked pulmonary oedema, increased in the concentration of bronchoalveolar proteins, cellular infiltration and increased cytokine levels. Moreover, the animals also showed a loss of body weight 24 h after LPS administration. This weight loss was not related to the surgical procedure on the trachea, as the animals that received the intratracheal injection of the vehicle did not show body weight loss.

The results obtained in this study confirmed and extended the role of TRPV1 channel protein in the development of LPS-induced ALI in mice. Several studies have highlighted the role of TRPV1 in respiratory diseases. The increased expression and activity of TRPV1 in pulmonary afferent nerves and bronchial epithelial cells have been reported in pathological conditions, such as allergic rhinitis, viral infections, chronic cough, asthma, COPD, and other pulmonary diseases, including lung injury in the cases of sepsis, burn injury, and exposure to LPS [7,44,45]. Moreover, using the same LPS-induced ALI model, some researchers reported that inhibiting or desensitising TRPV1 channels attenuates the effects of pulmonary injury [13,46,47].

Our results demonstrate physiological alterations in LPS-induced ALI, characterised by histopathological alterations, collagen deposition, increased inflammatory cell infiltration, protein extravasation, pulmonary oedema, and changes in body weight. All the above-mentioned effects were reversed or abolished by the pharmacological blockade of TRPV1 channels with capsazepine. Although administered systemically, capsazepine reduced pulmonary oedema and prevented weight loss, likely through local actions mediated by TRPV1 in pulmonary sensory nerves, as the injury was induced via intratracheal instillation. However, systemic effects cannot be ruled out, as TRPV1 is also present in structures [7] involved in appetite and metabolic control,

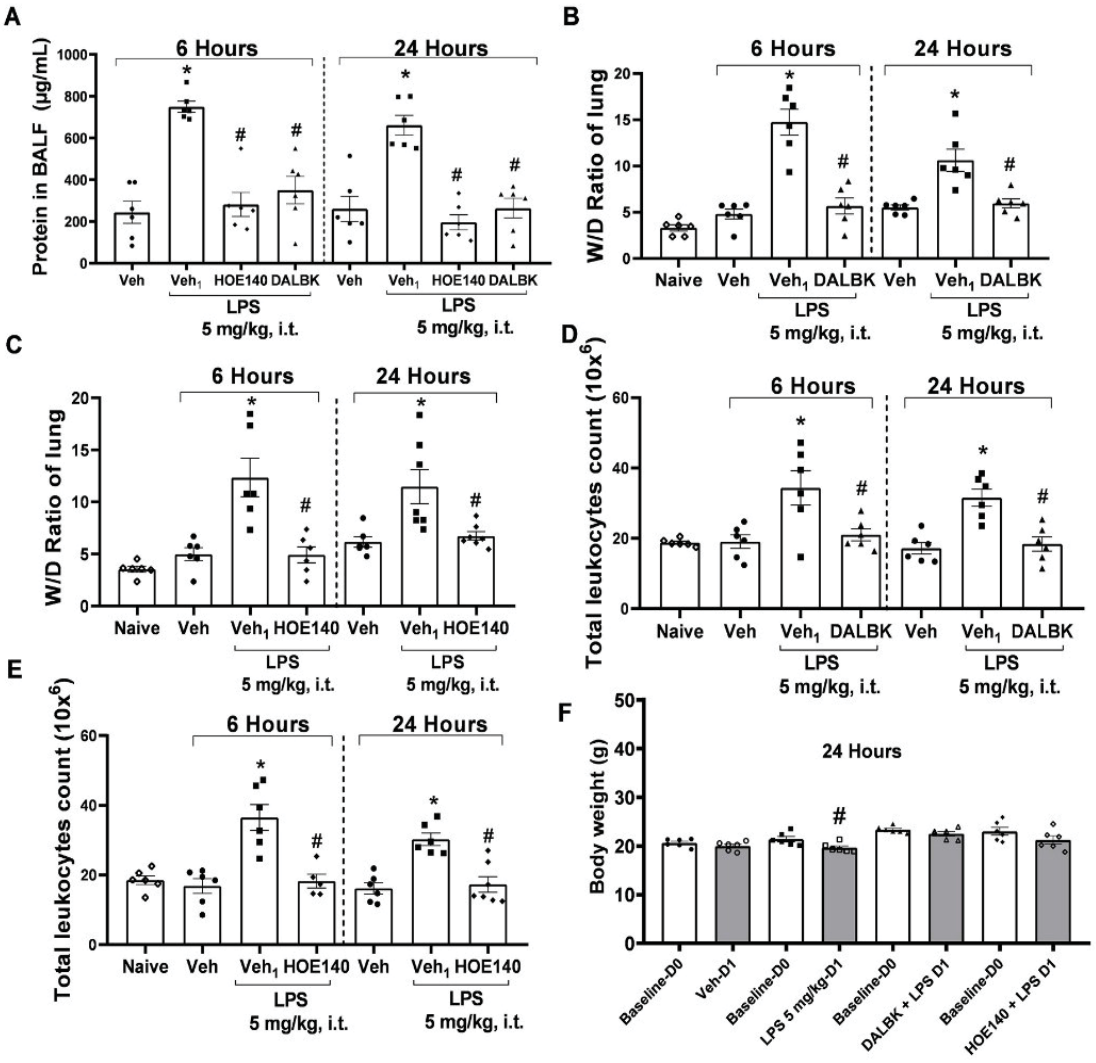


Fig. 6. Effects of B_1 (DALBK) and B_2 (HOE 140) receptor antagonists on protein content, lung weight, leukocyte infiltration and body weight at 6 and 24 h after the intratracheal administration of lipopolysaccharide (LPS) in mice. (A) Quantification of total protein content in bronchoalveolar lavage fluid (BALF). (B, C) Wet/Dry (W/D) weight ratio of lung. (D, E) Leukocyte infiltration in BALF. (F) Alteration in body weight in mice pretreated with DALBK, HOE 140, or their vehicles and collected at 6 or 24 h after treatment with LPS. The W/D ratio was estimated using the following formula: weight of the wet lung (mg) – weight of the dry lung (mg)/ body weight (g). Each point represents the mean (standard error of the mean, SEM) of six mice. $^{*}P \leq 0.05$ compared with the vehicle-treated group (Veh), $^{\#}P \leq 0.05$ compared with the vehicle of antagonists + LPS-treated group (Veh1) (one-way ANOVA followed by Student–Newman–Keuls test) or compared with the LPS baseline-D0 group, (two-way ANOVA applied to body weight changes, followed by Tukey's test for post-hoc analysis).

which may explain the observed weight recovery. Additionally, we observed an increase in TRPV1 expression in the lungs of mice subjected LPS-induced ALI, which was prevented by capsazepine pretreatment. These findings suggest that TRPV1 upregulation is involved in the pathophysiological changes associated with this model.

Although we did not directly assess TRPV1 functional activation, the observed upregulation, together with its established role in inflammation, supports its contribution to ALI and highlights its potential as a therapeutic target.

Previous studies have also shown that endogenous mediators, such as bradykinin, histamine, calcitonin gene-related peptide (CGRP), and substance P, can sensitise TRPV1 channels or induce the expression of TRPV1 channel protein (for review see: [46]). Once upregulated, the receptor becomes relatively more susceptible to endogenous stimuli because of a lowered activation threshold.

Bradykinin does not directly activate TRPV1 but sensitizes it through B_1 and B_2 receptor activation, which initiates intracellular signaling via PLC, PKC, and PKA, leading to phosphorylation of TRPV1 and a reduction in its activation threshold [20–22,55]. Additionally, PKC may stimulate phospholipase A_2, generating lipoxygenase-derived metabolites that further activate TRPV1. Once activated, TRPV1 promotes calcium influx and activate signaling pathways such as PKC, MAPK, and NF-κB, which lead to the production of pro-inflammatory cytokines like TNF-α, IL-6, and IL-1β [12,48] and the release of neuropeptides such as substance P and CGRP, increasing vascular permeability and leukocyte infiltration that can further stimulate bradykinin release. This creates a self-sustaining inflammatory cycle, potentially upregulating TRPV1 expression, further amplifying the response.

In the airways, this mechanism may be associated with a risk of respiratory disease complications [48]. Based on both the literature and our findings, we hypothesize that this mechanism plays a role in the development of ALI and may also be relevant in the context of ARDS. Thus, TRPV1 represents a promising therapeutic target for patients with ARDS.

In addition to TRPV1, other mediators in this inflammatory cascade, such as bradykinin, may represent additional therapeutic targets. Since it is established that bradykinin can lead to the sensitisation of TRPV1 channels or the alteration of TRPV1 expression, we hypothesized that B_1

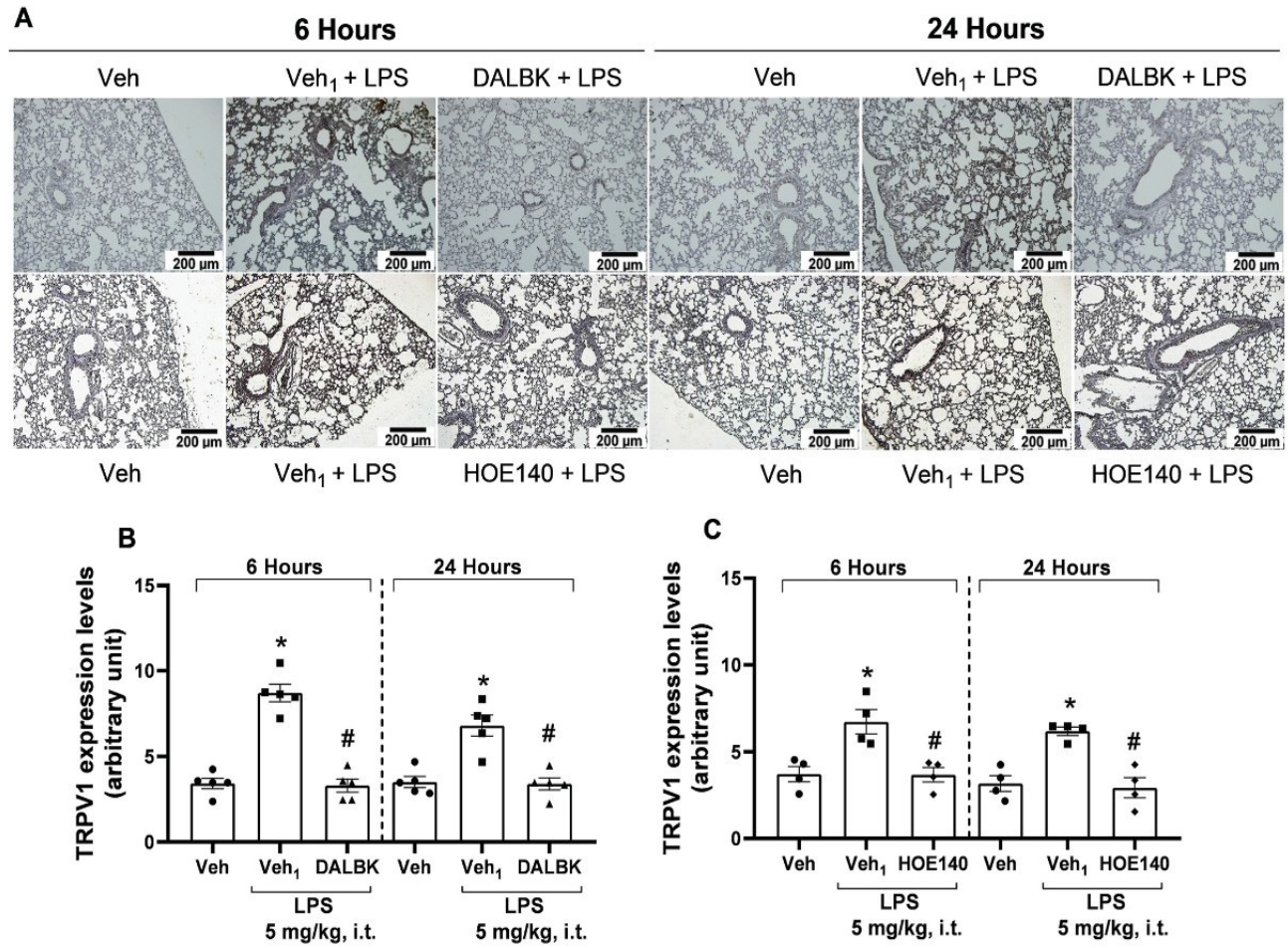


Fig. 7. Effects of DALBK and HOE 140 in the immunoreactivity of TRPV1 in the lungs of mice at 6 and 24 h after the intratracheal administration of lipopolysaccharide (LPS) in mice. (A) Representative light microphotograph of immunohistochemical staining and (B, C) quantification of TRPV1 channel expression in the lungs of mice pretreated with DALBK or HOE 140, collected at 6 and 24 h after LPS administration. Each point represents the mean (standard error of the mean, SEM) of five to six mice. $^{*}P \leq 0.05$ compared with the vehicle-treated group (Veh), $^{\#}P \leq 0.05$ compared with the vehicle of antagonists + LPS-treated group (Veh1) (one-way ANOVA followed by Student–Newman–Keuls test).

and B_2 bradykinin receptors could be involved in mediating the increased TRPV1 levels in mice subjected LPS-induced ALI. The present results clearly demonstrate that both B_1 and B_2 bradykinin receptors play significant roles in the development of LPS-induced ALI. This argument is supported by the results demonstrating that pretreatment with antagonists for these receptors not only reduced inflammatory marker concentrations and lung injury but also prevented weight loss in mice. In addition, we report that both the pharmacological blockade of TRPV1 channels and the inhibition of B_1 and B_2 receptors inhibited the upregulation of TRPV1 expression.

In this context, it has been shown that TRPV1 can be activated by inflammatory mediators including kinins, which play a key role in vascular leakage and oedema formation in pulmonary diseases [21,22,24,50]. Therefore, it is plausible that the beneficial effects observed with B_1 and B_2 receptor antagonists may, at part, be attributed to a reduced TRPV1 activation, as supported by our data demonstrating that these antagonists prevent the rise in TRPV1 expression. Although the definitive confirmation of pharmacological target specificity would require additional approaches such as the use of alternative antagonists, knockout models, or genetic manipulation, our findings, supported by the use of doses within the selective range described in the literature [17,51–53], the consistent effects observed, and the reduction in TRPV1 expression after antagonist treatment point to the functional involvement of these receptors. In addition, previous studies suggest that TRPV1 is activated as a result of bradykinin receptor stimulation during inflammation. Therefore, even if intermediate signaling pathways are involved, B_1 and B_2 receptors and TRPV1 likely act early in this inflammatory cascade, supporting their relevance as primary therapeutic targets.

On the other hand, although the present study demonstrates overlapping anti-inflammatory effects of TRPV1 and bradykinin receptor antagonists, these findings should be interpreted as evidence of an indirect functional relationship rather than a direct molecular interaction. Indeed, according to Calzetta et al. [54], demonstrating pharmacological interaction or synergy requires formal analyses using predictive models and mathematical validation. In contrast, a functional association refers to a scenario in which receptors or pathways influence each other's activity or jointly contribute to a biological outcome, even in the absence of direct binding or synergistic amplification.

In this sense, our results are consistent with this proposed mechanism, suggesting that bradykinin receptor activation contributes to TRPV1 expression and activity during LPS-induced lung injury. Critically, this cycle is reinforced by cytokine release (e.g., IL-1β, TNF-α) triggered by TRPV1 and bradykinin receptor activation, which further sensitizes TRPV1 channels [49,56,57].

The elevated levels of these cytokines are associated with lung injuries, such as ARDS, and increased disease severity [58–61]. It has been proposed that cytokine and bradykinin storms could be among the main causes of ARDS and multiple organ failure [60–62].

In our experimental model, cytokine profile alterations were

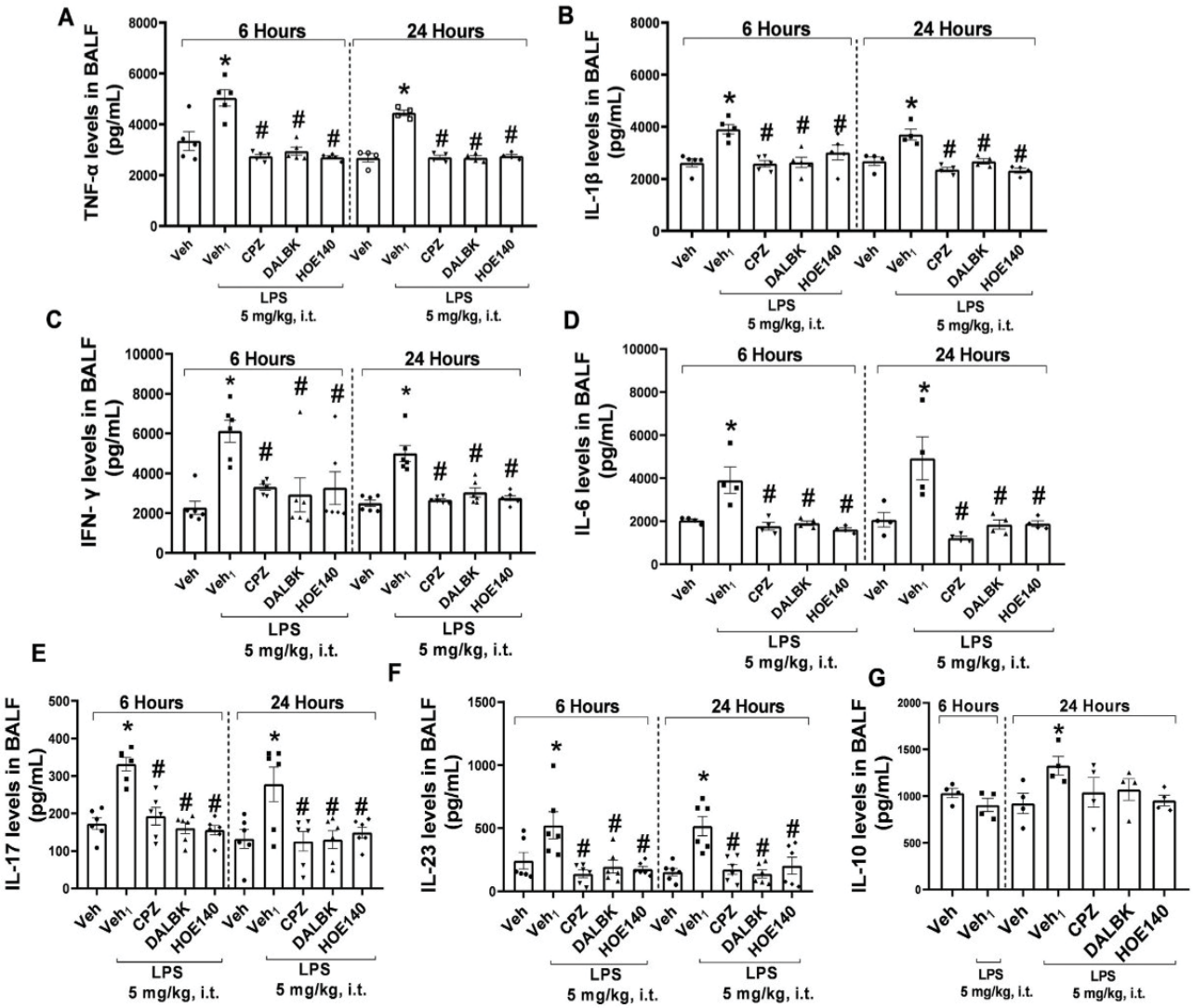


Fig. 8. Effects of capsazepine, DALBK, or HOE140 treatments on the levels of inflammatory cytokines (interleukin [IL]-1β, IL-6, IL-10, IL-17, IL-23, tumour necrosis factor alpha [TNF-α], and interferon gamma [IFN-γ]) and anti-inflammatory IL-10 in the bronchoalveolar space in mice with lipopolysaccharide LPS-induced acute lung injury. Cytokine concentrations are expressed in pg/mL, and the assay was performed on the BALF. Each point represents the mean (standard error of the mean, SEM) of four to six mice. *$P \leq 0.05$ compared with the vehicle-treated group (Veh), #$P \leq 0.05$ compared with the LPS-treated group (Veh1) (one-way ANOVA followed by Student–Newman–Keuls test).

observed, demonstrating that LPS-induced ALI leads to increased levels of IL-1β, IL-6, IL-10, IL-17, IL-23, TNF-α, IFN-γ. On the other hand, our data showed that IL-10 levels did not increase significantly 6 h after LPS administration but increased at 24 h. This pattern is consistent with previous studies reporting a delayed IL-10 response, with a small increase around 6–7 h and peaking between 24 and 48 h [63,64]. Therefore, we believe that the lack of change at early time points reflects a delayed anti-inflammatory response. Furthermore, the antagonists we tested reduced the IL-10 increase at 24 h, suggesting that they also affect the resolution phase of inflammation. Overall, our findings support a role for IL-10 in modulating the inflammatory response, although with a delayed onset.

It has been demonstrated that cytokines such as TNF-α rapidly sensitise TRPV1 activity, enhancing Ca^{2+} influx. This effect appears to be mediated by p38 mitogen-activated protein kinase and the c-Jun N-terminal kinase signaling pathway [49,65]. Accordingly, it can be hypothesized that these molecular events contribute to a self-sustaining inflammatory loop, potentially explaining why the bradykinin and cytokine storms characteristic of clinical ARDS [58] exert such profound pathological effects.

This self-sustaining inflammatory cycle, mediated by the functional interaction between bradykinin and TRPV1, suggests that therapeutic targeting of these pathways may interrupt the pathological cascade by reducing vascular permeability, leukocyte infiltration, and the release of pro-inflammatory cytokines and other mediators.

Thus, as illustrated in Fig. 9, our results show that pharmacological blockade of TRPV1 channels and inhibition of B_1 and B_2 receptors not only prevented the increase in inflammatory cytokines but also attenuated other inflammatory parameters. Although our study did not explore direct molecular interactions (e.g., co-immunoprecipitation) or pharmacological synergy (e.g., isobolographic analysis), the observed functional link between bradykinin receptors and TRPV1 channels highlights their potential relevance in pathophysiology in lung injury. These findings suggest that simultaneous targeting of both receptors may be beneficial not only in ALI but also in ARDS. On the other hand, we cannot rule out that TRPV1 interacts with other receptors, since TRPV1 does not act alone but works together with other ion channels like TRPA1 and TRPV4 [66,67], as well as intracellular pathways triggered by mediators such as prostaglandins, leukotrienes, and neurotrophic factors, which amplify inflammatory responses [68].

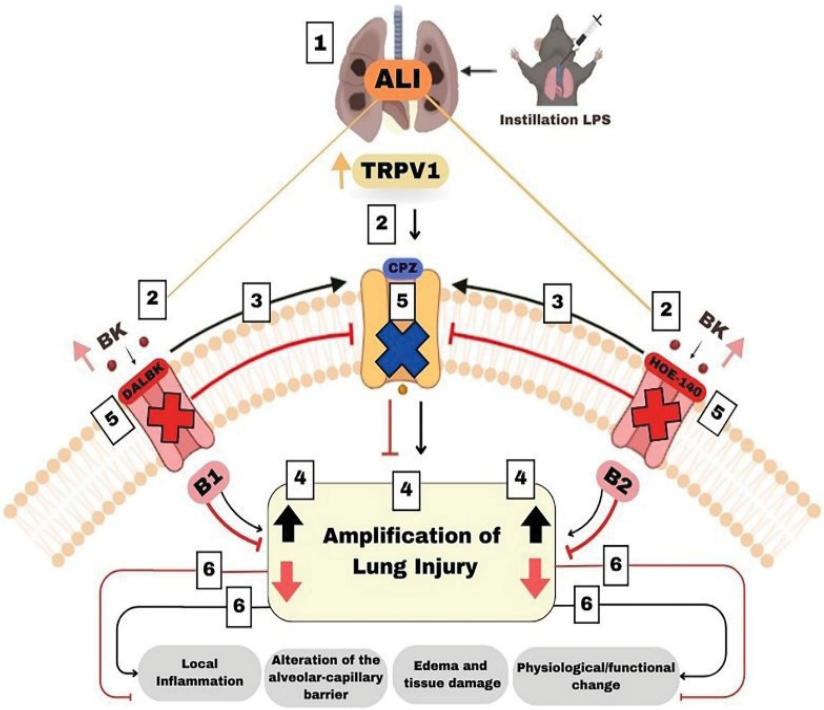


Fig. 9. Schematic illustration of the role of TRPV1 channel protein and B1 and B2 receptors in LPS-induced acute lung injury (ALI) in mice. The intratracheal administration of LPS can induce ALI (1). During ALI, B_1 and B_2 receptors and TRPV1 channels are activated (2), and this activation contributes to the development of ALI. Once activated, B_1 and B_2 receptors can indirectly sensitise and activate TRPV1 channels (3), lowering its activation threshold and promoting continuous activation, which contributes to the exacerbation and sustainment of the inflammatory response (4). Blocking these receptors (5), prevents the aggravation of lung injury (6), and attenuates the pathophysiological changes observed in LPS-induced ALI (6).

5. Conclusion

These findings reinforce previous evidence of a functional association between kinin B_1/B_2 receptor activation and TRPV1 channel activity, which may also depend on cytokine release, potentially establishing a feedback loop that sustains mediator production and recurrent TRPV1 stimulation.

In this context, the increased levels of these cytokines may additionally contribute to the maintenance and sustenance of the inflammatory response in our LPS-induced ALI mouse model. These observations suggest that simultaneous targeting of kinin receptors and TRPV1 channels may offer a promising pharmacological strategy to mitigate lung injury progression. Nonetheless, this study has certain limitations. Although the LPS-induced acute lung injury (ALI) model is widely used, it does not fully replicate the complexity of acute respiratory distress syndrome (ARDS) in humans, which is multifactorial and often associated with sepsis, trauma, or pneumonia. The acute inflammatory response triggered by LPS may not encompass the full spectrum of human ARDS, which also involves proliferative and fibrotic phases. To enhance the translational relevance of these findings, future studies could incorporate comparisons with other experimental models, such as mechanical ventilation, ischemia-reperfusion, or acid-induced lung injury. Furthermore, to better investigate the potential additive or synergistic effects between TRPV1 antagonists and B_1/B_2 receptor blockers, future studies could use LPS-induced lung injury models with combined administration of these inhibitors. The individual and combined effects on inflammatory outcomes could then be compared. Using isobolographic analysis, as suggested by Ref. [54], would allow a quantitative assessment of whether the interaction between treatments is additive or synergistic.

CRediT authorship contribution statement

Mayara Alves Amorim: Writing – original draft, Validation, Methodology, Formal analysis. **Vitor Hélio Souza Oliveira:** Methodology, Formal analysis. **João B. Calixto:** Project administration, Funding acquisition. **Eunice André:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization.

Ethics approval

All experimental protocols were conducted in accordance with the guidelines approved by the Ethics and Animal Experimentation Committee (CEUA) of the UFPR under number 1432/2021 - 1602/2024 and were compliant with the ARRIVE guidelines, as previously outlined by Percie du Sert et al. (2020).

Funding

This work was supported by CNPq (Productivity in Research (PQ) fellowship number 307183/2021-1) and grants from INCT-INOVAMED (465430/2014-7).

Declaration of competing interest

The authors have no conflicts of interest to disclose.

Acknowledgements

Amorim M.A. and Oliveira V.H.S. are PhD students in Pharmacology and thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for their fellowship support. Authors would like to thank to the Centro de Tecnologias Avançadas em Fluorescência (CTAF-UFPR) for their microscopy services.

Data availability

Data will be made available on request.

References

[1] M.A. Matthay, R.L. Zemans, G.A. Zimmerman, Y.M. Arabi, J.R. Beitler, A. Mercat, M. Herridge, A.G. Randolph, C.S. Calfee, Acute respiratory distress syndrome, Nat. Rev. Dis. Primers 5 (2019) 18, https://doi.org/10.1038/s41572-019-0069-0.

[2] F.R. D'Alessio, Mouse models of acute lung injury and ARDS, Methods Mol. Biol. 1809 (2018) 341–350, https://doi.org/10.1007/978-1-4939-8570-8_22.

[3] M. Diamond, H.L.P. Feliciano, D. Sanghavi, S. Mahapatra, Acute Respiratory Distress Syndrome (ARDS), StatsPearls. Treasure Island (FL), StatPearls Publishing, 2023.

[4] K. Raghavendran, L.M. Napolitano, Definition of ALI/ARDS, Crit. Care Clin. 27 (2011) 429–437, https://doi.org/10.1016/j.ccc.2011.05.006.

[5] A. Saguil, M.V. Fargo, Acute respiratory distress syndrome: diagnosis and management, Am. Fam. Physician 101 (2020) 730–738. PMID: 32538594.

[6] M.A. Wortley, M.A. Birrell, M.G. Belvisi, Drugs affecting TRP channels, Handb. Exp. Pharmacol. 237 (2017) 213–241, https://doi.org/10.1007/164_2016_63.

[7] F. De Logu, R. Patacchini, G. Fontana, G. Geppetti, TRP functions in the broncho-pulmonary system, Semin. Immunopathol. 38 (2016) 321–329, https://doi.org/10.1007/s00281-016-0557-1.

[8] J.H. Kim, Allergy Asthma Immunol. Res. 10 (2018) 187–188, https://doi.org/10.4168/aair.2018.10.3.187.

[9] Q. Du, Q. Liao, C. Chen, X. Yang, R. Xie, J. Xu J, The role of transient receptor potential vanilloid 1 in common diseases of the digestive tract and the cardiovascular and respiratory system, Front. Physiol. 21 (2019) 1064, https://doi.org/10.3389/fphys.2019.01064.11.

[10] A. Dietrich, Transient receptor potential (TRP) channels in health and disease, Cells 8 (2019) 413, https://doi.org/10.3390/cells8050413.

[11] H. Wallace, Airway pathogenesis is linked to TRP channels, in: T.L.R. Emir (Ed.), Neurobiology of TRP Channels, vol. 13, 2017, pp. 251–264, https://doi.org/10.4324/9781315152837-13, 10.4324/9781315152837-13.

[12] A.P. Koivisto, M.G. Belvisi, R. Gaudet, A. Szallasi, Advances in TRP channel drug discovery: from target validation to clinical studies, Nat. Rev. Drug Discov. 21 (2022) 41–59, https://doi.org/10.1038/s41573-021-00268-4.

[13] L.D. Cabral, A. Giusti-Paiva, The transient receptor potential vanilloid 1 antagonist capsazepine improves the paired lung mechanics during endotoxemia, Basic Clin. Pharmacol. Toxicol. 119 (2016) 421–427, https://doi.org/10.1111/bcpt.12605.

[14] D.A.B. Rex, N. Vaid, K. Deepak, S. Dagamajalu, T.S.K. Prasad, A comprehensive review on current understanding of bradykinin in COVID-19 and inflammatory diseases, Mol. Biol. Rep. 49 (2022) 9915–9927, https://doi.org/10.1007/s11033-022-07539-2.

[15] C.P. Sodhi, C. Wohlford-Lenane, Y. Yamaguchi, T. Prindle, W.B. Fulton, S. Wang, P. B. McCray, P.B. Jr, M. Chappell, D.J. Hackam, H. Jia, Attenuation of pulmonary ACE2 activity impairs inactivation of des-Arg9 bradykinin/BKB1R axis and facilitates LPS-induced neutrophil infiltration, Am. J. Physiol. Lung Cell. Mol. Physiol. 314 (2018) 17–31, https://doi.org/10.1152/ajplung.00498.2016.

[16] W.M. Abraham, M. Scuri, S.G. Farmer, Peptide and non-peptide bradykinin receptor antagonists: role in allergic airway disease, Eur. J. Pharmacol. 533 (2006) 215–221, https://doi.org/10.1016/j.ejphar.2005.12.071.

[17] J.B. Calixto, D.A. Cabrini, J. Ferreira, M.M. Campos, Inflammatory pain: kinins and antagonists, Curr. Opin. Anaesthesiol. 14 (2001) 519–526, https://doi.org/10.1097/00001503-200110000-00010.

[18] J.B. Calixto, R. Medeiros, E.S. Fernandes, J. Ferreira, D.A. Cabrini, M.M. Campos, Kinin B1 receptors: key G-protein-coupled receptors and their role in inflammatory and painful processes, Br. J. Pharmacol. 143 (2004) 803–818, https://doi.org/10.1038/sj.bjp.0706012.

[19] B. Székács, S. Várbíró, L. Debreczeni L, High-dose ACEi might be harmful in COVID 19 patients with serious respiratory distress syndrome by leading to excessive bradykinin receptor activation, Phys. Int. 108 (2021) 1–9, https://doi.org/10.1556/2060.2021.00007.

[20] J. Ferreira, G.L. da Silva, J.B. Calixto, Contribution of vanilloid receptors to the overt nociception induced by B2 kinin receptor activation in mice, Br. J. Pharmacol. 141 (2004) 787–794, https://doi.org/10.1038/sj.bjp.0705546.

[21] F. Al-Shamlan, A.Z. El-Hashim, Bradykinin sensitizes the cough reflex via a B_2 receptor dependent activation of TRPV1 and TRPA1 channels through metabolites of cyclooxygenase and 12-lipoxygenase, Respir. Res. 20 (2019) 110, https://doi.org/10.1186/s12931-019-1060-8.

[22] V. Cernit, J. Sénécal, R. Othman, R. Couture, Reciprocal regulatory interaction between TRPV1 and Kinin B1 receptor in a rat neuropathic pain model, Int. J. Mol. Sci. 21 (2020) 821, https://doi.org/10.3390/ijms21030821.

[23] M.A. Amorim, J.R. Jentsch Matias de Oliveira, V.H.S. Oliveira, D.A. Cabrini, M. F. Otuki, E. André, Role of nitric oxide, bradykinin B_2 receptor, and TRPV1 in the airway alterations caused by simvastatin in rats, Eur. J. Pharmacol. 912 (2021) 174591, https://doi.org/10.1016/j.ejphar.2021.174591.

[24] J.R. de Oliveira, M.F. Otuki, D.A. Cabrini, I. Brusco, S.M. Oliveira, J. Ferreira, E. André, Involvement of the TRPV1 receptor in plasma extravasation in airways of rats treated with an angiotensin-converting enzyme inhibitor, Pulm. Pharmacol. Ther. 41 (2016) 25–33, https://doi.org/10.1016/j.pupt.2016.09.001.

[25] N.P. Sert, A. Ahluwalia, S. Alam, M.T. Avey, M. Baker, W.J. Browne, A. Clark, I. C. Cuthill, U. Dirnagl, M. Emerson, P. Garner, S.T. Holgate, D.W. Howells, V. Hurst, N.A. Karp, S.E. Lazic, K. Lidster, C.J. MacCallum, M. Macleod, E.J. Pearl, O. H. Petersen, F. Rawle, P. Reynolds, K. Rooney, E.S. Sena, S.D. Silberberg, T. Steckler, H. Würbel, Reporting animal research: explanation and elaboration for the arrive guidelines 2.0, PLoS Biol. (2020), https://doi.org/10.1371/journal.pbio.3000411.

[26] Y. Duan, J. Learoyd, A.Y. Meliton, A.R. Leff, X. Zhu, Inhibition of Pyk2 blocks lung inflammation and injury in a mouse model of acute lung injury, Respir. Res. 13 (2012) 4, https://doi.org/10.1186/1465-9921-13-4.

[27] N. Honari, P. Shaban, S. Nasseri, M. Hosseini, Ethanolic extract of *Achillea wilhelmsii* C. Koch improves pulmonary function and inflammation in LPS-induced acute lung injury mice, J. Compl. Integr. Med. 19 (2021) 261–267, https://doi.org/10.1515/jcim-2021-0045.

[28] N. Nguyen, S. Xu, T.Y.W. Lam, W. Liao, W.S.F. Wong, R. Ge, ISM1 suppresses LPS-induced acute lung injury and post-injury lung fibrosis in mice, Mol. Med. 28 (2022) 72, https://doi.org/10.1186/s10020-022-00500-w.

[29] R. Rafikov, C. Dimitropoulou, S. Aggarwal, A. Kangath, C. Gross, D. Pardo, S. Sharma, A. Jezierska Drutel, V. Patel, C. Snead, R. Lucas, A. Verin, D. Fulton, J. D. Catravas, S.M. Black, Lipopolysaccharide-induced lung injury involves the nitration-mediated activation of RhoA, J. Biol. Chem. 289 (2014) 4710–4722, https://doi.org/10.1074/jbc.M114.547596.

[30] L. Tao, F. Cao, G. Xu, H. Xie, M. Zhang, C. Zhang, Mogroside IIIE attenuates LPS-Induced acute lung injury in mice partly through regulation of the TLR4/MAPK/NF-κB axis via AMPK activation, Phytother Res. 7 (2017) 1097–1106, https://doi.org/10.1002/ptr.5833.

[31] M. Rigoni, M. Trevisani, D. Gazzieri, R. Nadaletto, M. Tognetto, C. Creminon, J. B. Davis, B. Campi, S. Amadesi, P. Geppetti, S. Harrison, Neurogenic responses mediated by vanilloid receptor-1 (TRPV1) are blocked by the high affinity antagonist, iodo-resiniferatoxin, Br. J. Pharmacol. 138 (2003) 977–985, https://doi.org/10.1038/sj.bjp.0705110.

[32] R.O. De Campos, R.V. Alves, D.J. Kyle, S. Chakravarty, B.J. Mavunkel, J.B. Calixto, Antioedematogenic and antinociceptive actions of NPC 18521, a novel bradykinin B2 receptor antagonista, Eur. J. Pharmacol. 316 (1996) 277–286, https://doi.org/10.1016/s0014-2999(96)00661-9.

[33] R.C. Dutra, D.F. Leite, A.F. Bento, M.N. Manjavachi, E.S. Patrício, C.P. Figueiredo, J.B. Pesquero, J.B. Calixto, The role of kinin receptors in preventing neuroinflammation and its clinical severity during experimental autoimmune encephalomyelitis in mice, PLoS One 6 (2011) 27875, https://doi.org/10.1371/journal.pone.0027875.

[34] X. An, X. Sun, Y. Hou, X. Yang, H. Chen, P. Zhang, J. Wu, Protective effect of oxytocin on LPS-induced acute lung injury in mice, Sci. Rep. 9 (2019) 2836, https://doi.org/10.1038/s41598-019-393491.

[35] Y. Geng, S. Su, L. Cao, Y. Yang, Effect of PD 1 inhibitor combined with X-Ray irradiation on the inflammatory microenvironment and lung tissue injury in mice, J. Inflamm. Res. 15 (2022) 545–556, https://doi.org/10.2147/JIR.S350112.

[36] S. Seger, M. Stritt, E. Vezzali, O. Nayler, P. Hess, P.M.A. Groenen, A.K. Stalder, A fully automated image analysis method to quantify lung fibrosis in the bleomycin-induced rat model, PLoS One 13 (2018) 193057, https://doi.org/10.1371/journal.pone.0193057.

[37] K. Inoue, H. Takano, R. Yanagisawa, S. Hirano, T. Kobayashi, T. Ichinose, T. Yoshikawa, Effects of organic chemicals derived from ambient particulate matter on lung inflammation related to lipopolysaccharide, Arch. Toxicol. 80 (12) (2006) 833–838, https://doi.org/10.1007/s00204-006-0105-1.

[38] H.H. Yang, J.X. Duan, S.K. Liu, J.B. Xiong, X.X. Guan, W.J. Zhong, C.C. Sun, C. Y. Zhang, X.Q. Luo, Y.F. Zhang, P. Chen, B.D. Hammock, S.H. Hwang, J.X. Jiang, Y. Zhou, C.X. Guan, A COX-2/sEH dual inhibitor PTUPB alleviates lipopolysaccharide-induced acute lung injury in mice by inhibiting NLRP3 inflammasome activation, Theranostics 10 (2020) 4749–4761, https://doi.org/10.7150/thno.43108.

[39] A.R. Crowe, W. Yue, Semi-quantitative determination of protein expression using immunohistochemistry staining and analysis: an integrated protocol, Bio. Protoc. 9 (2019) 3465, https://doi.org/10.21769/BioProtoc.3465.

[40] J.R.M.O. Jentsch, M.A. Amorim, E. André, The role of TRPA1 and TRPV4 channels in bronchoconstriction and plasma extravasation in airways of rats treated with captopril, Pulm. Pharmacol. Ther. 65 (2020) 102004, https://doi.org/10.1016/j.pupt.2021.102004.

[41] G. Matute Bello, G. Downey, B.B. Moore, S.D. Groshong, M.A. Matthay, A. S. Slutsky, W.M. Kuebler, Acute lung injury in animals study group. An official American thoracic society workshop report: features and measurements of experimental acute lung injury in animals, Am. J. Respir. Cell Mol. Biol. 44 (2011) 725–738, https://doi.org/10.1165/rcmb.2009-0210ST.

[42] H.S. Kulkarni, J.S. Lee, J.A. Bastarache, W.M. Kuebler, G.P. Downey, G. M. Albaiceta, W.A. Altemeier, A. Artigas, J.H.T. Bates, C.S. Calfee, C.S. Dela Cruz, R.P. Dickson, J.A. Englert, J.I. Everitt, M.B. Fessler, A.E. Gelman, K.M. Gowdy, S. D. Groshong, S. Herold, R.J. Homer, J.C. Horowitz, C.C.W. Hsia, K. Kurahashi, V. E. Laubach, M.R. Looney, R. Lucas, N.S. Mangalmurti, A.M. Manicome, T. R. Martin, S. Matalon, M.A. Matthay, D.F. McAuley, S.A. McGrath-Morrow, J. P. Mizgerd, S.A. Montgomery, B.B. Moore, A. Noël, C.E. Perlman, J.P. Reilly, E. P. Schmidt, S.J. Skerrett, T.L. Suber, C. Summers, B.T. Suratt, M. Takata, R. Tuder, S. Uhlig, M. Witzenrath, R.L. Zemans, G. Matute-Bello, Update on the features and measurements of experimental acute lung injury in animals: an official American thoracic society workshop report, Am. J. Respir. Cell Mol. Biol. 66 (2022) 1–14, https://doi.org/10.1165/rcmb.2021-0531ST.

[43] H. Fan, J.A. Cook, Molecular mechanisms of endotoxin tolerance, J. Endotoxin Res. 10 (2004) 71–84, https://doi.org/10.1179/096805104225003997.

[44] K.C. Thomas, J.K. Roberts, C.E. Deering-Rice, E.G. Romero, R.O. Dull, J. Lee, G. S. Yost, C.A. Reilly, Contributions of TRPV1, endovanilloids, and endoplasmic reticulum stress in lung cell death in vitro and lung injury, Am. J. Physiol. Lung

Cell. Mol. Physiol. 302 (2012) 111–119, https://doi.org/10.1152/ajplung.00231.2011.

[45] A. Nahama, R. Ramachandran, A.F. Cisternas, H. Ji, The role of afferent pulmonary innervation in ARDS associated with COVID-19 and potential use of resiniferatoxin to improve prognosis: a review, Med. Drug Discov. 5 (2020) 100033, https://doi.org/10.1016/j.medidd.2020.100033.

[46] M. Liu, X. Jia, H. Liu, R. He, X. Zhang, Y. Shao, Role of TRPV1 in respiratory disease and association with traditional Chinese medicine: a literature review, Biomed. Pharmacother. 155 (2022) 113676, https://doi.org/10.1016/j.biopha.2022.113676.

[47] Q. Hu, H. Liu, R. Wang, L. Yao, S. Chen, Y. Wang, Capsaicin attenuates LPS-induced acute lung injury by inhibiting inflammation and autophagy through regulation of the TRPV1/AKT pathway, J. Inflamm. Res. 17 (2024) 153–170, https://doi.org/10.2147/JIR.S441141.

[48] Z. Liu, P. Wang, S. Lu, R. Guo, W. Gao, H. Tong, Y. Yin, X. Han, T. Liu, X. Chen, M. X. Zhu, Z. Yang, Liquiritin, a novel inhibitor of TRPV1 and TRPA1, protects against LPS-induced acute lung injury, Cell Calcium 88 (2020) 102198, https://doi.org/10.1016/j.ceca.2020.102198.

[49] I. Devesa, R. Planells-Cases, G. Fernández-Ballester, J.M. González-Ros, A. Ferrer-Montiel, A. Fernández-Carvajal, Role of the transient receptor potential vanilloid 1 in inflammation and sepsis, J. Inflamm. Res. 4 (2011) 67–81, https://doi.org/10.2147/JIR.S12978.

[50] J. Chen, W. Sun, Y. Zhu, F. Zhao, S. Deng, M. Tian, Y. Wang, Y. Gong, TRPV1: the key bridge in neuroimmune interactions, J. Intens. Med. 4 (2024) 442–452, https://doi.org/10.1016/j.jointm.2024.01.008.

[51] M.M. Campos, G.E. Souza, J.B. Calixto, Role of bradykinin B1 and B2 receptors in inflammatory nociception: insights from experimental and clinical studies, Curr. Drug Targets 13 (2012) 312–323.

[52] R.J. Docherty, J.C. Yeats, S. Bevan, H.W. Boddeke, Inhibition of calcineurin inhibits the desensitization of capsaicin-evoked currents in cultured dorsal root ganglion neurons, Neuroscience 78 (1997) 877–886, https://doi.org/10.1007/s004240050074.

[53] K. Wirth, F.J. Hock, U. Albus, W. Linz, P.A. Martorana, B.A. Scholkens, J.P. Stasch, Hoe 140 a new potent and long acting bradykinin-antagonist: in vitro studies, Br. J. Pharmacol. 102 (1991) 774–777, https://doi.org/10.1111/j.1476-5381.1991.tb12249.x.

[54] L. Calzetta, C. Page, M.G. Matera, M. Cazzola, P. Rogliani, Drug-drug interactions and synergy: from pharmacological models to clinical application, Pharmacol. Rev. 76 (2024) 1159–1220, https://doi.org/10.1124/pharmrev.124.000951.

[55] H.H. Chuang, E.D. Prescott, H. Kong, S. Shields, S.E. Jordt, A.I. Basbaum, M. V. Chao, D. Julius, Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns (4,5) P2-mediated inhibition, Nature 411 (2001) 957–962, https://doi.org/10.1038/35082088.

[56] S.A. Wilczynski, C.F. Wenceslau, C.G. McCarthy, R.C. Webb, A cytokine/bradykinin storm comparison: what is the relationship between hypertension and COVID-19? Am. J. Hypertens. 34 (2021) 304–306, https://doi.org/10.1093/ajh/hpaa217.

[57] Y. Hu, Q. Gu, R.L. Lin, R. Kryscio, L.Y. Lee, Calcium transient evoked by TRPV1 activators is enhanced by tumor necrosis factor-{alpha} in rat pulmonary sensory neurons, Am. J. Physiol. Lung Cell. Mol. Physiol. 299 (2010) 483–492, https://doi.org/10.1152/ajplung.00111.2010.

[58] N. Potere, M.G. Del Buono, R. Caricchio, P.C. Cremer, A. Vecchié, E. Porreca, D. D. Gasperina, F. Dentali, A. Abbate, A. Bonaventura, Interleukin-1 and the NLRP3 inflammasome in COVID-19: pathogenetic and therapeutic implications, EBioMedicine 85 (2022) 104299, https://doi.org/10.1016/j.ebiom.2022.104299.

[59] W.Y. Park, R.B. Goodman, K.P. Steinberg, J.T. Ruzinski, F. Radella 2and, D.R. Park, J. Pugin, S.J. Skerrett, L.D. Hudson, T.R. Martin, Cytokine balance in the lungs of patients with acute respiratory distress syndrome, Am. J. Respir. Crit. Care Med. 164 (2001) 1896–1903, https://doi.org/10.1164/ajrccm.164.10.2104013.

[60] J. Wang, X. Yang, Y. Li, J.A. Huang, J. Jiang, N. Su, Specific cytokines in the inflammatory cytokine storm of patients with COVID-19-associated acute respiratory distress syndrome and extrapulmonary multiple-organ dysfunction, Virol. J. 18 (2021) 117, https://doi.org/10.1186/s12985-021-01588-y.

[61] G.P. Fadanni, J.B. Calixto, Recent progress and prospects for anti-cytokine therapy in preclinical and clinical acute lung injury, Cytokine Growth Factor Rev. 71 (2023) 13–25, https://doi.org/10.1016/j.cytogfr.2023.07.002.

[62] Q. Ye, B. Wang, J. Mao, The pathogenesis and treatment of the 'cytokine storm' in COVID-19, J. Infect. 80 (2020) 607–613, https://doi.org/10.1016/j.jinf.2020.03.037.

[63] H. Chanteux, A.C. Guisset, C. Pilette, Y. Sibille, LPS induces IL-10 production by human alveolar macrophages via MAPKinases- and Sp1-dependent mechanisms, Respir. Res. 8 (2007) 71, https://doi.org/10.1186/1465-9921-8-7.

[64] R. de Waal Malefyt, J. Abrams, B. Bennett, C.G. Figdor, J.E. de Vries, Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes, J. Exp. Med. 174 (1991) 1209–1220, https://doi.org/10.1084/jem.174.5.1209.

[65] M. Schäfers, L. Sorkin, Effect of cytokines on neuronal excitability, Neurosci. Lett. 437 (2008) 188–193, https://doi.org/10.1016/j.neulet.2008.03.052.

[66] L.Y. Lee, C.C. Hsu, Y.J. Lin, R.L. Lin, M. Khosravi, Interaction between TRPA1 and TRPV1: synergy on pulmonary sensory nerves, Pulm. Pharmacol. Ther. 35 (2015) 87–93, https://doi.org/10.1016/j.pupt.2015.08.003.

[67] S.T. O'Leary, K.J. Narwaney, N.M. Wagner, C.R. Kraus, S.B. Omer, J.M. Glanz, Efficacy of a web-based intervention to increase uptake of maternal vaccines: an RCT, Am. J. Prev. Med. 57 (2019) 125–133, https://doi.org/10.1016/j.amepre.2019.05.018. Epub 2019 Aug 27. PMID: 31471001.

[68] J.J. Adcock, TRPV1 receptors in sensitisation of cough and pain reflexe, Pulm. Pharmacol. Ther. 22 (2009) 65–70, https://doi.org/10.1016/j.pupt.2008.12.014.

3.2 MANUSCRITO ORIGINAL 2

A ser submetido.

TRPA1 and TRPV4 respond to bradykinin signaling in experimental acute lung injury

Mayara Alves Amorim[1], Vitor Hélio Souza Oliveira[1], João B. Calixto[2], Eunice André[1]

[1]Pharmacology, Federal University of Paraná, Curitiba, Brazil.

[2]Centro de Inovação e Ensaios Pré-Clínicos-CIEnP, Florianópolis, SC, Brazil.

Corresponding author:

Eunice André, professor of pharmacology,

Department of Pharmacology,

Federal University of Paraná,

E-mail address: eunice.andre@ufpr.br

Telephone: (55) 413361-1693

Curitiba, PR, Brazil – 81531-980

ABSTRACT

Although Transient Receptor Potential Ankyrin 1 (TRPA1) and Transient Receptor Potential Vanilloid 4 (TRPV4) are recognized as amplifiers of lung inflammation, and bradykinin has been shown to modulate TRP channels in other systems, their regulation by bradykinin signaling in acute lung injury (ALI) is not well established. In this study, we examined the involvement of TRPA1 and TRPV4, and their modulation by bradykinin B_1 and B_2 receptors, in a murine model of lipopolysaccharide (LPS)-induced ALI (5 mg/kg, intratracheally). Mice were pretreated intraperitoneally with TRPA1 (HC030031, 100 mg/kg) or TRPV4 (HC067047, 10 mg/kg) antagonists, or with B_1 (DALBK, 50 nmol/kg) and B_2 (HOE140, 10 nmol/kg) receptor antagonists, 15 minutes before LPS instillation, and evaluated after 6 and 24 hours. Lung tissue was analyzed for edema, histology, collagen deposition, and TRP expression by immunohistochemistry. Bronchoalveolar lavage fluid (BALF) was assessed for leukocyte infiltration, protein content, and cytokine levels. LPS exposure induced epithelial barrier damage, edema, cell infiltration, and elevated IL-1β, IFN-γ, IL-6, IL-17, IL-23, and TNF-α levels in BALF. These changes were significantly reduced by TRPA1 or TRPV4 inhibition. Notably, LPS upregulated TRPA1 and TRPV4 expressions, which was suppressed by B_1 or B_2 receptor blockade, suggesting that these channels are downstream targets of bradykinin signaling. Although direct interaction between TRPA1 and TRPV4 has not been evaluated, their parallel modulation by bradykinin receptor antagonists suggests they may participate in a convergent inflammatory pathway. These findings support a model in which bradykinin receptors contribute to lung injury through activation of distinct pro-inflammatory TRP channels.

Keywords: TRP; inflammation, lung injury; bronchoalveolar lavage fluid; edema; airways.

1. Introduction

Acute lung injury (ALI) is a critical clinical syndrome characterized by intense pulmonary inflammation and disruption of the alveolar–capillary barrier (Verma et al., 2025). Among the various mediators involved, bradykinin (BK) plays a key role in amplifying the inflammatory response (Nasseri et al., 2015). However, the specific molecular effectors that transduce BK signaling in lung tissue remain incompletely understood. Acute respiratory distress syndrome (ARDS) is triggered by a complex interaction of inflammatory, mechanical stress, and oxidative stimuli (Bos and Ware, 2022). Although current therapies target isolated pathways, the multifactorial nature of ARDS requires strategies that act simultaneously on multiple mechanisms.

In this context, Transient Receptor Potential (TRP) channels have emerged as promising targets, as they respond to diverse stimuli and can act in a coordinated manner to amplify pulmonary inflammation (Achanta and Jordt, 2020; Müller et al., 2022). TRP channels are increasingly recognized as molecular integrators of noxious and inflammatory signals in multiple tissues (Zhang et al., 2023). Based on previous findings implicating TRPV1 modulation by BK in ALI, we investigated whether additional TRP channels also participate in this pathway. In particular, TRPA1 and TRPV4, both expressed in pulmonary tissues and implicated in barrier dysfunction and cytokine release, have not yet been explored in the context of bradykinin signaling during lung injury (Achanta and Jordt, 2020). Given this gap, it is important to further characterize the role of other TRP channels in BK-driven pulmonary inflammation.

TRPA1 is a non-selective cation channel permeable to Ca^{2+} that functions as both a chemical and thermal sensor, responding to a variety of noxious and pro-inflammatory stimuli (Naert et al., 2021; Zhang et al., 2022). In the lung, TRPA1 is expressed in both neuronal cells, such as vagal sensory neurons (Lee et al., 2015), and non-neuronal cells, including airway epithelial cells, smooth muscle cells, lymphocytes, and fibroblasts (Nassini et al., 2012; Li et al., 2020; Luostarinen et al., 2021; Yap et al., 2020). Thus, in the airways, TRPA1 functions as a sensor of noxious stimuli and plays a pivotal role in amplifying inflammatory responses (Bautista et al., 2013). Its activation has been associated with local inflammation and may contribute to the progression of lung injury, while modulation of this channel has been shown to improve outcomes in various pulmonary disorders, such as asthma (Balestrini et al.,

2021), chronic obstructive pulmonary disease (COPD) (Kocot et al., 2025), and ALI (Liu et al., 2020).

TRPV4 is also involved in sensing noxious stimuli and acts as a detector of elevated temperatures, acidic conditions, mechanical pressure, and osmotic changes (Koskimäki et al., 2024). It contributes to endothelial barrier dysfunction and pulmonary edema (Weber et al., 2020). Substantial evidence indicates that activation of TRPV4 channels promotes the breakdown of the pulmonary endothelial and epithelial barriers, a fundamental aspect of lung damage (Lu et al., 2021; Wang et al., 2024; Weber et al., 2020). Moreover, pharmacological inhibition of TRPV4 has shown protective effects in different models of pulmonary injury, suggesting that this channel contributes to lung damage through multiple pathological pathways (Balakrishna et al., 2014; Cai et al., 2022; Toumpanakis et al., 2022).

On the other hand, although there is considerable evidence that both channels are involved in lung injury, their specific roles in ALI and ARDS remain poorly defined. These conditions continue to represent a major clinical challenge, with high mortality rates and no effective treatments currently available (Peck et al., 2019). Because there are still no appropriate therapies, there is growing interest in identifying novel molecular targets involved in the progression of ARDS. Importantly, the effects of bradykinin are not limited to TRPV1. Emerging evidence suggests that BK modulates distinct TRP channels, such as TRPA1 and TRPV4, pointing to a broader signaling network underlying the multifactorial pathology of ALI. While our prior work established TRPV1 as a key mediator of bradykinin-driven inflammation (Amorim et al., 2025), TRPV1 alone cannot explain the full spectrum of ALI pathophysiology. This gap suggests that bradykinin receptors recruit a broader TRP channel network (TRPA1/TRPV4) to sustain lung injury via parallel pathways.

Since TRPA1 and TRPV4 also respond to pro-inflammatory stimuli and have been shown to interact with the BK system (Jentsch et al., 2020), it is reasonable to hypothesize that they contribute, alongside other TRP channels, to the amplification of lung inflammation. Thus, we hypothesize that bradykinin receptors independently regulate TRPA1 and TRPV4, creating a self-reinforcing inflammatory loop through convergent but distinct mechanisms. While direct interactions between TRP channels remain unexplored, their modulation via BK receptors suggests that these channels collectively exacerbate ALI. Taken together, these observations support a model in

which BK receptors modulate both TRPA1 and TRPV4, expanding the role of TRPV1 and offering new targets for multi-mechanism therapies in ALI.

2. Material and methods

2.1 Ethical Procedures and Animal Model

Experiments were performed on male and female C57BL/6 mice (20-35g) aged between 7 and 8 weeks, sourced from the Bioterium Complex of the Biological Sciences Sector at the Federal University of Paraná. The animals were housed at 22 ± 2°C under a 12 h light/dark cycle. Food and water were provided *ad libitum*, with a maximum of four mice per cage. The study was conducted in accordance with the ARRIVE guidelines, as previously reported by Sert et al. (2020), and all experimental procedures were approved by the local Ethics Committee of Animal Experimentation of the Federal University of Paraná (Protocol number 1602, 1427, and 1486). For a period of at least 24 hours before the beginning of the experiments the animals acclimated to the laboratory environment for at least 24 h before the experiments. As no significant differences were identified between male and female mice, both sexes were analyzed together to enhance statistical power and minimize variability. Subsequently, the animals were randomly assigned to experimental groups using a computer-generated randomization (random.org), ensuring a minimum of six animals per group, ensuring at least six animals per group to allow reliable data generation and appropriate statistical analyses.

2.2 Experimental Design and Treatment Groups

Although no animal model fully replicates ARDS, the method of inducing ALI by intratracheal instillation of LPS in animals has been widely accepted as a valid experimental model to study ARDS (D'Alessio, 2018; Zhang et al., 2024). Accordingly, mice were anesthetized with ketamine (50 mg/kg) and xylazine (10 mg/kg). Following anesthesia induction, the animals were maintained in an upright position, and under aseptic conditions, a 5-mm midline incision was made in the cervical region. Using a sterile 30-gauge needle, lipopolysaccharide (LPS) from *Escherichia coli* (O111:B4, Sigma Aldrich) was administered intratracheally at a dose of 5 mg/kg in 50 µl, or its

vehicle (50 µl of saline) (Yang et al., 2020). Immediately after administration, the incision was closed by suturing. The animals were then monitored and euthanized at 6 and 24 hours post-instillation by intraperitoneal injection of thiopental (50 mg/kg), preceded by lidocaine administration (10 mg/mL, i.p.). Subsequently, the thoracic cavity was opened, and a cannula was inserted into the left ventricle and directed toward the aorta to perform transcardiac perfusion with 0.9% NaCl, following confirmation of adequate anesthesia. The lungs were then collected for histological evaluation, wet-to-dry (W/D) weight ratio analysis, and immunohistochemistry. In addition, bronchoalveolar lavage fluid (BALF) was obtained for further assessment of cellular infiltrates and cytokine quantification.

Following the establishment of the ALI model, specific receptor antagonists were administered to assess their contribution to disease modulation. To evaluate the role of TRPA1 and TRPV4 receptors in LPS-induced ALI in mice, 15 minutes prior to the administration of LPS (5 mg/kg, i.t.), animals were pretreated intraperitoneally with either the TRPA1 receptor antagonist HC030031 (100 mg/kg), the TRPV4 receptor antagonist HC067047 (10 mg/kg), or their respective vehicles (Descoeur et al., 2011; Dong et al., 2017). Another group of mice received Des-Arg^{9}-Leu^{8}-bradykinin (DALBK; 50 nmol/kg, i.p.) or icatibant (HOE 140; 10 nmol/kg, i.p.), B_1 and B_2 receptor antagonists, respectively, or their respective vehicles (Correa et al., 1996; Dutra et al., 2013). At 6 and 24 hours after ALI induction, the mice were euthanized by an overdose of thiopental (50 mg/kg, i.p.), preceded by lidocaine administration (10 mg/mL, i.p.). Subsequently, the lungs were harvested for histological and immunohistochemical analyses, and BALF was collected for further evaluation. As an additional parameter for assessing general health status, body weight was recorded prior to LPS administration and again at 6 and 24 hours following treatment with LPS and receptor antagonists (Piirsalu et al., 2020).

2.3. Morphological Assessments of the Lungs

2.3.1. Histological Analysis and Injury Scoring

After the experimental protocols, the lungs were removed, fixed in 4% paraformaldehyde for 24 hours, and subsequently embedded in paraffin. The blocks were sectioned at a thickness of 4 µm and stained with hematoxylin and eosin (H&E) for histological evaluation. Analysis was performed by a pathologist blinded to

experimental groups using optical microscopy. Histological parameters evaluated included pulmonary edema, alveolar congestion, inflammatory cell infiltration, and alveolar hemorrhage, each scored on a four-point scale: (0) absent, (1) mild, (2) moderate, and (3) severe (An et al., 2019).

2.3.2. Collagen Staining (Masson's Trichrome)

To quantify collagen deposition in lung tissue, histological sections were stained with Masson's trichrome. The slides were analyzed under an optical microscope, and images were processed using ImageJ software (version 1.4d). The area occupied by collagen was quantified as the percentage of the field area, allowing estimation of the degree of tissue fibrosis. This methodology was based on previously validated protocols (Geng et al., 2022; Seger et al., 2018).

2.3.3. Wet/Dry Weight Ratio (Pulmonary Edema)

Mice were euthanized 6 and 24 h after LPS-induced injury, and the lungs were promptly excised and weighed using an analytical balance to obtain wet weight. The degree of pulmonary edema was assessed by the ratio between the wet and dry lung weights (W/D ratio). The lungs were then oven-dried at 60 °C for 72 h to determine the dry weight. The W/D ratio was calculated as an indicator of pulmonary edema. This parameter reflects fluid accumulation in lung tissue, characteristic of ALI (Ali et al., 2020; Bernardo et al., 2022).

2.4. Analysis of Bronchoalveolar Lavage Fluid (BALF)

2.4.1. Collection and Processing

The procedure was adapted from Van Hoecke et al. (2017), with minor modifications. For BALF collection, the trachea was exposed via a small cervical incision, followed by lavage performed three times with 1 mL of phosphate-buffered saline (PBS). The recovered fluid was collected in microcentrifuge tubes kept on ice. The entire volume of exudate was centrifuged at 290 × g for 8 minutes at 4 °C. The resulting cell pellet was resuspended in 100 µL PBS for total and differential cell count analysis, while the supernatant was used for protein concentration measurement and cytokine quantification.

2.4.2. Cell Count and Protein Concentration

Total inflammatory cell counts were performed using a Neubauer chamber under an optical microscope (400× magnification) after the addition of Türk's solution (0.5 mg/mL in PBS). The results were expressed as total cell number (×10^6) according to Kadam and Schnitzer (2024). Protein concentration in BALF was determined by the Bradford assay (Bio-Rad, California, USA) using bovine serum albumin (BSA) as a standard (Yu et al., 2016). The protein concentration was determined by interpolation from a standard curve (0.1–1.0 mg/mL) prepared with bovine serum albumin (BSA). Sample absorbance was measured at 595 nm using a spectrophotometer.

2.4.3. Quantification of Inflammatory Cytokines (ELISA)

In addition to cellular and protein analyses, the BALF supernatant was collected after centrifugation at 290 × g for 8 minutes at 4 °C for the quantification of inflammatory cytokines. The cytokines evaluated included IL-6, IL-10, IL-17, IL-23, IL-1β, IFN-γ, and TNF-α. Concentrations were determined by enzyme-linked immunosorbent assay (ELISA) using commercial kits (PeproTech, BioLegend), following the manufacturers' instructions. Absorbance was measured at 405 nm, according to the methodology described by Zhou et al. (2017).

2.5 Immunohistochemistry for TRPA1 and TRPV4 receptors

Immunohistochemistry was performed to evaluate TRPA1 and TRPV4 receptor expression in mouse lung tissue. Following transcardiac perfusion with 0.9% NaCl, the lungs were removed, fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned at a thickness of 5 μm. Tissue sections were blocked and incubated overnight at 4 °C with primary antibodies against TRPA1 (1:100) or TRPV4 (1:100). After four washes with PBS, the sections were incubated with a goat anti-rabbit secondary antibody (1:100) for 1 hour, followed by four additional washes with PBS containing 1% BSA. Positive staining was visualized using a 3,3′-diaminobenzidine (DAB) substrate kit (BD Pharmingen, San Jose, CA, USA).

Immunoreactivity for TRPA1 and TRPV4 was observed under an Olympus microscope equipped with Cell^F capture software (2008, Olympus Soft Imaging

Solutions GmbH). Images were analyzed using ImageJ software (version 1.4d), and receptor expression levels were quantified as the mean gray value normalized to the number of nuclei in 2–4 randomly selected images per lung (Crowe and Yue, 2019; Jentsch et al., 2020).

2.6 Drugs and reagents

The experimental protocols were performed with the following drugs: LPS from *Escherichia coli* (O111:B4), HC030031, HC067047, DALBK acetate salt, HOE140, goat anti-rabbit secondary antibody, all of which were purchased from Sigma Chemical Co., St. Louis, USA. TRPA1 polyclonal antibody (PA1-46159) and TRPV4 polyclonal antibody (PA5-34292) were purchased by Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA. DAB Substrate Kit was purchased from BD Pharmingen, San Jose, CA, USA. The Kits of cytokines IL1-β, IL-6, IL-10, TNF-α were purchased from PeproTech, Rocky Hill, NJ, USA. The kits of cytokines IL-17, IL-23, IFN-γ were purchased from BioLegend San Diego, CA, USA. LPS, DALBK acetate salt and HOE140 were dissolved in 0.9% of NaCl. HC030031 solution was made using 0.9% NaCl composed of 10% DMSO and 10% Tween 80 and HC067047 solution was made using 0.9% NaCl composed of 10% DMSO and 5% Tween 80. The solutions were diluted on the day of the experiment just prior to use.

2.7 Statistical analysis

All data were described as mean ± standard error of mean (S.E.M) of a minimum of six animals per group. Statistical evaluation of the data was carried out using the One Way Analysis of Variance (ANOVA) followed by Student-Newman Keuls (GraphPad Prism software version 8). Body weight data were analyzed using two-way ANOVA followed by Tukey’s post-hoc test, considering 'treatment' (vehicle vs. LPS) and 'time' (0, 6, or 24 hours) as independent variables. A P value lower than 0.05 was significant. All statistical analyses were performed with the aid of GraphPad Prism software version 8.

3. Results

3.1 TRPA1 and TRPV4 antagonists attenuate and collagen deposition on LPS-induced ALI in mice

Histopathological alterations were assessed using H&E staining to determine the protective effects of TRPA1 and TRPV4 antagonists against LPS-induced ALI. Intratracheal instillation of LPS (5 mg/kg) significantly increased the lung injury score at both 6 and 24 hours compared to the vehicle group (*p < 0.05). At 6 hours, the histological score in the LPS group (Veh_1) was 7 ± 0.63, and at 24 hours 6.33 ± 0.21, whereas the vehicle group showed scores of 2.66 ± 0.21 and 2.5 ± 0.22, respectively. Pretreatment with the TRPA1 antagonist HC030031 (100 mg/kg) significantly reduced the lung injury score to 2.5 ± 0.22 and 2.33 ± 0.21 at 6 and 24 hours, respectively (#p < 0.05 vs. Veh_1). Similarly, treatment with the TRPV4 antagonist HC067047 (10 mg/kg) decreased the scores to 2.33 ± 0.21 at both time points (#p < 0.05 vs. Veh_1) (Figure 1A,B).

Lung tissue from LPS-treated mice also exhibited a marked increase in collagen deposition at 6 and 24 hours, reaching 40.05% ± 5.41% and 42.31% ± 4.16% of the total area, respectively, compared to 25.16% ± 2.89% and 21.74% ± 1.37% in the vehicle group (*p < 0.05). HC030031 significantly reduced collagen accumulation to 24.04% ± 3.32% and 20.67% ± 1.06%, and HC067047 produced a similar effect (25.09% ± 1.11% and 22.25% ± 3.82%) at 6 and 24 hours, respectively (#p < 0.05 vs. Veh_1) (Figure 1C,D).

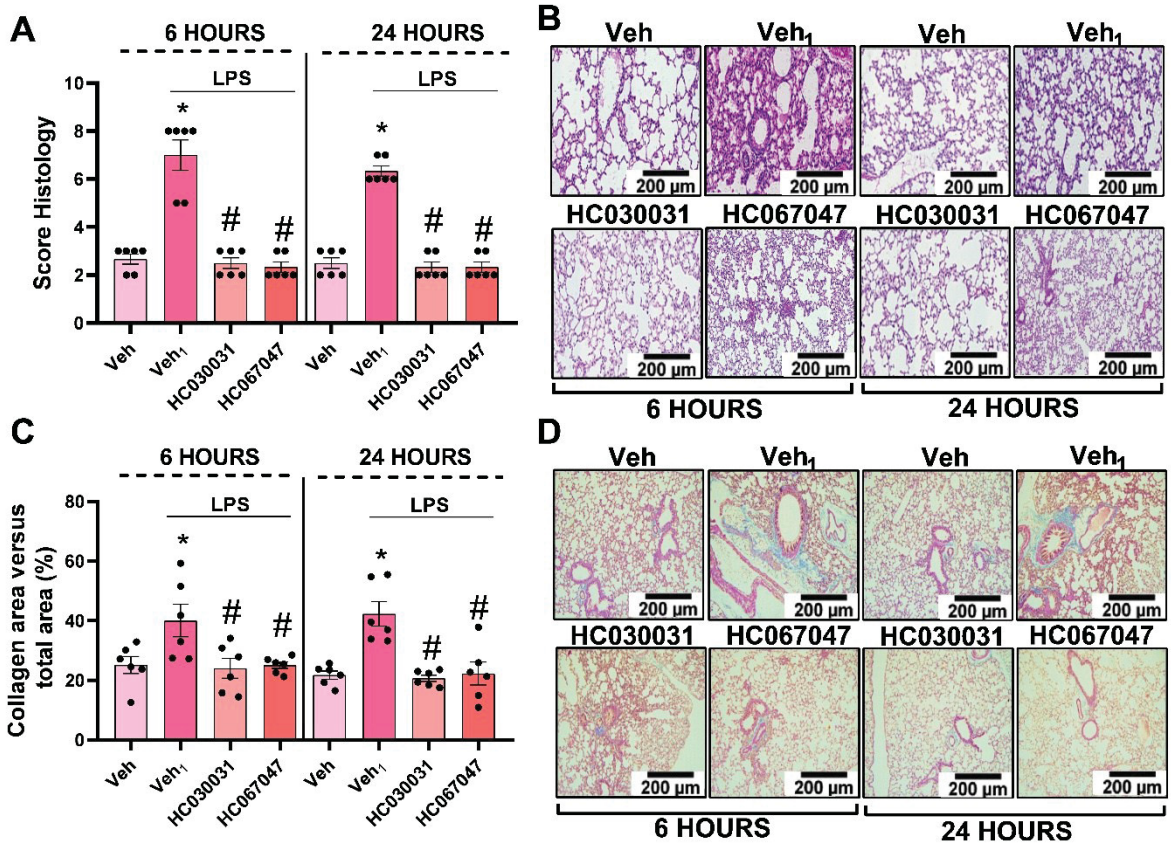


Fig. 1 TRPA1 and TRPV4 antagonists reduce LPS-induced ALI in mice at 6 and 24 hours post-administration. (A) Lung histological scores based on inflammation, alveolar thickening, and leukocyte infiltration at 6 and 24 hours after intratracheal LPS (5 mg/kg) administration. (B) Representative images of H&E-stained lung sections showing inflammatory cell infiltration and alveolar damage. (C) Quantification of collagen deposition area expressed as a percentage of the total lung area. (D) Representative images of lung sections stained with Masson's trichrome, highlighting collagen deposition (blue). Pretreatment with the TRPA1 antagonist HC030031 (100 mg/kg, i.p.) or the TRPV4 antagonist HC067047 (10 mg/kg, i.p.) significantly reduced histological damage and collagen deposition compared to the LPS group (Veh_1). Data is presented as mean ± SEM (n = 6 per group). *P ≤ 0.05 vs. vehicle control (Veh); #P ≤ 0.05 vs. LPS group (Veh_1), analyzed by one-way ANOVA followed by Student–Newman–Keuls post hoc test.

3.2 TRPA1 and TRPV4 antagonists reduce pulmonary edema, vascular permeability, and inflammation on LPS-induced ALI in mice

To further investigate the effects of TRPA1 and TRPV4 antagonists on pulmonary edema and inflammation, we evaluated protein concentration in BALF, lung W/D ratio, leukocyte infiltration, and body weight (Figure 2). Intratracheal administration of LPS (5 mg/kg) significantly increased protein levels in BALF,

indicating alveolar-capillary barrier disruption and increased vascular permeability. At 6 and 24 hours, the LPS group (Veh_1) exhibited protein concentrations of 679.20 ± 47.54 µg/mL and 657.30 ± 61.10 µg/mL, respectively, compared to the vehicle group (289.70 ± 48.47 µg/mL and 213.30 ± 49.15 µg/mL, *p < 0.05). Pretreatment with HC030031 (100 mg/kg) markedly reduced these levels to 359.90 ± 42.67 µg/mL and 298.00 ± 60.24 µg/mL, and HC067047 (10 mg/kg) produced similar effects (374.00 ± 74.75 µg/mL and 223.40 ± 60.53 µg/mL) at 6 and 24 hours, respectively (#p < 0.05 vs. Veh_1) (Figure 2A).

LPS also caused a significant increase in lung W/D ratio, indicative of pulmonary edema, with values of 9.95 ± 0.79 and 8.50 ± 0.32 at 6 and 24 hours, respectively, compared to the vehicle group (4.92 ± 0.21, 4.65 ± 0.27, *p < 0.05). This increase was significantly attenuated by pretreatment with HC030031 (5.05 ± 0.58 and 4.60 ± 0.37) and HC067047 (4.88 ± 0.35 and 4.22 ± 0.16) (#p < 0.05 vs. Veh_1) (Figure 2B). Furthermore, LPS markedly elevated the total leukocyte count in BALF at both 6 and 24 hours (40.76 ± 3.19 ×10⁶ cells and 42.16 ± 2.08 ×10⁶ cells) compared to the vehicle group (19.89 ± 1.57 ×10⁶ cells and 18.94 ± 1.08 ×10⁶ cells, *p < 0.05). Both antagonists effectively reduced leukocyte recruitment, HC030031 to 22.10 ± 1.49 ×10⁶ and 20.29 ± 0.75 ×10⁶, and HC067047 to 22.11 ± 1.30 ×10⁶ and 21.13 ± 1.34 ×10⁶ (#p < 0.05 vs. Veh_1) (Figure 2C).

Body weight measurements showed no significant differences after 6 hours (data not shown). However, after 24 hours, mice treated with LPS alone exhibited significant weight loss (20.55 ± 0.24 g) compared to baseline (22.31 ± 0.53 g, *p < 0.05), whereas animals treated with saline showed no weight loss compared to baseline (21.51 ± 0.48 g and 22.07 ± 0.69 g, respectively). Animals pretreated with HC030031 (17.59 ± 0.63 g) maintained weights close to baseline (18.23 ± 0.89 g), with no significant reductions. The same was observed in animals pretreated with HC067047, where weight after 24 hours (16.87 ± 0.36 g) did not show a significant decrease compared to baseline (18.09 ± 0.64 g) (Figure 2D).

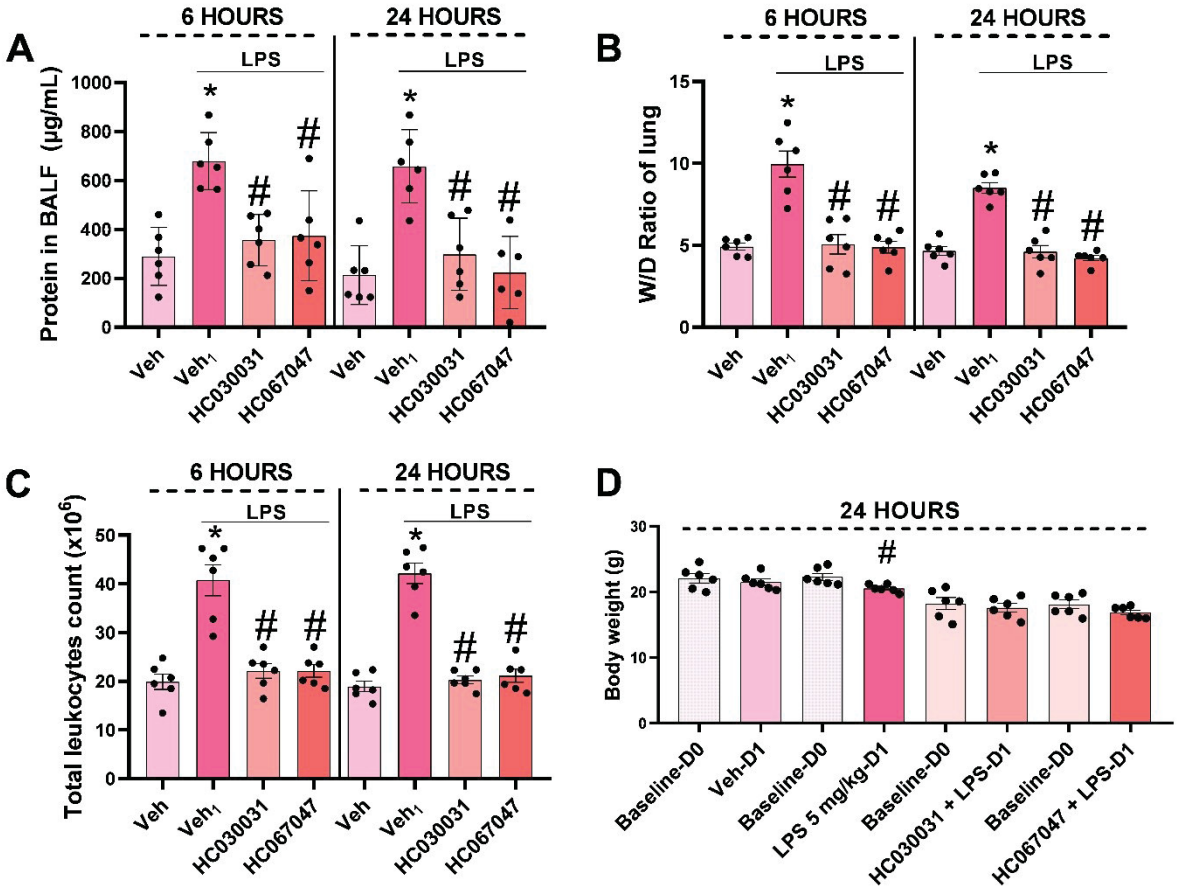


Fig. 2 TRPA1 and TRPV4 antagonists attenuate inflammation and weight loss in LPS-induced ALI. (A) Total protein concentration in BALF, indicating alveolar-capillary barrier disruption, measured at 6 and 24 hours after LPS (5 mg/kg, i.t.) administration. (B) Lung wet-to-dry (W/D) weight ratio, representing pulmonary edema, at the same time points. (C) Total leukocytes count in BALF, reflecting inflammatory cell recruitment. (D) Body weight measured before (D0) and 24 hours after (D1) vehicle or LPS administration. Pretreatment with the TRPA1 antagonist HC030031 (100 mg/kg, i.p.) or the TRPV4 antagonist HC067047 (10 mg/kg, i.p.) significantly reduced LPS-induced protein leakage, pulmonary edema, leukocyte infiltration, and body weight loss. Data is presented as mean ± SEM (n = 6 per group). *P ≤ 0.05 vs. vehicle control (Veh); #P ≤ 0.05 vs. LPS group (Veh_1) or vs. LPS baseline-D0 group for body weight changes. Data were analyzed by one-way ANOVA followed by Student–Newman–Keuls post hoc test, except for body weight changes, which were analyzed by two-way ANOVA followed by Tukey's post hoc test.

3.3 Increased TRPA1 and TRPV4 expression and pro-inflammatory cytokine production following LPS-induced ALI in mice

Quantitative analysis showed that intratracheal LPS administration significantly increased TRPA1 expression in lung tissue at 6 and 24 hours compared with the

vehicle group (Figure 3A). At 6 hours, TRPA1 expression levels were 2.45 ± 0.42 in the vehicle group (Veh), 6.88 ± 0.42 in the LPS + vehicle group (Veh_1), and 2.91 ± 0.41 in the HC030031-treated group ($p < 0.05$ vs. Veh; # $p < 0.05$ vs. Veh_1). This increase persisted at 24 hours, with values of 2.80 ± 0.50 (Veh), 6.81 ± 0.63 (Veh_1), and 3.39 ± 0.38 (HC030031) (Figure 3A,C).

Similarly, TRPV4 expression was significantly upregulated following LPS exposure at both 6 and 24 hours compared with the vehicle group (Figure 3B). At 6 hours, the values were 1.93 ± 0.36 (Veh), 6.35 ± 0.34 (Veh_1), and 2.65 ± 0.12 (HC067047). At 24 hours, the values were 2.28 ± 0.21 (Veh), 5.64 ± 0.26 (Veh_1), and 3.33 ± 0.31 (HC067047) (Figure 3B,D).

To evaluate the inflammatory response induced by LPS, the concentrations of TNF-α, IL-6, IL-1β, IL-10, IL-17, IL-23, and IFN-γ were measured in BALF at 6 and 24 hours. LPS administration markedly increased all cytokines compared with the vehicle group at both time points. For TNF-α, levels rose from 3208 ± 331 pg/mL (Veh) to 4905 ± 295 pg/mL (Veh_1) at 6 hours and from 2512 ± 155 pg/mL to 4363 ± 95 pg/mL at 24 hours ($p < 0.05$). Pretreatment with HC030031 and HC067047 reduced TNF-α to 2644 ± 73 pg/mL and 2841 ± 163 pg/mL (6 h) and to 2582 ± 152 pg/mL and 2464 ± 136 pg/mL (24 h), respectively (# $p < 0.05$ vs. Veh_1) expression (Figure 4 A).

IL-6 increased from 2016 ± 48 pg/mL to 4043 ± 396 pg/mL at 6 hours and from 1939 ± 235 pg/mL to 4872 ± 642 pg/mL at 24 hours. After treatment with HC030031 or HC067047, values decreased to 3299 ± 1748 pg/mL and 1792 ± 240 pg/mL (6 h) and 2226 ± 338 pg/mL and 2001 ± 159 pg/mL (24 h) (Figure 4 B). IL-1β followed a similar trend, rising from 2521 ± 159 pg/mL (Veh) to 4147 ± 280 pg/mL (Veh_1) at 6 hours and from 2581 ± 139 pg/mL to 3687 ± 141 pg/mL at 24 hours, with reductions to 2734 ± 114 pg/mL and 2697 ± 242 pg/mL (6 h) and 2497 ± 138 pg/mL and 2631 ± 185 pg/mL (24 h) after antagonists (Figure 4 C). IL-10 did not increase 6 hours after LPS administration but increased from 915 ± 84 pg/mL to 1379 ± 93 pg/mL (24 h); The reductions observed after administration of the antagonists were modest, occurring only with the HC067047), which caused a reduction of 930 ± 48. In contrast, the antagonist HC030031 did not show a statistically significant effect (Figure 4 D).

IL-17 increased from 104 ± 15 pg/mL to 283 ± 20 pg/mL at 6 hours and from 130 ± 11 pg/mL to 242 ± 40 pg/mL at 24 hours, with reductions to 145 ± 21 pg/mL and 115 ± 18 pg/mL (6 h) and 107 ± 2 pg/mL and 133 ± 19 pg/mL (24 h) after antagonists (HC030031 and HC067047, respectively) (Figure 4 E). IL-23 enhances from 131 ± 7

pg/mL to 389 ± 25 pg/mL (6 h) and from 128 ± 17 pg/mL to 279 ± 38 pg/mL (24 h), and treatment with antagonists (HC030031 and HC067047, respectively) values decreased to 86 ± 28 pg/mL and 141 ± 45 pg/mL (6 h) and 101 ± 24 pg/mL and 128 ± 36 pg/mL (24 h) (Figure 4 F). While IFN-γ levels rose significantly from 2161 ± 395 pg/mL (Veh) to 7141 ± 724 pg/mL (Veh_1) at 6 hours and from 2386 ± 306 pg/mL to 6617± 1003 pg/mL at 24 hours, decreasing to 3975 ± 719 pg/mL and 4502 ± 834 pg/mL (6 h) and 2820 ± 103 pg/mL and 2300 ± 249 pg/mL (24 h) after antagonist treatment (Figure 4 G).

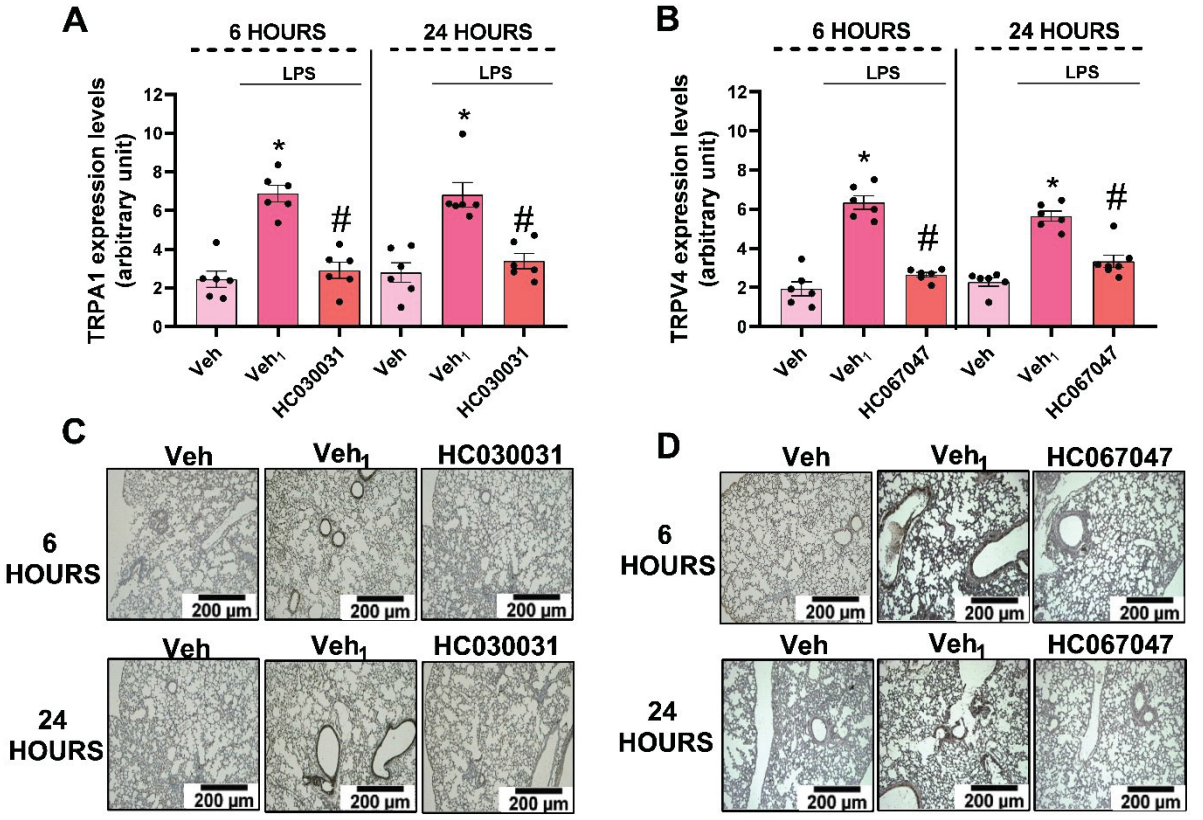


Fig. 3 TRPA1 and TRPV4 antagonists reduce upregulation of TRPA1 and TRPV4 expression in LPS-induced ALI. (A,B) Relative expression levels of TRPA1 and TRPV4 in lung tissue measured at 6 and 24 hours after intratracheal administration of LPS (5 mg/kg), with or without pretreatment using the TRPA1 antagonist HC030031 (100 mg/kg, i.p.) or the TRPV4 antagonist HC067047 (10 mg/kg, i.p.). (C,D) Representative immunohistochemical images showing TRPA1 and TRPV4 expression in lung sections from mice with LPS-induced ALI. Both antagonists significantly reduced the LPS-induced increase in receptor expression. Data is presented as mean ± SEM (n = 6 per group). *P ≤ 0.05 vs. vehicle control (Veh); #P ≤ 0.05 vs. LPS group (Veh_1), analyzed by one-way ANOVA followed by Student–Newman–Keuls post hoc test.

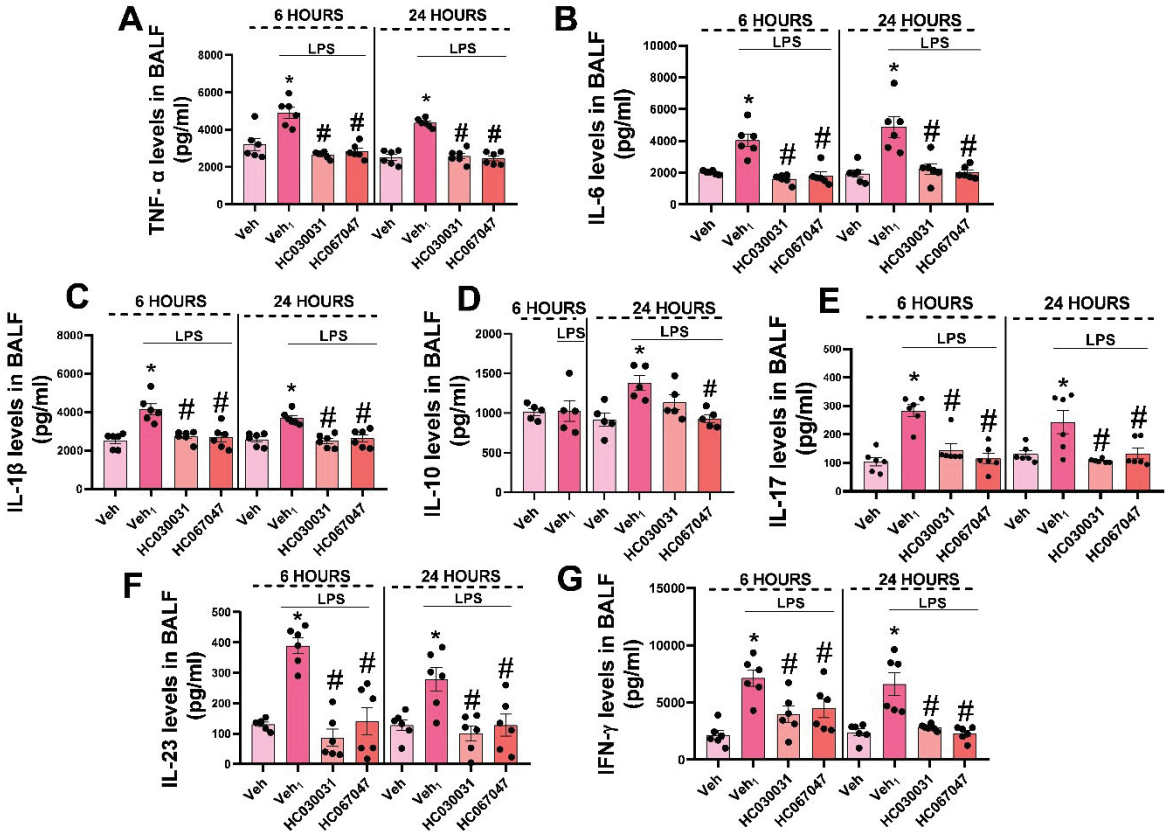


Fig. 4 TRPA1 and TRPV4 antagonists reduce proinflammatory cytokine levels in BALF after LPS-induced ALI. Levels of inflammatory cytokines were measured in BALF at 6 and 24 hours after intratracheal LPS (5 mg/kg) administration, with or without pretreatment using the TRPA1 antagonist HC030031 (100 mg/kg, i.p.) or the TRPV4 antagonist HC067047 (10 mg/kg, i.p.). (A) TNF-α, (B) IL-6, (C) IL-1β, (D) IL-10, (E) IL-17, (F) IL-23, and (G) IFN-γ levels. LPS administration significantly increased the levels of all measured cytokines. Pretreatment with TRPA1 or TRPV4 antagonists markedly reduced the LPS-induced increase in both proinflammatory (e.g., TNF-α, IL-6, IL-1β, IL-17, IL-23, IFN-γ) and anti-inflammatory (IL-10) cytokines in BALF. Data is presented as mean ± SEM (n = 6 per group). *P ≤ 0.05 vs. vehicle control (Veh); #P ≤ 0.05 vs. LPS-treated group (Veh_1), analyzed by one-way ANOVA followed by Student–Newman–Keuls post hoc test.

3.4 Involvement of B_1 and B_2 receptors in the expression of TRPA1 and TRPV4 receptors in LPS-induced ALI in mice

The results depicted in Figure 5 demonstrated a significant increase in the relative expression of TRPA1 and TRPV4 at 6 and 24 hours after LPS treatment. However, pretreatment with DALBK (50 nmol/kg, i.p.) completely abolished this increased. In addition, the expression of TRPA1 receptors was significantly reduced to

2.46 ± 0.30 in 6 hours and 1.83 ± 0.32 at 24 hours in DALBK-treated animals, compared to vehicle-treated animals, which showed values of 5.54 ± 0.70 and 4.20 ± 0.52, respectively (Figures 5A and 5B). Similarly, the TRPV4 expression was also significantly reduced by DALBK, with values of 1.97 ± 0.33 at 6 hours and 2.47 ± 0.45 at 24 hours, compared to the vehicle group, which showed 5.86 ± 0.72 and 6.26 ± 0.31, respectively (Figures 5C and 5D).

Similarly, pretreatment with the B_2 receptor antagonist (HOE140, 10 nmol/kg, i.p.) also abolished the elevated expression of TRPA1 (Figure 6A,B), and TRPV4 channels (Figure 6C,D), 6 (relative expression TRPA1: 1.86 ± 0.32, TRPV4: 2.90 ± 0.24) and 24 hours after treatment with LPS (relative expression TRPA1: 1.88 ± 0.36, TRPV4: 2.81 ± 0.58, respectively), when compared to the treated vehicle group (6 hours: TRPA 1: 5.30 ± 0.45, TRPV4: 6.22 ± 0.71; and 24 hours TRPA 1: 5.30 ± 0.45, TRPV4: 5.34 ± 0.69).

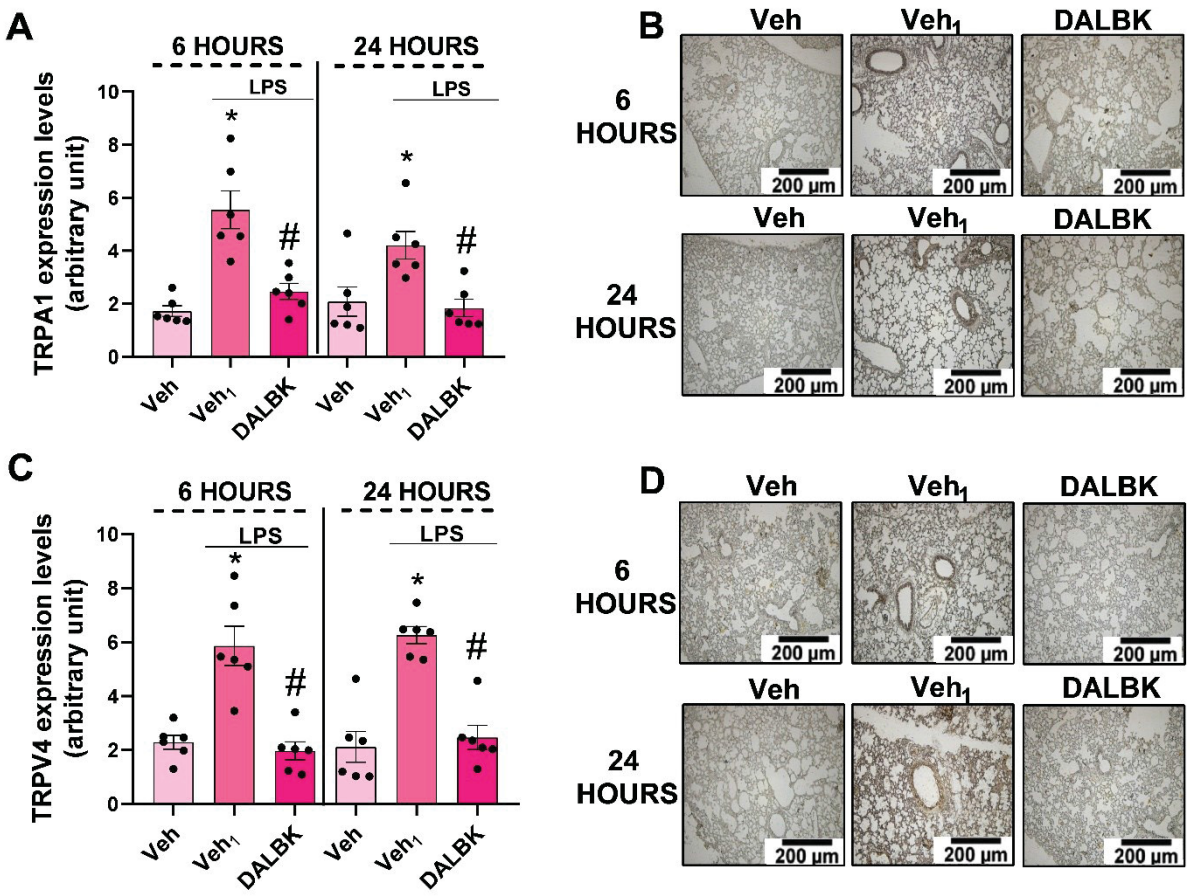


Fig. 5 B_1 receptor antagonism with DALBK reduces TRPA1 and TRPV4 expression in LPS-induced ALI. (A,B) Quantification and representative immunohistochemistry images of TRPA1 expression levels in lung tissue at 6 and 24 hours after intratracheal LPS (5 mg/kg) administration, with or without pretreatment with the B_1 receptor antagonist DALBK (50 nmol/kg, i.p.). (C,D) Quantification and representative immunohistochemistry images of TRPV4 expression in lung tissue at corresponding time points following the same treatments. Pretreatment with DALBK significantly reduced the LPS-induced upregulation of both TRPA1 and TRPV4 in lung tissue. Data is presented as mean ± SEM (n = 6 per group). *P ≤ 0.05 vs. vehicle control (Veh); #P ≤ 0.05 vs. LPS-treated group (Veh_1), analyzed by one-way ANOVA followed by Student–Newman–Keuls post hoc test.

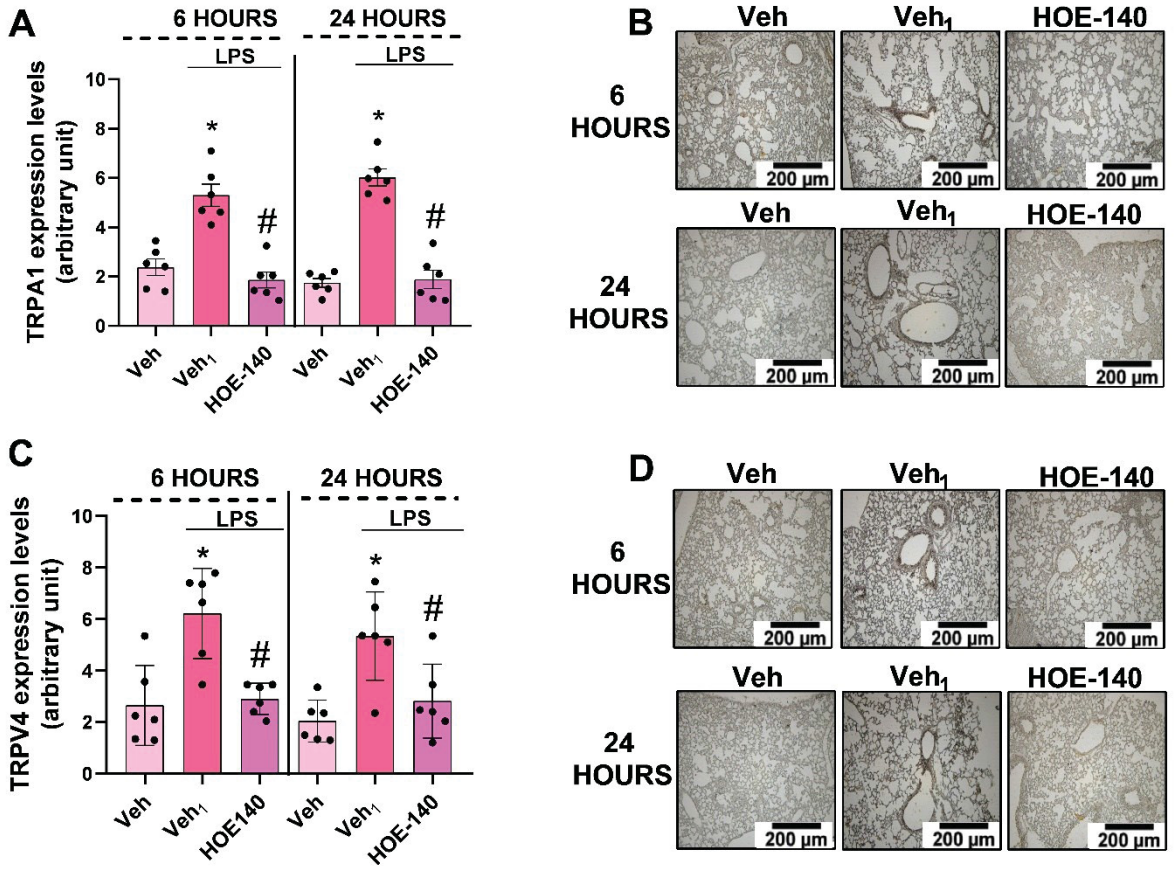


Fig. 6 B_2 receptor antagonism with HOE-140 reduces TRPA1 and TRPV4 expression in LPS-induced ALI. (A,B) Quantification and representative immunohistochemistry images of TRPA1 expression levels in lung tissue at 6 and 24 hours after intratracheal LPS (5 mg/kg) administration, with or without pretreatment with the B_2 receptor antagonist HOE-140 (10 nmol/kg, i.p.). (C,D) Quantification and representative immunohistochemistry images of TRPV4 expression at corresponding time points following the same treatments. Pretreatment with HOE-140 significantly reduced the LPS-induced upregulation of both TRPA1 and TRPV4 in lung tissue. Data is presented as mean ± SEM (n = 6 per group). *P ≤ 0.05 vs. vehicle control (Veh); #P ≤ 0.05 vs. LPS-treated group (Veh_1), analyzed by one-way ANOVA followed by Student–Newman–Keuls post hoc test.

4. Discussion

Our results reinforce the concept that modulation of TRPA1 and TRPV4 channels exerts significant protective effects in ALI, expanding current understanding of their role in pulmonary inflammation. Although a previous study from our group established a functional link between bradykinin receptors and TRPV1 in LPS-induced ALI, the current results demonstrate that TRPA1 and TRPV4 are also modulated by

the same system. This extends the reach of bradykinin signaling to other TRP channels involved in this condition.

In the current study, we observed that pharmacological inhibition of TRPA1 and TRPV4 led to a significant reduction in classic markers of lung inflammation, including epithelial barrier disruption, alveolar septal thickening, edema, cellular infiltrate, and collagen deposition (Matthay et al., 2011). TRPV4 has been extensively implicated in endothelial permeability (Alvarez et al., 2006), alveolar epithelial cell disruption (Scheraga et al., 2020), pulmonary edema formation (Thorneloe et al., 2012), macrophage phagocytosis (Hamanaka et al., 2010), modulation of cytokine secretion (Dalsgaard et al., 2016), and pulmonary inflammation (Balakrishna et al., 2014; Scheraga et al., 2016).

In our model, TRPV4 inhibition resulted in a reduction of the lung W/D ratio, supporting its role in promoting vascular permeability. Interestingly, TRPA1 has been implicated in epithelial barrier dysfunction through mechanisms involving neurogenic inflammation (Andrè et al., 2008), oxidative stress pathways (Ko et al., 2020), and the release of inflammatory mediators (Lin et al., 2015). These processes contribute to edema formation and airway inflammation (Gouin et al., 2017; Weng et al., 2024), reinforcing the relevance of TRPA1 in the pathophysiology of lung injury.

In parallel, both channels have been associated with fibroblast activation and tissue remodeling. TRPA1 regulates calcium influx essential for the release of pro-fibrotic cytokines such as TGF-β, which promotes extracellular matrix production and fibroblast activation (Yang et al., 2024). Meanwhile, TRPV4 senses increased tissue stiffness, triggering mechanosensitive pathways that sustain the myofibroblast phenotype (Adapala et al., 2021; Rahaman et al., 2014), suggesting that they may act in concert to amplify lung injury and fibrotic progression (Ratnasingham et al., 2025). These overlapping mechanisms may help explain the observed attenuation of both edema and collagen deposition after blocking either channel.

In addition, we observed a marked reduction in both TRPA1 and TRPV4 expression levels and in the release of pro-inflammatory cytokines such as IL-1β, IFN-γ, IL-6, IL-17, IL-23, TNF-α, suggesting that TRP modulation may contribute to the overall anti-inflammatory effect. These findings highlight the anti-inflammatory potential of TRPA1 and TRPV4 antagonism in ALI and raise the possibility of therapeutic relevance in chronic lung diseases such as COPD and pulmonary fibrosis, where persistent inflammation and tissue remodeling are hallmarks.

Thus, the reduction in TRP channel expression and cytokine release suggested that blockade of these pathways may interrupt an inflammatory amplification loop in the lung. Although we did not assess direct or synergistic interaction between TRPA1, TRPV4, and TRPV1, the overlapping anti-inflammatory effects observed with inhibition of each support the idea of convergent or complementary roles. Notably, functional interactions between bradykinin receptors and TRP channels, such as TRPA1 and TRPV1, have been described in the lung, particularly in models of inflammation and airway hyperresponsiveness, in which bradykinin-induced activation of these channels contributes to neurogenic inflammation, increased vascular permeability, and bronchoconstriction (Al-Shalam and El-Hashim, 2019; Amorim et al., 2021; Bandell et al., 2004; de Oliveira et al., 2016; de Oliveira et al., 2024; Jentsch et al., 2020). However, these interactions remain unexplored in the context of ALI or ARDS. Future studies should evaluate whether B_1 or B_2 receptors physically or functionally interact with TRPA1 and TRPV4 in lung inflammation, for example, through coimmunoprecipitation, proximity ligation assays, or colocalization approaches.

Previous studies have demonstrated coexpression of these channels in sensory neurons and lung cells, raising the possibility of functional cooperation (Al-Shalam and El-Hashim, 2019; Bessac and Jordt, 2008). Cross signaling could occur through shared intracellular cascades involving calcium influx, kinase activation, or transcription of inflammatory mediators (Bessac and Jordt, 2008). Supporting this, TRPV1 and TRPV4 have been shown to form heteromeric complexes in vascular endothelium, while TRPA1 and TRPV1 display calcium-dependent synergism in pulmonary neurons (Lee et al., 2015). Together, these findings suggest that TRP channels may operate as an integrated pro-inflammatory network.

Another fundamental aspect of this study is the demonstration that the inflammatory effects mediated by TRPA1 and TRPV4 are modulated by signaling via bradykinin B_1 and B_2 receptors. Here, we also found that these receptors, B_1 and B_2, exert an indirect modulatory role on TRPA1 and TRPV4 channels during LPS-induced ALI.

The significant reduction in the expression of both TRP channels after treatment with the specific antagonists DALBK (B_1) and HOE140 (B_2) suggests a functional interaction between these signaling pathways, indicating that bradykinin receptor activation may promote TRPA1 and TRPV4 activation or expression in an inflammatory context.

Studies have shown that TRPA1 antagonists were able to inhibit the cough response induced by BK in guinea pigs, indicating that TRPA1 directly mediates some sensory responses to BK (Grace et al., 2012). Additionally, the activation of the TRPV4 channel can be potentiated by BK in HEK293 cells (Fan et al., 2009), and BK sensitizes TRPV4 to induce mechanical hyperalgesia in murine models (Costa et al., 2018), suggesting a mechanism by which BK amplifies the TRPV4 response to mechanical stimuli. Complementing these findings, TRPA1 and TRPV4, via a mechanism dependent on B_2 receptor activation, are involved as common effectors in plasma extravasation and bronchoconstriction induced by captopril (de Oliveira et al., 2024).

Together, these data indicate that bradykinin-mediated signaling is fundamental for the activation and sensitization of pro-inflammatory TRP channels, such that inhibition of bradykinin signaling attenuates the inflammatory cascade, possibly by preventing the activation of upstream pathways that would lead to the induction of TRP channels and amplification of the pulmonary inflammatory response.

This hypothesis is based on our findings suggesting that bradykinin receptors modulate multiple TRP channels, each contributing to the pathogenesis of ALI through distinct but complementary mechanisms. Although the functional interaction between TRPA1 and TRPV4 was not directly assessed in this study, their similar modulation by bradykinin receptor antagonists supports the hypothesis that bradykinin signaling contributes to their activation during lung inflammation (Figure 7).

A limitation of this study is that although our data demonstrate that TRPA1 and TRPV4 are modulated by B_1/B_2 receptors in the ALI model, no experiments were performed to directly assess the physical or functional interaction between these TRP channels. Although our results suggest a possible interaction between the bradykinin receptors and TRP channels, the existence of a coordinated signaling axis remains to be clarified. Further studies are needed to determine its functional relevance in the context of ALI.

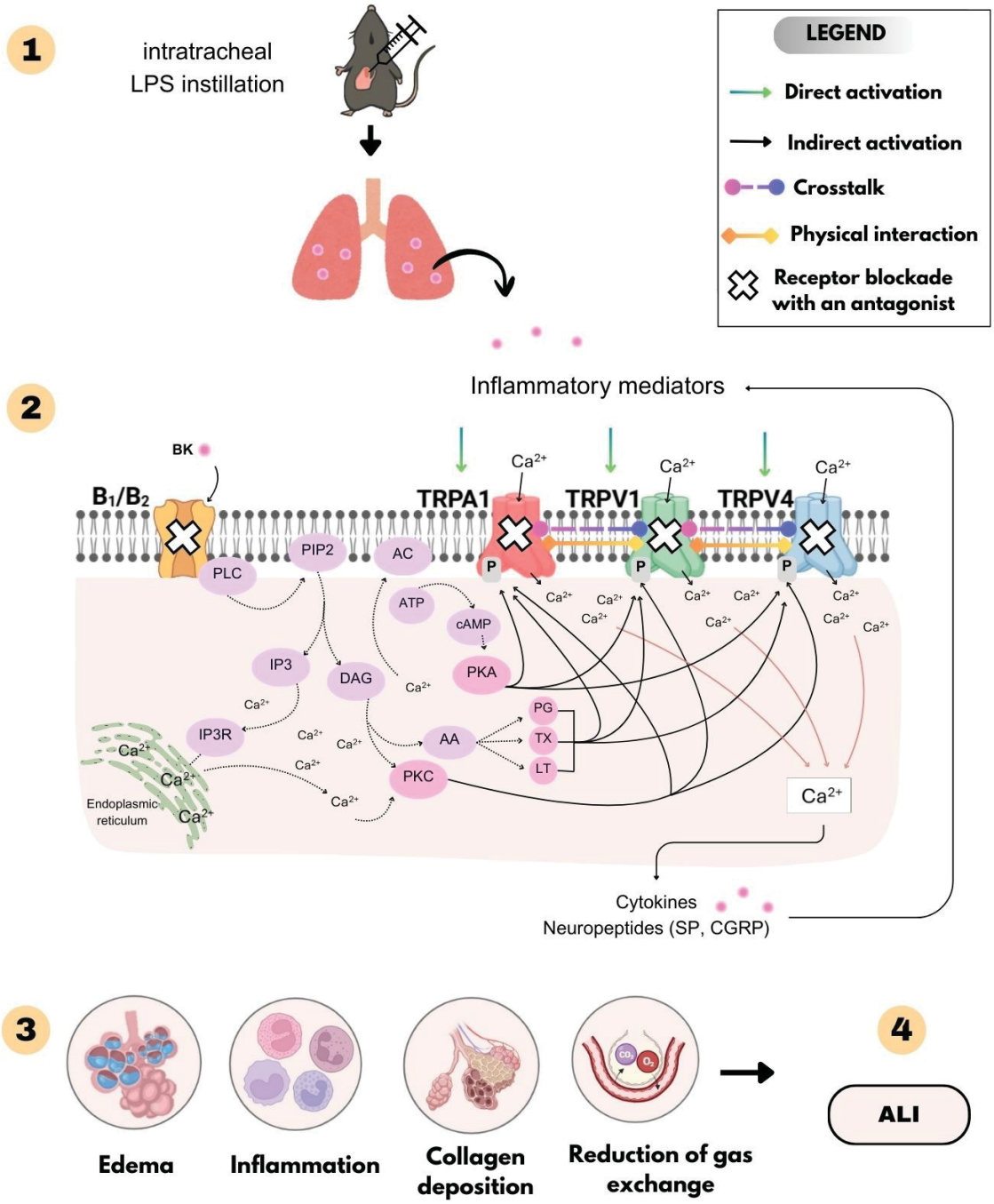


Fig. 7 Schematic representation of the involvement of bradykinin receptors (B_1 and B_2) and TRP channels (TRPV1, TRPA1, and TRPV4) in the pathophysiology of LPS-induced ALI in mice. (1) Intratracheal instillation of LPS triggers a pulmonary inflammatory response. (2) This response involves both the direct activation of B1 and B2 receptors and TRPV1, TRPA1, and TRPV4 channels, as well as the indirect activation of TRP channels mediated by bradykinin B_1/B_2 receptors, modulating inflammatory processes and endothelial permeability. TRP channel modulation by bradykinin receptors occurs when bradykinin (BK) binds to B_1/B_2 receptors, activating phospholipase C (PLC) and promoting the hydrolysis of phosphatidylinositol

bisphosphate (PIP2) into inositol trisphosphate (IP3) and diacylglycerol (DAG). IP3 induces Ca^{2+} release from the endoplasmic reticulum, increasing intracellular Ca^{2+} concentration. This increase activates protein kinase C (PKC), which phosphorylates TRPV1, TRPA1 and TRPV4 channels, and also stimulates adenylyl cyclase (AC), elevating cAMP levels and activating protein kinase A (PKA), which phosphorylates TRP (TRPV1, TRPA1 and TRPV4). DAG is converted into arachidonic acid (AA), a precursor of inflammatory mediators such as prostaglandins (PG), thromboxanes (TX), and leukotrienes (LT), which also activate TRPV1, TRPA1 and TRPV4. Crosstalk and physical interactions between TRP channels further increase Ca^{2+} influx, enhancing cytokine and neuropeptide production. This mechanism generates a self-sustaining inflammatory cycle, potentially upregulating TRP channel expression and amplifying the inflammatory response. Pharmacological blockade of these targets with specific antagonists (indicated by “X”) highlights their contribution to inflammation. (3) Activation of these pathways results in pulmonary inflammation, edema, collagen deposition, and impaired gas exchange. (4) Collectively, these events lead to ALI development, while pharmacological inhibition of these mediators attenuates inflammation and reduces the severity of lung injury (Duitama et al., 2022; Lee et al., 2015).

Acknowledgements

Amorim M.A. and Oliveira V.H.S. are PhD students in Pharmacology and thank Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for their fellowship support. Authors would like to thank to the Centro de Tecnologias Avançadas em Fluorescência (CTAF-UFPR) for their microscopy services.

CRediT authorship contribution statement

MAA: methodology, validation, formal analysis, writing- Original draft preparation. VHSO: methodology, formal analysis. JBC: review& editing, project administration. EA: conceptualization, writing – review & editing, project administration. All the authors approved the final version of the manuscript. The authors declare that all data were generated in-house and that no paper mill was used.

Funding

This work was supported by CNPq (Productivity in Research (PQ) fellowship number 307183/2021-1) and grants from INCT-INOVAMED (465430/2014-7).

Data availability

No datasets were generated or analyzed during the current study.

Ethics approval

All experimental protocols were conducted in accordance with the guidelines approved by the Ethics and Animal Experimentation Committee (CEUA) of the UFPR under number 1602, 1427 and 1486 and were compliant with the ARRIVE guidelines, as previously outlined by Percie du Sert et al. (2020).

Declaration of Competing Interest

The authors declare no conflict of interest.

References

Achanta S, Jordt SE. Transient receptor potential channels in pulmonary chemical injuries and as countermeasure targets. Ann N Y Acad Sci. 2020;1480(1):73-103. https://doi:10.1111/nyas.14472

Adapala RK, Katari V, Teegala LR, Thodeti S, Paruchuri S, Thodeti CK. TRPV4 Mechanotransduction in Fibrosis. Cells. 2021;10(11):3053. https://doi:10.3390/cells10113053

Ali H, Khan A, Ali J, Ullah H, Khan A, Ali H, Irshad N, Khan S. Attenuation of LPS-induced acute lung injury by continentalic acid in rodents through inhibition of inflammatory mediators correlates with increased Nrf2 protein expression. BMC Pharmacol Toxicol. 2021;21(1):81. https://doi:10.1186/s40360-020-00458-7

Al-Shamlan F, El-Hashim AZ. Bradykinin sensitizes the cough reflex via a B2 receptor dependent activation of TRPV1 and TRPA1 channels through metabolites of cyclooxygenase and 12-lipoxygenase. Respir Res. 2019; 20:110. https://doi:10.1186/s12931-019-1060-8

Alvarez DF, King JA, Weber D, Addison E, Liedtke W, Townsley MI. Transient receptor potential vanilloid 4-mediated disruption of the alveolar septal barrier: a novel mechanism of acute lung injury. Circ Res. 2006; 99:988–995. https://doi:10.1161/01.RES.0000247065.11756.19

Amorim MA, Souza Oliveira VH, Calixto JB, André E. Functional interplay between bradykinin receptors and transient receptor potential vanilloid-1 in lipopolysaccharide-induced acute lung injury in mice. Pulm Pharmacol Ther. 2025; 90:102384. https://doi:10.1016/j.pupt.2025.102384

Amorim MA, Jentsch Matias de Oliveira JR, Souza Oliveira VH, Cabrini DA, Otuki MF, André E. Role of nitric oxide, bradykinin B2 receptor, and TRPV1 in the airway alterations caused by simvastatin in rats. Eur J Pharmacol. 2021; 912:174591. https://doi:10.1016/j.ejphar.2021.174591

An X, Sun X, Hou Y, Yang X, Chen H, Zhang P, Wu J. Protective effect of oxytocin on LPS-induced acute lung injury in mice. Sci Rep. 2019; 9:2836. https://doi:10.1038/s41598-019-39349-1

Andrè E, Campi B, Materazzi S, Trevisani M, Amadesi S, Massi D, Creminon C, Vaksman N, Nassini R, Civelli M, Baraldi PG, Poole DP, Bunnett NW, Geppetti P, Patacchini R. Cigarette smoke-induced neurogenic inflammation is mediated by alpha,beta-unsaturated aldehydes and the TRPA1 receptor in rodents. J Clin Invest. 2008; 118(7):2574-2582. https://doi:10.1172/JCI34886

Balakrishna S, Song W, Achanta S, Doran SF, Liu B, Kaelberer MM, Yu Z, Sui A, Cheung M, Leishman E, Eidam HS, Ye G, Willette RN, Thorneloe KS, Bradshaw HB, Matalon S, Jordt SE. TRPV4 inhibition counteracts edema and inflammation and improves pulmonary function and oxygen saturation in chemically induced acute lung injury. Am J Physiol Lung Cell Mol Physiol. 2014; 307(2):158-172. https://doi:10.1152/ajplung.00065.2014

Balestrini A, Joseph V, Dourado M, Reese RM, Shields SD, Rougé L, Bravo DD, Chernov-Rogan T, Austin CD, Chen H, Wang L, Villemure E, Shore DGM, Verma VA, Hu B, Chen Y, Leong L, Bjornson C, Hötzel K, Gogineni Lee WP, Suto E, Wu X, Liu J, Zhang J, Gandham V, Wang J, Payandeh J, Ciferri C, Estevez A, Arthur CP, Kortmann J, Wong RL, Heredia JE, Doerr J, Jung M, Vander Heiden JA, Roose-Girma M, Tam L, Barck KH, Carano RAD, Ding HT, Brillantes B, Tam C, Yang X, Gao SS, Ly JQ, Liu L, Chen L, Liederer BM, Lin JH, Magnuson S, Chen J, Hackos DH, Elstrott J, Rohou A, Safina BS, Volgraf M, Bauer RN, Riol-Blanco L. A TRPA1 inhibitor suppresses neurogenic inflammation and airway contraction for asthma treatment. J Exp Med. 2021; 218(4):20201637. https://doi:10.1084/jem.20201637

Bandell M, Story GM, Hwang SW, Viswanath V, Eid SR, Petrus MJ, Earley TJ, Patapoutian A. Noxious cold ion channel TRPA1 is activated by pungent compounds

and bradykinin. Neuron. 2004; 41(6):849-857. https://doi:10.1016/s0896-6273(04)00150-3

Bautista DM, Pellegrino M, Tsunozaki M. TRPA1: A gatekeeper for inflammation. Annu Rev Physiol. 2013; 75:181-200. https://doi:10.1146/annurev-physiol-030212-183811

Bernardo LR, Ferreira LKDP, Ferreira LAMP, Vieira CID, Oliveira JB, Lima LM, Alves AF, Araújo RS, Maia MS, Scotti MT, Barbosa Filho JM, Piuvezam MR. Milonine attenuates the lipopolysaccharide-induced acute lung injury in mice by modulating the Akt/NF-κB signaling pathways. *An Acad Bras Cienc*. 2022 28;94 (suppl 4):e20211327. https://doi: 10.1590/0001-3765202220211327.

Bessac BF, Jordt SE. Breathtaking TRP channels: TRPA1 and TRPV1 in airway chemosensation and reflex control. *Physiology (Bethesda)*. 2008; 23:360-70. https://doi: 10.1152/physiol.00026.2008.

Bos LDJ, Ware LB. Acute respiratory distress syndrome: causes, pathophysiology, and phenotypes. *Lancet*. 2022; 400(10358):1145-1156. https://doi: 10.1016/S0140-6736(22)01485-4.

Cai J, Xu G, Lin Y, Zhou B, Luo Z, Yu S, Lu J. Inhibition of TRPV4 attenuates ferroptosis against LPS-induced ALI via Ca2+ pathway. *Turk J Biol*. 2022; 46(6):465-474. https://doi: 10.55730/1300-0152.2632.

Correa CR, Kyle DJ, Chakraverty S, Calixto JB. Antinociceptive profile of the pseudopeptide B2 bradykinin receptor antagonist NPC 18688 in mice. *Br J Pharmacol*. 1996; 117:552-558. https://doi: 10.1111/j.1476-5381.1996.tb15226.x.

Costa R, Bicca MA, Manjavachi MN, Segat GC, Dias FC, Fernandes ES, Calixto JB. Kinin receptors sensitize TRPV4 channel and induce mechanical hyperalgesia: relevance to paclitaxel-induced peripheral neuropathy in mice. *Mol Neurobiol*. 2018. https://doi: 10.1007/s12035-017-0475-9.

Crowe AR, Yue W. Semi-quantitative determination of protein expression using immunohistochemistry staining and analysis: an integrated protocol. *Bio Protoc*. 2019; 9:3465. https://doi: 10.21769/BioProtoc.3465.

D'Alessio FR. Mouse models of acute lung injury and ARDS. *Methods Mol Biol*. 2018; 1809:341-350. https://doi: 10.1007/978-1-4939-8570-8_22.

Dalsgaard T, Sonkusare SK, Teuscher C, Poynter ME, Nelson MT. Pharmacological inhibitors of TRPV4 channels reduce cytokine production, restore endothelial function and increase survival in septic mice. *Sci Rep*. 2016; 6:33841. https://doi: 10.1038/srep33841.

de Oliveira JR, Otuki MF, Cabrini DA, Brusco I, Oliveira SM, Ferreira J, André E. Involvement of the TRPV1 receptor in plasma extravasation in airways of rats treated

with an angiotensin-converting enzyme inhibitor. *Pulm Pharmacol Ther*. 2016; 41:25-33. https://doi: 10.1016/j.pupt.2016.09.001.

de Oliveira JRJM, Amorim MA, Oliveira VHS, Cabrini DA, Otuki MF, Galindo CM, da Luz BB, Werner MFP, Calixto JB, André E. Repeated doses of captopril induce airway hyperresponsiveness by modulating the TRPV1 receptor in rats. *Pulm Pharmacol Ther*. 2024; 86:102302. https://doi: 10.1016/j.pupt.2024.102302.

Descoeur J, Pereira V, Pizzoccaro A, Francois A, Ling B, Maffre V, Couette B, Busserolles J, Courteix C, Noel J, Lazdunski M, Eschalier A, Authier N, Bourinet E. Oxaliplatin-induced cold hypersensitivity is due to remodelling of ion channel expression in nociceptors. *EMBO Mol Med*. 2011; 3(5):266-278. https://doi: 10.1002/emmm.201100134.

Dong Q, Li J, Wu QF, Zhao N, Qian C, Ding D, Wang BB, Chen L, Guo KF, Fu D, Han B, Liao YH, Du YM. Blockage of transient receptor potential vanilloid 4 alleviates myocardial ischemia/reperfusion injury in mice. *Sci Rep*. 2017; 7:42678. https://doi: 10.1038/srep42678.

Duitama M, Moreno Y, Santander SP, Casas Z, Sutachan JJ, Torres YP, Albarracín SL. TRP Channels as Molecular Targets to Relieve Cancer Pain. *Biomolecules* 2022, *12*, 1. https://doi.org/10.3390/biom12010001

Dutra RC, Bento AF, Leite DF, Manjavachi MN, Marcon R, Bicca MA, Pesquero JB, Calixto JB. The role of kinin B1 and B2 receptors in the persistent pain induced by experimental autoimmune encephalomyelitis (EAE) in mice: evidence for the involvement of astrocytes. *Neurobiol Dis*. 2013; 54:82-93. https://doi: 10.1016/j.nbd.2013.02.007.

Fan HC, Zhang X, McNaughton PA. Activation of the TRPV4 ion channel is enhanced by phosphorylation. *J Biol Chem*. 2009. https://doi: 10.1074/jbc.M109.028803.

Geng Y, Su S, Cao L, Yang T. Effect of PD-1 inhibitor combined with X-ray irradiation on the inflammatory microenvironment and lung tissue injury in mice. *J Inflamm Res*. 2022; 15:545-556. https://doi: 10.2147/JIR.S350112.

Gouin O, L'Herondelle K, Lebonvallet N, Le Gall-Ianotto C, Sakka M, Buhé V, Plée-Gautier E, Carré JL, Lefeuvre L, Misery L, Le Garrec R. TRPV1 and TRPA1 in cutaneous neurogenic and chronic inflammation: pro-inflammatory response induced by their activation and their sensitization. *Protein Cell*. 2017; 8(9):644-661. https://doi: 10.1007/s13238-017-0395-5.

Grace M, Birrell MA, Dubuis E, Maher SA, Belvisi MG. Transient receptor potential channels mediate the tussive response to prostaglandin E2 and bradykinin. *Thorax*. 2012. https://doi: 10.1136/thoraxjnl-2011-201443.

Hamanaka K, Jian MY, Townsley MI, King JA, Liedtke W, Weber DS, et al. TRPV4 channels augment macrophage activation and ventilator-induced lung injury. *Am J Physiol Lung Cell Mol Physiol*. 2010; 299(3):353–62. https://doi: 10.1152/ajplung.00315.2009.

Jentsch M, de Oliveira JR, Amorim MA, André E. The role of TRPA1 and TRPV4 channels in bronchoconstriction and plasma extravasation in airways of rats treated with captopril. *Pulm Pharmacol Ther*. 2020; 65:102004. https://doi: 10.1016/j.pupt.2021.102004.

Kadam AH, Schnitzer JE. Insights into disease progression of translational preclinical rat model of interstitial pulmonary fibrosis through endpoint analysis. *Cells*. 2024; 13(6):515. https://doi: 10.3390/cells13060515.

Ko HK, Lin AH, Perng DW, Lee TS, Kou YR. Lung epithelial TRPA1 mediates lipopolysaccharide-induced lung inflammation in bronchial epithelial cells and mice. *Front Physiol*. 2020; 11:596314. https://doi: 10.3389/fphys.2020.596314.

Kocot N, Pękala E, Koczurkiewicz-Adamczyk P, Chłoń-Rzepa G, Łapa A, Wójcik-Pszczoła K. Airway and cardiovascular remodeling in chronic obstructive pulmonary disease (COPD) as a target for transient receptor potential ankyrin 1 (TRPA1) channel modulators. *Bioorg Chem*. 2025; 158:108301. https://doi: 10.1016/j.bioorg.2025.108301.

Koskimäki S, Tojkander S. TRPV4-A multifunctional cellular sensor protein with therapeutic potential. *Sensors (Basel)*. 2024; 24(21):6923. https://doi: 10.3390/s24216923.

Lee LY, Hsu CC, Lin YJ, Lin RL, Khosravi M. Interaction between TRPA1 and TRPV1: synergy on pulmonary sensory nerves. *Pulm Pharmacol Ther*. 2015; 35:87-93. https://doi: 10.1016/j.pupt.2015.08.003.

Li M, Fan X, Yue Q, Hu F, Zhang Y, Zhu C. The neuro-immune interaction in airway inflammation through TRPA1 expression in CD4+ T cells of asthmatic mice. *Int Immunopharmacol*. 2020; 86:1–9. https://doi: 10.1016/j.intimp.2020.106696.

Lin AH, Liu MH, Ko HK, Perng DW, Lee TS, Kou YR. Lung epithelial TRPA1 transduces the extracellular ROS into transcriptional regulation of lung inflammation induced by cigarette smoke: the role of influxed Ca^{2+}. *Mediators Inflamm*. 2015; 2015:148367. https://doi: 10.1155/2015/148367.

Liu Z, Wang P, Lu S, Guo R, Gao W, Tong H, Yin Y, Han X, Liu T, Chen X, Zhu MX, Yang Z. Liquiritin, a novel inhibitor of TRPV1 and TRPA1, protects against LPS-induced acute lung injury. *Cell Calcium*. 2020; 88:102198. https://doi: 10.1016/j.ceca.2020.102198.

Lu Q, Zemskov EA, Sun X, Wang H, Yegambaram M, Wu X, Garcia-Fores A, Song S, Tang H, Kangath A, Cabanillas GZ, Yuan JX, Wang T, Fineman JR, Black SM. Activation of the mechanosensitive Ca(2+) channel TRPV4 induces endothelial barrier permeability via the disruption of mitochondrial bioenergetics. *Redox Biol*. 2021; 38:101785. https:// doi: 10.1016/j.redox.2020.101785.

Luostarinen S, Hämäläinen M, Hatano N, Muraki K, Moilanen E. The inflammatory regulation of TRPA1 expression in human A549 lung epithelial cells. *Pulm Pharmacol Ther*. 2021; 70:102059. https://doi: 10.1016/j.pupt.2021.102059.

Matthay MA, Zemans RL. The acute respiratory distress syndrome: pathogenesis and treatment. *Annu Rev Pathol*. 2011; 6:147-63. https://doi: 10.1146/annurev-pathol-011110-130158.

Müller I, Alt P, Rajan S, Schaller L, Geiger F, Dietrich A. Transient receptor potential (TRP) channels in airway toxicity and disease: an update. *Cells*. 2022; 11(18):2907. https://doi: 10.3390/cells11182907.

Naert R, López-Requena A, Talavera K. TRPA1 expression and pathophysiology in immune cells. *Int J Mol Sci*. 2021; 22(21):11460. https://doi: 10.3390/ijms222111460.

Nasseri S, Gurusamy M, Jung B, Lee D, Khang G, Doods H, Wu D. Kinin B1 receptor antagonist BI113823 reduces acute lung injury. *Crit Care Med*. 2015; 43(11):e499-507. https://doi: 10.1097/CCM.0000000000001268.

Nassini R, Pedretti P, Moretto N, Fusi C, Carnini C, Facchinetti F, Viscomi AR, Pisano AR, Stokesberry S, Brunmark C, Svitacheva N, McGarvey L, Patacchini R, Damholt AB, Geppetti P, Materazzi S. Transient receptor potential ankyrin 1 channel localized to non-neuronal airway cells promotes non-neurogenic inflammation. *PLoS One*. 2012; 7(8):e42454. https://doi: 10.1371/journal.pone.0042454.

O'Leary ST, Narwaney KJ, Wagner NM, Kraus CR, Omer SB, Glanz JM. Efficacy of a web-based intervention to increase uptake of maternal vaccines: an RCT. *Am J Prev Med*. 2019; 57(4):e125-e133. https://doi: 10.1016/j.amepre.2019.05.018.

Peck TJ, Hibbert KA. Recent advances in the understanding and management of ARDS. *F1000Res*. 2019; 8:1000 Faculty Rev-1959. https://doi: 10.12688/f1000research.20411.1.

Piirsalu M, Taalberg E, Lilleväli K, Tian L, Zilmer M, Vasar E. Treatment with lipopolysaccharide induces distinct changes in metabolite profile and body weight in 129Sv and Bl6 mouse strains. *Front Pharmacol*. 2020; 11:371. https://doi: 10.3389/fphar.2020.00371.

Rahaman SO, Grove LM, Paruchuri S, Southern BD, Abraham S, Niese KA, Scheraga RG, Ghosh S, Thodeti CK, Zhang DX, Moran MM, Schilling WP, Tschumperlin DJ,

Olman MA. TRPV4 mediates myofibroblast differentiation and pulmonary fibrosis in mice. *J Clin Invest*. 2014; 124(12):5225-38. https://doi: 10.1172/JCI75331.

Ratnasingham M, Bradding P, Roach KM. The role of TRP channels in lung fibrosis: mechanisms and therapeutic potential. *Int J Biochem Cell Biol*. 2025; 180:106728. https://doi: 10.1016/j.biocel.2024.106728.

Scheraga RG, Abraham S, Grove LM, Southern BD, Crish JF, Perelas A, McDonald C, Asosingh K, Hasday JD, Olman MA. TRPV4 protects the lung from bacterial pneumonia via MAPK molecular pathway switching. *J Immunol*. 2020; 204(5):1310–1321. https://doi: 10.4049/jimmunol.1901033.

Scheraga RG, Abraham S, Niese KA, Southern BD, Grove LM, Hite RD, et al. TRPV4 mechanosensitive ion channel regulates lipopolysaccharide-stimulated macrophage phagocytosis. *J Immunol*. 2016; 196(1):428–36. https://doi: 10.4049/jimmunol.1501688.

Seger S, Stritt M, Vezzali E, Nayler O, Hess P, Groenen PMA, Stalder AK. A fully automated image analysis method to quantify lung fibrosis in the bleomycin-induced rat model. *PLoS One*. 2018; 13:193057. https://doi: 10.1371/journal.pone.0193057.

Sert NP, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U, Emerson M, Garner P, Holgate ST, Howells DW, Hurst V, Karp NA, Lazic SE, Lidster K, MacCallum CJ, Macleod M, Pearl EJ, Petersen OH, Rawle F, Reynolds P, Rooney K, Sena ES, Silberberg SD, Steckler T, Würbel H. Reporting animal research: explanation and elaboration for the ARRIVE guidelines 2.0. *PLoS Biol*. 2020. https://doi: 10.1371/journal.pbio.3000411.

Thorneloe KS, Cheung M, Bao W, Alsaid H, Lenhard S, Jian MY, et al. An orally active TRPV4 channel blocker prevents and resolves pulmonary edema induced by heart failure. *Sci Transl Med*. 2012; 4(159):ra48–48. https://doi: 10.1126/scitranslmed.3004276.

Toumpanakis D, Chatzianastasiou A, Vassilakopoulou V, Mizi E, Dettoraki M, Perlikos F, Giatra G, Mikos N, Theocharis S, Vassilakopoulos T. TRPV4 inhibition exerts protective effects against resistive breathing induced lung injury. *Int J Chron Obstruct Pulmon Dis*. 2022; 17:343-353. https://doi: 10.2147/COPD.S336108.

Van Hoecke L, Job ER, Saelens X, Roose K. Bronchoalveolar lavage of murine lungs to analyze inflammatory cell infiltration. *J Vis Exp*. 2017; 123:55398. https://doi: 10.3791/55398.

Verma N, Hochhegger B, Mukhopadhyay S, Teixeira E Silva Torres PP, Mohammed TL. Acute lung injury. *J Thorac Imaging*. 2025; 40(3):e0820. https://doi: 10.1097/RTI.0000000000000820.

Wang M, Zhang Y, Cai X, Yang S, Sun S, Zhou S, Lv W, Du N, Li Y, Ma C, Ren K, Liu M, Tang B, Wang A, Chen X, Li P, Lv K, Zheng Z. Exploration and structure-activity relationship research of benzenesulfonamide derivatives as potent TRPV4 inhibitors for treating acute lung injury. *Bioorg Chem*. 2024; 147:107396. https://doi: 10.1016/j.bioorg.2024.107396.

Weber J, Rajan S, Schremmer C, Chao YK, Krasteva-Christ G, Kannler M, Yildirim AÖ, Brosien M, Schredelseker J, Weissmann N, Grimm C, Gudermann T, Dietrich A. TRPV4 channels are essential for alveolar epithelial barrier function as protection from lung edema. *JCI Insight*. 2020; 5(20):e134464. https://doi: 10.1172/jci.insight.134464.

Weng J, Liu Q, Li C, Feng Y, Chang Q, Xie M, Wang X, Li M, Zhang H, Mao R, Zhang N, Yang X, Chung KF, Adcock IM, Huang Y, Li F. TRPA1-PI3K/Akt-OPA1-ferroptosis axis in ozone-induced bronchial epithelial cell and lung injury. *Sci Total Environ*. 2024; 918:170668. https://doi: 10.1016/j.scitotenv.2024.170668.

Yang HH, Duan JX, Liu SK, Xiong JB, Guan XX, Zhong WJ, Sun CC, Zhang CY, Luo XQ, Zhang YF, Chen P, Hammock BD, Hwang SH, Jiang JX, Zhou Y, Guan CX. A COX-2/sEH dual inhibitor PTUPB alleviates lipopolysaccharide-induced acute lung injury in mice by inhibiting NLRP3 inflammasome activation. *Theranostics*. 2020; 10(11):4749-4761. https://doi: 10.7150/thno.43108.

Yang Y, Xiao Z, Yang W, Sun Y, Sui X, Lin X, Yang X, Bao Z, Cui Z, Ma Y, Li W, Wang S, Yang J, Wang Y, Luo Y. Role of transient receptor potential ankyrin 1 in idiopathic pulmonary fibrosis: modulation of M2 macrophage polarization. *Cell Mol Life Sci*. 2024; 81(1):187. https://doi: 10.1007/s00018-024-05219-x.

Yap JMG, Ueda T, Takeda N, Fukumitsu K, Fukuda S, Uemura T, Tajiri T, Ohkubo H, Maeno K, Ito Y, Kanemitsu Y, Niimi A. An inflammatory stimulus sensitizes TRPA1 channel to increase cytokine release in human lung fibroblasts. *Cytokine*. 2020; 129:155027. https://doi: 10.1016/j.cyto.2020.155027.

Yu Y, Li X, Qu L, Chen Y, Dai Y, Wang M, Zou W. DXXK exerts anti-inflammatory effects by inhibiting the lipopolysaccharide-induced NF-κB/COX-2 signalling pathway and the expression of inflammatory mediators. *J Ethnopharmacol*. 2016; 178:199–208. https://doi: 10.1016/j.jep.2015.11.016.

Zhang H, Wang C, Zhang K, Kamau PM, Luo A, Tian L, Lai R. The role of TRPA1 channels in thermosensation. *Cell Insight*. 2022; 1(6):100059. https://doi: 10.1016/j.cellin.2022.100059.

Zhang J, Guo Y, Mak M, Tao Z. Translational medicine for acute lung injury. *J Transl Med*. 2024; 22:25. https://doi: 10.1186/s12967-023-04828-7.

Zhang M, Ma Y, Ye X, Zhang N, Pan L, Wang B. TRP (transient receptor potential) ion channel family: structures, biological functions and therapeutic interventions for

diseases. *Sig Transduct Target Ther*. 2023; 8:261. https://doi: 10.1038/s41392-023-01464-x.

Zhou W, Li H, Zhang Y, Huang M, He Q, Li P, Zhang F, Shi Y, Su X. Diagnostic value of galactomannan antigen test in serum and bronchoalveolar lavage fluid samples from patients with nonneutropenic invasive pulmonary aspergillosis. *J Clin Microbiol*. 2017; 55:2153-2161. https://doi: 10.1128/JCM.00345-17.

4. CONCLUSÃO FINAL

A SDRA é uma condição grave e multifatorial, caracterizada por inflamação pulmonar difusa, aumento da permeabilidade da barreira alveolo-capilar, acúmulo de líquido no espaço alveolar e comprometimento severo das trocas gasosas. Clinicamente, a SDRA está associada a elevadas taxas de mortalidade e morbidade, sendo frequentemente desencadeada por sepse, trauma, pneumonia ou aspiração de conteúdo gástrico. Sua fisiopatologia envolve uma complexa rede de mediadores inflamatórios, células imunes e vias de sinalização que atuam de forma coordenada na lesão e disfunção pulmonar, progredindo por fases inflamatórias, proliferativas e fibróticas.

Apesar dos avanços no suporte ventilatório e cuidados intensivos, não existem terapias farmacológicas específicas capazes de modificar significativamente o curso da SDRA, o que torna o desenvolvimento de novas estratégias terapêuticas uma necessidade urgente. Assim, compreender os mecanismos moleculares que regulam a resposta inflamatória e a progressão da lesão pulmonar é fundamental para identificar alvos terapêuticos inovadores.

Neste contexto, a sinalização mediada pelos receptores de bradicinina B_1 e B_2 emerge como um elemento central no desencadeamento e na manutenção dessa resposta. A ativação desses receptores metabotrópicos ocorre em resposta ao aumento dos níveis de bradicinina durante o processo inflamatório, promovendo uma série de eventos intracelulares que culminam na sensibilização e ativação dos canais TRP, especialmente TRPV1, TRPA1 e TRPV4, expressos em neurônios sensoriais e células pulmonares.

A ativação dos canais TRP resulta na entrada de íons cálcio e sódio, despolarizando as membranas celulares e desencadeando a liberação de mediadores pró-inflamatórios, incluindo citocinas. Esses mediadores ampliam a resposta inflamatória local, promovendo recrutamento de células imunes e perpetuando a ativação dos receptores de bradicinina, configurando assim um ciclo de retroalimentação que sustenta e exacerba a inflamação pulmonar. Além disso, a ativação dos canais TRP está associada à neuroinflamação e à liberação de substâncias neurogênicas que contribuem para alterações vasculares, aumento da permeabilidade capilar e edema alveolar.

Os resultados desta tese demonstram que a inibição dos receptores B_1/B_2 e dos canais TRP são capazes de interromper esse ciclo vicioso, reduzindo a ativação dos canais TRP, a liberação de citocinas e, consequentemente, a intensidade da resposta inflamatória e o dano pulmonar em modelo experimental de LPA induzida por LPS. A modulação paralela dos canais TRPV1, TRPA1 e TRPV4 sugere que esses canais atuam de maneira complementar dentro dessa rede inflamatória fortalecendo a hipótese de um eixo coordenado de sinalização.

Portanto, a sinalização de bradicinina atua como um ponto de convergência na ativação de múltiplos canais TRP, cuja regulação integrada se mostra fundamental para a progressão da LPA. Esta compreensão abre caminhos para o desenvolvimento de estratégias farmacológicas que visem o bloqueio combinado destes receptores e canais, com o objetivo de interromper a cascata inflamatória e melhorar os desfechos clínicos da SDRA.

Apesar de o modelo de LPA induzido por LPS ser amplamente utilizado e aceito como ferramenta experimental, ele não reproduz integralmente a complexidade e heterogeneidade da SDRA humana, ressaltando a necessidade de estudos adicionais com modelos complementares, como ventilação mecânica, isquemia-reperfusão e lesão pulmonar induzida por ácido, capazes de reproduzir diferentes aspectos da fisiopatologia humana.

Futuras abordagens experimentais que explorem a administração combinada de antagonistas de canais TRP e bloqueadores de receptores B_1/B_2, associadas a métodos quantitativos como a análise isobolográfica, poderão elucidar de forma precisa o potencial aditivo ou sinérgico dessas estratégias. A validação dessa interação em modelos pré-clínicos mais complexos poderá fortalecer a aplicabilidade translacional e abrir caminho para o desenvolvimento de terapias inovadoras direcionadas à modulação da resposta inflamatória pulmonar.

Em síntese, os achados desta tese indicam que a sinalização mediada por bradicinina constitui um ponto-chave na ativação e sensibilização de canais TRP pró-inflamatórios e que sua modulação representa uma via estratégica e inovadora para conter a progressão da LPA e, potencialmente, da SDRA. A compreensão mais aprofundada dessa rede de sinalização poderá, no futuro, contribuir para a formulação de terapias direcionadas capazes de reduzir a mortalidade e melhorar o prognóstico de pacientes acometidos por essa grave síndrome.

REFERÊNCIAS

Abraham WM, Scuri M, Farmer SG. Peptide and non-peptide bradykinin receptor antagonists: role in allergic airway disease. Eur J Pharmacol. 2006; 533:215-21. https://doi: 10.1016/j.ejphar.2005.12.071

Achanta S, Jordt SE. Transient receptor potential channels in pulmonary chemical injuries and as countermeasure targets. Ann N Y Acad Sci. 2020; 1480(1):73-103. https://doi:10.1111/nyas.14472

Adapala RK, Katari V, Teegala LR, Thodeti S, Paruchuri S, Thodeti CK. TRPV4 Mechanotransduction in Fibrosis. Cells. 2021; 10(11):3053. https://doi:10.3390/cells10113053

Adcock JJ. TRPV1 receptors in sensitisation of cough and pain reflexes. Pulm Pharmacol Ther. 2009; 22:65-70. https://doi: 10.1016/j.pupt.2008.12.014

Ali H, Khan A, Ali J, Ullah H, Khan A, Ali H, Irshad N, Khan S. Attenuation of LPS-induced acute lung injury by continentalic acid in rodents through inhibition of inflammatory mediators correlates with increased Nrf2 protein expression. BMC Pharmacol Toxicol. 2021; 21(1):81. https://doi:10.1186/s40360-020-00458-7

Al-Shamlan F, El-Hashim AZ. Bradykinin sensitizes the cough reflex via a B2 receptor dependent activation of TRPV1 and TRPA1 channels through metabolites of cyclooxygenase and 12-lipoxygenase. Respir Res. 2019; 20:110. https://doi:10.1186/s12931-019-1060-8

Alvarez DF, King JA, Weber D, Addison E, Liedtke W, Townsley MI. Transient receptor potential vanilloid 4-mediated disruption of the alveolar septal barrier: a novel mechanism of acute lung injury. Circ Res. 2006; 99:988–995. https://doi:10.1161/01.RES.0000247065.11756.19

Amarato MBP, Carvalho CRR, Vieira S, Isola A, Rotman V, Moock M, José A, Franca SA. Mechanical ventilation in the acute lung injury/acute respiratory distress syndrome. Rev. bras. ter. intensiva. 2007; 19. https://doi.org/10.1590/S0103-507X2007000300020

Amorim MA, Jentsch Matias de Oliveira JR, Souza Oliveira VH, Cabrini DA, Otuki MF, André E. Role of nitric oxide, bradykinin B2 receptor, and TRPV1 in the airway alterations caused by simvastatin in rats. Eur J Pharmacol. 2021; 912:174591. https://doi:10.1016/j.ejphar.2021.174591

Amorim MA, Souza Oliveira VH, Calixto JB, André E. Functional interplay between bradykinin receptors and transient receptor potential vanilloid-1 in lipopolysaccharide-induced acute lung injury in mice. Pulm Pharmacol Ther. 2025; 90:102384. https://doi:10.1016/j.pupt.2025.102384

An X, Sun X, Hou Y, Yang X, Chen H, Zhang P, Wu J. Protective effect of oxytocin on LPS-induced acute lung injury in mice. Sci Rep. 2019; 9:2836. https://doi:10.1038/s41598-019-39349-1

Andrè E, Campi B, Materazzi S, Trevisani M, Amadesi S, Massi D, Creminon C, Vaksman N, Nassini R, Civelli M, Baraldi PG, Poole DP, Bunnett NW, Geppetti P, Patacchini R. Cigarette smoke-induced neurogenic inflammation is mediated by alpha,beta-unsaturated aldehydes and the TRPA1 receptor in rodents. J Clin Invest. 2008; 118(7):2574-2582. https://doi:10.1172/JCI34886

ARDS Definition Task Force; Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E, Fan E, Camporota L, Slutsky AS. Acute respiratory distress syndrome: the Berlin Definition. JAMA. 2012; 307(23):2526-33. https://doi: 10.1001/jama.2012.5669. PMID: 22797452

Balakrishna S, Song W, Achanta S, Doran SF, Liu B, Kaelberer MM, Yu Z, Sui A, Cheung M, Leishman E, Eidam HS, Ye G, Willette RN, Thorneloe KS, Bradshaw HB, Matalon S, Jordt SE. TRPV4 inhibition counteracts edema and inflammation and improves pulmonary function and oxygen saturation in chemically induced acute lung injury. Am J Physiol Lung Cell Mol Physiol. 2014; 307(2):158-172. https://doi:10.1152/ajplung.00065.2014

Balestrini A, Joseph V, Dourado M, Reese RM, Shields SD, Rougé L, Bravo DD, Chernov-Rogan T, Austin CD, Chen H, Wang L, Villemure E, Shore DGM, Verma VA, Hu B, Chen Y, Leong L, Bjornson C, Hötzel K, Gogineni Lee WP, Suto E, Wu X, Liu J, Zhang J, Gandham V, Wang J, Payandeh J, Ciferri C, Estevez A, Arthur CP, Kortmann J, Wong RL, Heredia JE, Doerr J, Jung M, Vander Heiden JA, Roose-Girma M, Tam L, Barck KH, Carano RAD, Ding HT, Brillantes B, Tam C, Yang X, Gao SS, Ly JQ, Liu L, Chen L, Liederer BM, Lin JH, Magnuson S, Chen J, Hackos DH, Elstrott J, Rohou A, Safina BS, Volgraf M, Bauer RN, Riol-Blanco L. A TRPA1 inhibitor suppresses neurogenic inflammation and airway contraction for asthma treatment. J Exp Med. 2021; 218(4):20201637. https://doi:10.1084/jem.20201637

Ballard-Croft C, Wang D, Sumpter LR, Zhou X, Zwischenberger JB. Large-animal models of acute respiratory distress syndrome. Ann Thorac Surg. 2012; 93(4):1331-1339. https://doi: 10.1016/j.athoracsur.2011.06.107

Bandell M, Story GM, Hwang SW, Viswanath V, Eid SR, Petrus MJ, Earley TJ, Patapoutian A. Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. Neuron. 2004; 41(6):849-857. https://doi:10.1016/s0896-6273(04)00150-3

Bautista DM, Pellegrino M, Tsunozaki M. TRPA1: A gatekeeper for inflammation. Annu Rev Physiol. 2013; 75:181-200. https://doi:10.1146/annurev-physiol-030212-183811

Baxter M, Eltom S, Dekkak B, Yew-Booth L, Dubuis ED, Maher SA, Belvisi MG, Birrell MA. Role of transient receptor potential and pannexin channels in cigarette smoke-

triggered ATP release in the lung. Thorax. 2014; 69:1080-1089. https://doi: 10.1136/thoraxjnl-2014-205467

Bellani G, Laffey JG, Pham T, Fan E; LUNG SAFE Investigators and the ESICM Trials Group. The LUNG SAFE study: a presentation of the prevalence of ARDS according to the Berlin Definition! Crit Care. 2016; 20(1):268. https://doi: 10.1186/s13054-016-1443-x

Belvisi MG, Birrell MA. The emerging role of transient receptor potential channels in chronic lung disease. Eur Respir J. 2017; 50(2):1601357. https://doi: 10.1183/13993003.01357-2016

Benemei S, Patacchini R, Trevisani M, Geppetti P. TRP channels. Curr Opin Pharmacol. 2015; 22:18-23. https://doi: 10.1016/j.coph.2015.02.006

Bernard GR, Artigas A, Brigham KL, Carlet J, Falke K, Hudson L, Lamy M, Legall JR, Morris A, Spragg R. The American-European Consensus Conference on ARDS. Definitions, mechanisms, relevant outcomes, and clinical trial coordination. Am J Respir Crit Care Med. 1994; 149:818-24. https://doi: 10.1164/ajrccm.149.3.7509706

Bernardo LR, Ferreira LKDP, Ferreira LAMP, Vieira CID, Oliveira JB, Lima LM, Alves AF, Araújo RS, Maia MS, Scotti MT, Barbosa Filho JM, Piuvezam MR. Milonine attenuates the lipopolysaccharide-induced acute lung injury in mice by modulating the Akt/NF-κB signaling pathways. *An Acad Bras Cienc*. 2022; 94:e20211327. https://doi: 10.1590/0001-3765202220211327

Bessac BF, Jordt SE. Breathtaking TRP channels: TRPA1 and TRPV1 in airway chemosensation and reflex control. *Physiology (Bethesda)*. 2008; 23:360-70. https://doi: 10.1152/physiol.00026.2008

Bonvini SJ, Belvisi MG. Cough and airway disease: The role of ion channels. Pulm Pharmacol Ther. 2017; 47:21-28. https://doi: 10.1016/j.pupt.2017.06.009

Bonvini SJ, Birrell MA, Grace MS, Maher SA, Adcock JJ, Wortley MA, Dubuis E, Ching YM, Ford AP, Shala F, Miralpeix M, Tarrason G, Smith JA, Belvisi MG. Transient receptor potential cation channel, subfamily V, member 4 and airway sensory afferent activation: Role of adenosine triphosphate. J Allergy Clin Immunol. 2016; 138(1):249-261.e12. https://doi: 10.1016/j.jaci.2015.10.044

Bos LDJ, Ware LB. Acute respiratory distress syndrome: causes, pathophysiology, and phenotypes. *Lancet*. 2022; 400(10358):1145-1156. https://doi: 10.1016/S0140-6736(22)01485-4

Boyle AJ, Mac Sweeney R, McAuley DF. Pharmacological treatments in ARDS; a state-of-the-art update. BMC Med. 2013; 20(11):166. https://doi: 10.1186/1741-7015-11-166

Cabral LD, Giusti-Paiva A. The Transient Receptor Potential Vanilloid 1 Antagonist Capsazepine Improves the Impaired Lung Mechanics during Endotoxemia. Basic Clin Pharmacol Toxicol. 2016;119(5):421-427. https://doi: 10.1111/bcpt.12605

Cai J, Xu G, Lin Y, Zhou B, Luo Z, Yu S, Lu J. Inhibition of TRPV4 attenuates ferroptosis against LPS-induced ALI via Ca2+ pathway. *Turk J Biol*. 2022; 46(6):465-474. https://doi: 10.55730/1300-0152.2632

Calixto JB, Cabrini DA, Ferreira J, Campos MM. Inflammatory pain: kinins and antagonists. Curr Opin Anaesthesiol. 2001; 14:519-26. https://doi: 10.1097/00001503-200110000-00010

Calixto JB, Medeiros R, Fernandes ES, Ferreira J, Cabrini DA, Campos MM. Kinin B1 receptors: key G-protein-coupled receptors and their role in inflammatory and painful processes. Br J Pharmacol. 2004; 143:803-18. https://doi: 10.1038/sj.bjp.0706012

Calzetta L, Page C, Matera MG, Cazzola M, Rogliani P. Drug–drug interactions and synergy: from pharmacological models to clinical application. *Pharmacol Rev*. 2024; 76:1159–1220. https://doi: 10.1124/pharmrev.124.000951

Campanholle G, Landgraf RG, Borducchi E, Semedo P, Wang PH, Amano MT, Russo M, Pacheco-Silva A, Jancar S, Camara NO. Bradykinin inducible receptor is essential to lipopolysaccharide-induced acute lung injury in mice. Eur J Pharmacol. 2010; 634(1-3):132-7. https://doi: 10.1016/j.ejphar.2010.02.002

Campos MM, Souza GE, Calixto JB. Role of bradykinin B1 and B2 receptors in inflammatory nociception: insights from experimental and clinical studies. Curr Drug Targets. 2012; 13:312-23

Cepkova M, Matthay MA. Pharmacotherapy of acute lung injury and the acute respiratory distress syndrome. J Intensive Care Med. 2006; 21(3):119-43. https://doi: 10.1177/0885066606287045

Cernit V, Sénécal J, Othman R, Couture R. Reciprocal Regulatory Interaction between TRPV1 and Kinin B1 Receptor in a Rat Neuropathic Pain Model. Int J Mol Sci. 2020; 21(3):821. https://doi: 10.3390/ijms21030821

Chanteux H, Guisset AC, Pilette C, Sibille Y. LPS induces IL-10 production by human alveolar macrophages via MAPKinases- and Sp1-dependent mechanisms. Respir Res. 2007; 8:71. https://doi: 10.1186/1465-9921-8-71

Chen H, Bai C, Wang X. The value of the lipopolysaccharide-induced acute lung injury model in respiratory medicine. Expert Rev Respir Med. 2010; 4(6):773-83. https://doi: 10.1586/ers.10.71

Chen J, Sun W, Zhu Y, Zhao F, Deng S, Tian M, Wang Y, Gong Y. TRPV1: The key bridge in neuroimmune interactions. J Intensive Med. 2024; 4:442-52. https://doi: 10.1016/j.jointm.2024.01.008

Choi JY, Lee HY, Hur J, Kim KH, Kang JY, Rhee CK, Lee SY. TRPV1 Blocking Alleviates Airway Inflammation and Remodeling in a Chronic Asthma Murine Model. Allergy Asthma Immunol Res. 2018; 10(3):216-224. https://doi: 10.4168/aair.2018.10.3.216

Chuang HH, Prescott ED, Kong H, Shields S, Jordt SE, Basbaum AI, Chao MV, Julius D. Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)P2-mediated inhibition. *Nature*. 2001; 411:957–962. https://doi: 10.1038/35082088

Confalonieri M, Salton F, Fabiano F. Acute respiratory distress syndrome. Eur Respir Rev. 2017; 26(144):160116. https://doi: 10.1183/16000617.0116-2016

Correa CR, Kyle DJ, Chakraverty S, Calixto JB. Antinociceptive profile of the pseudopeptide B2 bradykinin receptor antagonist NPC 18688 in mice. *Br J Pharmacol*. 1996; 117:552-558. https://doi: 10.1111/j.1476-5381.1996.tb15226.x

Cosens D., Manning, A. Abnormal Electroretinogram from a *Drosophila* Mutant. *Nature* **224**, 285–287 (1969)

Costa R, Bicca MA, Manjavachi MN, Segat GC, Dias FC, Fernandes ES, Calixto JB. Kinin receptors sensitize TRPV4 channel and induce mechanical hyperalgesia: relevance to paclitaxel-induced peripheral neuropathy in mice. *Mol Neurobiol*. 2018. https://doi: 10.1007/s12035-017-0475-9

Crowe AR, Yue W. Semi-quantitative determination of protein expression using immunohistochemistry staining and analysis: an integrated protocol. *Bio Protoc*. 2019; 9:3465. https://doi: 10.21769/BioProtoc.3465

D'Alessio FR. Mouse models of acute lung injury and ARDS. *Methods Mol Biol*. 2018; 1809:341-350. https://doi: 10.1007/978-1-4939-8570-8_22

Dalsgaard T, Sonkusare SK, Teuscher C, Poynter ME, Nelson MT. Pharmacological inhibitors of TRPV4 channels reduce cytokine production, restore endothelial function and increase survival in septic mice. *Sci Rep*. 2016; 6:33841. https://doi: 10.1038/srep33841

De Campos RO, Alves RV, Kyle DJ, Chakravarty S, Mavunkel BJ, Calixto JB. Antioedematogenic and antinociceptive actions of NPC 18521, a novel bradykinin B2 receptor antagonist. Eur J Pharmacol. 1996; 316:277-86. https://doi: 10.1016/S0014-2999(96)00661-9

De Logu F, Patacchini R, Fontana G, Geppetti G. TRP functions in the broncho-pulmonary system. Semin Immunopathol. 2016; 38:321-9. https://doi: 10.1007/s00281-016-0557-1

De Oliveira JR, Otuki MF, Cabrini DA, Brusco I, Oliveira SM, Ferreira J, André E. Involvement of the TRPV1 receptor in plasma extravasation in airways of rats treated with an angiotensin-converting enzyme inhibitor. *Pulm Pharmacol Ther*. 2016; 41:25-33. https://doi: 10.1016/j.pupt.2016.09.001

De Oliveira JRJM, Amorim MA, Oliveira VHS, Cabrini DA, Otuki MF, Galindo CM, da Luz BB, Werner MFP, Calixto JB, André E. Repeated doses of captopril induce airway hyperresponsiveness by modulating the TRPV1 receptor in rats. *Pulm Pharmacol Ther*. 2024; 86:102302. https://doi: 10.1016/j.pupt.2024.102302

De Virgiliis F, Di Giovanni S. Lung innervation in the eye of a cytokine storm: neuroimmune interactions and COVID-19. Nat Rev Neurol. 2020; 16(11):645-652. https://doi: 10.1038/s41582-020-0402-y

De Waal Malefyt R, Abrams J, Bennett B, Figdor CG, De Vries JE. Interleukin-10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. J Exp Med. 1991; 174:1209-1220. https://doi: 10.1084/jem.174.5.1209

Descoeur J, Pereira V, Pizzoccaro A, Francois A, Ling B, Maffre V, Couette B, Busserolles J, Courteix C, Noel J, Lazdunski M, Eschalier A, Authier N, Bourinet E. Oxaliplatin-induced cold hypersensitivity is due to remodelling of ion channel expression in nociceptors. *EMBO Mol Med*. 2011; 3(5):266-278. https://doi: 10.1002/emmm.201100134

Devesa I, Planells-Cases R, Fernández-Ballester G, González-Ros JM, Ferrer-Montiel A, Fernández-Carvajal A. Role of the transient receptor potential vanilloid 1 in inflammation and sepsis. J Inflamm Res. 2011;4:67-81. https://doi: 10.2147/JIR.S12978

Diamond M, Peniston HL, Sanghavi DK, Mahapatra S. Acute Respiratory Distress Syndrome. 2024 Jan 31. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025 Jan–

Dietrich A. Transient Receptor Potential (TRP) Channels in Health and Disease. Cells. 2019; 8:413. https://doi: 10.3390/cells8050413

Docherty RJ, Yeats JC, Bevan S, Boddeke HW. Inhibition of calcineurin inhibits the desensitization of capsaicin-evoked currents in cultured dorsal root ganglion neurons. *Neuroscience*. 1997; 78:877–886. https://doi: 10.1007/s004240050074

Donavan C, Hansbro PM. TRPA1: A potential target for cold-induced airway disease? Respirology. 2019 24 (3) 193-194. doi: 10.1111/resp.13453

Dong Q, Li J, Wu QF, Zhao N, Qian C, Ding D, Wang BB, Chen L, Guo KF, Fu D, Han B, Liao YH, Du YM. Blockage of transient receptor potential vanilloid 4 alleviates myocardial ischemia/reperfusion injury in mice. *Sci Rep*. 2017; 7:42678. https://doi: 10.1038/srep42678

Du Q, Liao Q, Chen C, Yang X, Xie R, Xu J. The Role of Transient Receptor Potential Vanilloid 1 in Common Diseases of the Digestive Tract and the Cardiovascular and Respiratory System. Front Physiol. 2019; 10:1064. https://doi: 10.3389/fphys.2019.01064

Duan Y, Learoyd J, Meliton AY, Leff AR, Zhu X. Inhibition of Pyk2 blocks lung inflammation and injury in a mouse model of acute lung injury. Respir Res. 2012; 13:4. https://doi: 10.1186/1465-9921-13-4

Duitama M, Moreno Y, Santander SP, Casas Z, Sutachan JJ, Torres YP, Albarracín SL. TRP Channels as Molecular Targets to Relieve Cancer Pain. *Biomolecules* 2022, *12*, 1. https://doi.org/10.3390/biom12010001

Dushianthan A, Grocott MPW, Murugan GS, Wilkinson TMA, Postle AD. Pulmonary Surfactant in Adult ARDS: Current Perspectives and Future Directions. Diagnostics (Basel). 2023; 13(18):2964. https://doi: 10.3390/diagnostics13182964

Dutra RC, Bento AF, Leite DF, Manjavachi MN, Marcon R, Bicca MA, Pesquero JB, Calixto JB. The role of kinin B1 and B2 receptors in the persistent pain induced by experimental autoimmune encephalomyelitis (EAE) in mice: evidence for the involvement of astrocytes. *Neurobiol Dis*. 2013; 54:82-93. https://doi: 10.1016/j.nbd.2013.02.007

Dutra RC, Leite DF, Bento AF, Manjavachi MN, Patrício ES, Figueiredo CP, Pesquero JB, Calixto JB. The role of kinin receptors in preventing neuroinflammation and its clinical severity during experimental autoimmune encephalomyelitis in mice. PLoS One. 2011; 6:e27875. https://doi: 10.1371/journal.pone.0027875

Eberhardt M, Stueber T, de la Roche J, Herzog C, Leffler A, Reeh PW, Kistner K. TRPA1 and TRPV1 are required for lidocaine-evoked calcium influx and neuropeptide release but not cytotoxicity in mouse sensory neurons. PLoS One. 2017; 12(11):e0188008. https://doi: 10.1371/journal.pone.0188008

Estenssoro E, Dubin A. Síndrome de distrés respiratorio agudo [Acute respiratory distress syndrome]. Medicina (B Aires). 2016; 76(4):235-41

Fadanni GP, Calixto JB. Recent progress and prospects for anti-cytokine therapy in preclinical and clinical acute lung injury. Cytokine Growth Factor Rev. 2023; 71:13-25. https://doi: 10.1016/j.cytogfr.2023.07.002

Fan H, Cook JA. Molecular mechanisms of endotoxin tolerance. J Endotoxin Res. 2004; 10:71-84. https://doi: 10.1179/096805104225003997

Fan HC, Zhang X, McNaughton PA. Activation of the TRPV4 ion channel is enhanced by phosphorylation. *J Biol Chem*. 2009. https://doi: 10.1074/jbc.M109.028803

Ferreira J, da Silva GL, Calixto JB. Contribution of vanilloid receptors to the overt nociception induced by B2 kinin receptor activation in mice. Br J Pharmacol. 2004; 141:787-94. https://doi: 10.1038/sj.bjp.0705546

Fialho MFP, Brum ES, Becker G, Oliveira SM. TRPV4 Activation and its Intracellular Modulation Mediated by Kinin Receptors Contribute to Painful Symptoms Induced by Anastrozole. Mol Neurobiol. 2024 Mar;61(3):1627-1642. https://doi: 10.1007/s12035-023-03654-8

Fuller BM, Mohr NM, Skrupky L, Fowler S, Kollef MH, Carpenter CR. The use of inhaled prostaglandins in patients with ARDS: a systematic review and meta-analysis. Chest. 2015; 147(6):1510-1522. https://doi: 10.1378/chest.14-3161

Gebistorf F, Karam O, Wetterslev J, Afshari A. Inhaled nitric oxide for acute respiratory distress syndrome (ARDS) in children and adults. Cochrane Database Syst Rev. 2016; 2016(6):CD002787. https://doi: 10.1002/14651858.CD002787.pub3

Geng Y, Su S, Cao L, Yang T. Effect of PD-1 inhibitor combined with X-ray irradiation on the inflammatory microenvironment and lung tissue injury in mice. *J Inflamm Res*. 2022; 15:545-556. https://doi: 10.2147/JIR.S350112

Gouin O, L'Herondelle K, Lebonvallet N, Le Gall-Ianotto C, Sakka M, Buhé V, Plée-Gautier E, Carré JL, Lefeuvre L, Misery L, Le Garrec R. TRPV1 and TRPA1 in cutaneous neurogenic and chronic inflammation: pro-inflammatory response induced by their activation and their sensitization. *Protein Cell*. 2017; 8(9):644-661. https://doi: 10.1007/s13238-017-0395-5

Grace M, Birrell MA, Dubuis E, Maher SA, Belvisi MG. Transient receptor potential channels mediate the tussive response to prostaglandin E2 and bradykinin. *Thorax*. 2012. https://doi: 10.1136/thoraxjnl-2011-201443

Grace MS, Baxter M, Dubuis E, Birrell MA, Belvisi MG. Transient receptor potential (TRP) channels in the airway: role in airway disease. Br J Pharmacol. 2014; 171(10):2593-607. https://doi: 10.1111/bph.12538

Grace MS, Bonvini SJ, Belvisi MG, McIntyre P. Modulation of the TRPV4 ion channel as a therapeutic target for disease. Pharmacol Ther. 2017; 177:9-22. https://doi: 10.1016/j.pharmthera.2017.02.019

Gu Q, Lee LY. TRP channels in airway sensory nerves. Neurosci Lett. 2021; 748:135719. https://doi: 10.1016/j.neulet.2021.135719

Haeberle HA, Calov S, Martus P, Serna-Higuita LM, Koeppen M, Goll A, Bernard A, Zarbock A, Meersch M, Weiss R, Mehrländer M, Marx G, Putensen C, Bakchoul T, Magunia H, Nieswandt B, Mirakaj V, Rosenberger P. Inhaled prostacyclin therapy in the acute respiratory distress syndrome: a randomized controlled multicenter trial. Respir Res. 2023; 24(1):58. https://doi: 10.1186/s12931-023-02346-0

Hamanaka K, Jian MY, Townsley MI, King JA, Liedtke W, Weber DS, et al. TRPV4 channels augment macrophage activation and ventilator-induced lung injury. *Am J*

Physiol Lung Cell Mol Physiol. 2010; 299(3):353–62. https://doi: 10.1152/ajplung.00315.2009

Hendrickson KW, Peltan ID, Brown SM. The Epidemiology of Acute Respiratory Distress Syndrome Before and After Coronavirus Disease 2019. Crit Care Clin. 2021; 37(4):703-716. https:// https://doi: 10.1016/j.ccc.2021.05.001

Honari N, Shaban P, Nasseri S, Hosseini M. Ethanolic extract of Achillea wilhelmsii C. Koch improves pulmonary function and inflammation in LPS-induced acute lung injury mice. J Complement Integr Med. 2021; 19:261-7. https://doi: 10.1515/jcim-2021-0045

Hu Q, Liu H, Wang R, Yao L, Chen S, Wang Y. Capsaicin attenuates LPS-induced acute lung injury by inhibiting inflammation and autophagy through regulation of the TRPV1/AKT pathway. J Inflamm Res. 2024; 17:153-70. https://doi: 10.2147/JIR.S441141

Hu Y, Gu Q, Lin RL, Kryscio R, Lee LY. Calcium transient evoked by TRPV1 activators is enhanced by tumor necrosis factor-α in rat pulmonary sensory neurons. *Am J Physiol Lung Cell Mol Physiol*. 2010; 299:L483–L492. https://doi: 10.1152/ajplung.00111.2010

Huang X, Xiu H, Zhang S, Zhang G. The Role of Macrophages in the Pathogenesis of ALI/ARDS. Mediators Inflamm. 2018; 2018:1264913. https://doi: 10.1155/2018/1264913

Huppert LA, Matthay MA, Ware LB. Pathogenesis of Acute Respiratory Distress Syndrome. Semin Respir Crit Care Med. 2019; 40(1):31-39. https://doi: 10.1055/s-0039-1683996

Hussain H, Fadel A, Alwaeli H, Guardiola V. Coronavirus (COVID-19) Fulminant Myopericarditis and Acute Respiratory Distress Syndrome (ARDS) in a Middle-Aged Male Patient. Cureus. 2020; 12(6):e8808. https://doi: 10.7759/cureus.8808

Imai Y, Kuba K, Neely GG, Yaghubian-Malhami R, Perkmann T, van Loo G, Ermolaeva M, Veldhuizen R, Leung YH, Wang H, Liu H, Sun Y, Pasparakis M, Kopf M, Mech C, Bavari S, Peiris JS, Slutsky AS, Akira S, Hultqvist M, Holmdahl R, Nicholls J, Jiang C, Binder CJ, Penninger JM. Identification of oxidative stress and Toll-like receptor 4 signaling as a key pathway of acute lung injury. Cell. 2008; 133(2):235-49. https://doi: 10.1016/j.cell.2008.02.043

Inoue K, Takano H, Yanagisawa R, Hirano S, Kobayashi T, Ichinose T, Yoshikawa T. Effects of organic chemicals derived from ambient particulate matter on lung inflammation related to lipopolysaccharide. Arch Toxicol. 2006; 80:833-8. https://doi: 10.1007/s00204-006-0105-1

Jarczak D, Nierhaus A. Cytokine Storm-Definition, Causes, and Implications. Int J Mol Sci. 2022; 23(19):11740. https://doi: 10.3390/ijms231911740

Jentsch M, de Oliveira JR, Amorim MA, André E. The role of TRPA1 and TRPV4 channels in bronchoconstriction and plasma extravasation in airways of rats treated with captopril. *Pulm Pharmacol Ther*. 2020; 65:102004. https://doi: 10.1016/j.pupt.2021.102004

Jha A, Sharma P, Anaparti V, Ryu MH, Halayko AJ. A role for transient receptor potential ankyrin 1 cation channel (TRPA1) in airway hyper-responsiveness? Can J Physiol Pharmacol. 2015; 93(3):171-6. https://doi: 10.1139/cjpp-2014-0417

Jia Y, Lee LY. Role of TRPV receptors in respiratory diseases. Biochim Biophys Acta. 2007; 1772(8):915-27. https://doi: 10.1016/j.bbadis.2007.01.013

Kadam AH, Schnitzer JE. Insights into disease progression of translational preclinical rat model of interstitial pulmonary fibrosis through endpoint analysis. *Cells*. 2024; 13(6):515. https://doi: 10.3390/cells13060515

Kaku S, Nguyen CD, Htet NN, Tutera D, Barr J, Paintal HS, Kuschner WG. Acute Respiratory Distress Syndrome: Etiology, Pathogenesis, and Summary on Management. J Intensive Care Med. 2020; 35(8):723-737. https://doi: 10.1177/0885066619855021

Kim JH. Allergy Asthma Immunol Res. 2018; 10:187-8. https://doi: 10.4168/aair.2018.10.3.187

Ko HK, Lin AH, Perng DW, Lee TS, Kou YR. Lung epithelial TRPA1 mediates lipopolysaccharide-induced lung inflammation in bronchial epithelial cells and mice. *Front Physiol*. 2020; 11:596314. https://doi: 10.3389/fphys.2020.596314

Kocot N, Pękala E, Koczurkiewicz-Adamczyk P, Chłoń-Rzepa G, Łapa A, Wójcik-Pszczoła K. Airway and cardiovascular remodeling in chronic obstructive pulmonary disease (COPD) as a target for transient receptor potential ankyrin 1 (TRPA1) channel modulators. *Bioorg Chem*. 2025; 158:108301. https://doi: 10.1016/j.bioorg.2025.108301

Koivisto AP, Belvisi MG, Gaudet R, Szallasi A. Advances in TRP channel drug discovery: from target validation to clinical studies. Nat Rev Drug Discov. 2022; 21(1):41-59. https://doi: 10.1038/s41573-021-00268-4

Koskimäki S, Tojkander S. TRPV4-A multifunctional cellular sensor protein with therapeutic potential. *Sensors (Basel)*. 2024; 24(21):6923. https://doi: 10.3390/s24216923

Kulkarni HS, Lee JS, Bastarache JA, Kuebler WM, Downey GP, Albaiceta GM, Altemeier WA, Artigas A, Bates JHT, Calfee CS, Dela Cruz CS, Dickson RP, Englert JA, Everitt JI, Fessler MB, Gelman AE, Gowdy KM, Groshong SD, Herold S, Homer RJ, Horowitz JC, Hsia CCW, Kurahashi K, Laubach VE, Looney MR, Lucas R, Mangalmurti NS, Manicome AM, Martin TR, Matalon S, Matthay MA, McAuley DF, McGrath-Morrow SA, Mizgerd JP, Montgomery SA, Moore BB, Noël A, Perlman CE,

Reilly JP, Schmidt EP, Skerrett SJ, Suber TL, Summers C, Suratt BT, Takata M, Tuder R, Uhlig S, Witzenrath M, Zemans RL, Matute-Bello G. Update on the features and measurements of experimental acute lung injury in animals: An official American Thoracic Society workshop report. Am J Respir Cell Mol Biol. 2022; 66:1-14. https://doi: 10.1165/rcmb.2021-0531ST

Lee LY, Gu Q. Role of TRPV1 in inflammation-induced airway hypersensitivity. Curr Opin Pharmacol. 2009; 9(3):243-9. https://doi: 10.1016/j.coph.2009.02.002

Lee LY, Hsu CC, Lin YJ, Lin RL, Khosravi M. Interaction between TRPA1 and TRPV1: synergy on pulmonary sensory nerves. *Pulm Pharmacol Ther*. 2015; 35:87-93. https://doi: 10.1016/j.pupt.2015.08.003

Li M, Fan X, Yue Q, Hu F, Zhang Y, Zhu C. The neuro-immune interaction in airway inflammation through TRPA1 expression in CD4+ T cells of asthmatic mice. *Int Immunopharmacol*. 2020; 86:1–9. https://doi: 10.1016/j.intimp.2020.106696

Lin AH, Liu MH, Ko HK, Perng DW, Lee TS, Kou YR. Lung epithelial TRPA1 transduces the extracellular ROS into transcriptional regulation of lung inflammation induced by cigarette smoke: the role of influxed Ca^{2+}. *Mediators Inflamm*. 2015; 2015:148367. https://doi: 10.1155/2015/148367

Liu M, Jia X, Liu H, He R, Zhang X, Shao Y. Role of TRPV1 in respiratory disease and association with traditional Chinese medicine: A literature review. Biomed Pharmacother. 2022; 155:113676. https://doi: 10.1016/j.biopha.2022.113676

Liu Z, Wang P, Lu S, Guo R, Gao W, Tong H, Yin Y, Han X, Liu T, Chen X, Zhu MX, Yang Z. Liquiritin, a novel inhibitor of TRPV1 and TRPA1, protects against LPS-induced acute lung injury. *Cell Calcium*. 2020; 88:102198. https://doi: 10.1016/j.ceca.2020.102198

Lu Q, Zemskov EA, Sun X, Wang H, Yegambaram M, Wu X, Garcia-Fores A, Song S, Tang H, Kangath A, Cabanillas GZ, Yuan JX, Wang T, Fineman JR, Black SM. Activation of the mechanosensitive Ca(2+) channel TRPV4 induces endothelial barrier permeability via the disruption of mitochondrial bioenergetics. *Redox Biol*. 2021; 38:101785. https:// doi: 10.1016/j.redox.2020.101785

Luo L, Song Q, Dong Z, Yi S, Wang K, Zhuang X, Fu L, Yang X, Hei F. Transient receptor potential vanilloid 4 in acute lung injury: from mechanistic insights to therapeutic strategies. 2025 299, 118075. https://doi.org/10.1016/j.ejmech.2025.118075

Luostarinen S, Hämäläinen M, Hatano N, Muraki K, Moilanen E. The inflammatory regulation of TRPA1 expression in human A549 lung epithelial cells. *Pulm Pharmacol Ther*. 2021; 70:102059. https://doi: 10.1016/j.pupt.2021.102059

Luu DD, Owens AM, Mebrat MD, Van Horn WD. A molecular perspective on identifying TRPV1 thermosensitive regions and disentangling polymodal activation. Temperature (Austin). 2021; 10(1):67-101. https://doi: 10.1080/23328940.2021.1983354

Malagarie-Cazenave S, Olea-Herrero N, Vara D, Morell C, Díaz-Laviada I. The vanilloid capsaicin induces IL-6 secretion in prostate PC-3 cancer cells. Cytokine. 2011; 54(3):330-7. https://doi: 10.1016/j.cyto.2011.03.010

Matthay MA, Arabi Y, Arroliga AC, Bernard G, Bersten AD, Brochard LJ, Calfee CS, Combes A, Daniel BM, Ferguson ND, Gong MN, Gotts JE, Herridge MS, Laffey JG, Liu KD, Machado FR, Martin TR, McAuley DF, Mercat A, Moss M, Mularski RA, Pesenti A, Qiu H, Ramakrishnan N, Ranieri VM, Riviello ED, Rubin E, Slutsky AS, Thompson BT, Twagirumugabe T, Ware LB, Wick KD. A New Global Definition of Acute Respiratory Distress Syndrome. Am J Respir Crit Care Med. 2024; 209(1):37-47. https://doi: 10.1164/rccm.202303-0558WS

Matthay MA, Ware LB, Zimmerman GA. The acute respiratory distress syndrome. J Clin Invest. 2012; 122(8):2731-40. https://doi: 10.1172/JCI60331

Matthay MA, Zemans RL, Zimmerman GA, Arabi YM, Beitler JR, Mercat A, Herridge M, Randolph AG, Calfee CS. Acute respiratory distress syndrome. Nat Rev Dis Primers. 2019; 5(1):18. https://doi: 10.1038/s41572-019-0069-0

Matthay MA, Zemans RL. The acute respiratory distress syndrome: pathogenesis and treatment. *Annu Rev Pathol*. 2011; 6:147-63. https://doi: 10.1146/annurev-pathol-011110-130158

Matuschak GM, Lechner AJ. Acute lung injury and the acute respiratory distress syndrome: pathophysiology and treatment. Mo Med. 2010; 107(4):252-8

Matute-Bello G, Downey G, Moore BB, Groshong SD, Matthay MA, Slutsky AS, Kuebler WM; Acute Lung Injury in Animals Study Group. An official American Thoracic Society workshop report: features and measurements of experimental acute lung injury in animals. Am J Respir Cell Mol Biol. 2011 May;44(5):725-38. https://doi: 10.1165/rcmb.2009-0210ST

McAlexander MA, Luttmann MA, Hunsberger GE, Undem BJ. Transient receptor potential vanilloid 4 activation constricts the human bronchus via the release of cysteinyl leukotrienes. J Pharmacol Exp Ther. 2014; 349(1):118-25. https://doi: 10.1124/jpet.113.210203

McGonagle D, Sharif K, O'Regan A, Bridgewood C. The Role of Cytokines including Interleukin-6 in COVID-19 induced Pneumonia and Macrophage Activation Syndrome-Like Disease. Autoimmun Rev. 2020; 19(6):102537. https://doi: 10.1016/j.autrev.2020.102537

McNicholas BA, Madotto F, Pham T, Rezoagli E, Masterson CH, Horie S, Bellani G, Brochard L, Laffey JG; LUNG SAFE Investigators and the ESICM Trials Group. Demographics, management and outcome of females and males with acute respiratory distress syndrome in the LUNG SAFE prospective cohort study. Eur Respir J. 2019; 54(4):1900609. https://doi: 10.1183/13993003.00609-2019

Meotti FC, Figueiredo CP, Manjavachi M, Calixto JB. The transient receptor potential ankyrin-1 mediates mechanical hyperalgesia induced by the activation of B_1 receptor in mice. Biochem Pharmacol. 2017; 125:75-83. https://doi: 10.1016/j.bcp.2016.11.003

Meyer NJ, Gattinoni L, Calfee CS. Acute respiratory distress syndrome. Lancet. 2021; 398(10300):622-637. https://doi: 10.1016/S0140-6736(21)00439-6

Moore C, Gupta R, Jordt SE, Chen Y, Liedtke WB. Regulation of Pain and Itch by TRP Channels. Neurosci Bull. 2017; 34(1):120-142. https://doi: 10.1007/s12264-017-02008

Müller I, Alt P, Rajan S, Schaller L, Geiger F, Dietrich A. Transient receptor potential (TRP) channels in airway toxicity and disease: an update. *Cells*. 2022; 11(18):2907. https://doi: 10.3390/cells11182907

Naert R, López-Requena A, Talavera K. TRPA1 expression and pathophysiology in immune cells. *Int J Mol Sci*. 2021; 22(21):11460. https://doi: 10.3390/ijms222111460

Nahama A, Ramachandran R, Cisternas AF, Ji H. The role of afferent pulmonary innervation in ARDS associated with COVID-19 and potential use of resiniferatoxin to improve prognosis: A review. Med Drug Discov. 2020; 5:100033. https://doi: 10.1016/j.medidd.2020.100033

Nasseri S, Gurusamy M, Jung B, Lee D, Khang G, Doods H, Wu D. Kinin B1 Receptor Antagonist BI113823 Reduces Acute Lung Injury. Crit Care Med. 2015; 43(11):499-507. https://doi: 10.1097/CCM.0000000000001268

Nassini R, Pedretti P, Moretto N, Fusi C, Carnini C, Facchinetti F, Viscomi AR, Pisano AR, Stokesberry S, Brunmark C, Svitacheva N, McGarvey L, Patacchini R, Damholt AB, Geppetti P, Materazzi S. Transient receptor potential ankyrin 1 channel localized to non-neuronal airway cells promotes non-neurogenic inflammation. *PLoS One*. 2012; 7(8):42454. https://doi: 10.1371/journal.pone.0042454

Nazemi SS, Jomezadeh V, Khademi G, Rahsepar S, Hajzadeh G, Naseri M, BahramizadehM, Abedi F, Sezavar M, Mohammadpour AH. The effect of Pentoxifylline on the prevention of ARDS in PICU patients: a randomized, double-blind clinical trial. International Journal of Pediatrics. 2024 12(121) 18517-18529

Nguyen N, Xu S, Lam TYW, Liao W, Wong WSF, Ge R. ISM1 suppresses LPS-induced acute lung injury and post-injury lung fibrosis in mice. Mol Med. 2022; 28:72. https://doi: 10.1186/s10020-022-00500-w

O'Leary ST, Narwaney KJ, Wagner NM, Kraus CR, Omer SB, Glanz JM. Efficacy of a web-based intervention to increase uptake of maternal vaccines: an RCT. Am J Prev Med. 2019; 57:125-133. https://doi: 10.1016/j.amepre.2019.05.018

Papazian L, Pauly V, Hamouda I, Daviet F, Orleans V, Forel JM, Roch A, Hraiech S, Boyer L. National incidence rate and related mortality for acute respiratory distress syndrome in France. Anaesth Crit Care Pain Med. 2021; 40(1):100795. https://doi: 10.1016/j.accpm.2020.100795

Park WY, Goodman RB, Steinberg KP, Ruzinski JT, Radella 2nd F, Park DR, Pugin J, Skerrett SJ, Hudson LD, Martin TR. Cytokine balance in the lungs of patients with acute respiratory distress syndrome. *Am J Respir Crit Care Med*. 2001; 164:1896–1903. https://doi: 10.1164/ajrccm.164.10.2104013

Peck TJ, Hibbert KA. Recent advances in the understanding and management of ARDS. *F1000Res*. 2019; 8:1000 Faculty Rev-1959. https://doi: 10.12688/f1000research.20411.1

Piirsalu M, Taalberg E, Lilleväli K, Tian L, Zilmer M, Vasar E. Treatment with lipopolysaccharide induces distinct changes in metabolite profile and body weight in 129Sv and Bl6 mouse strains. *Front Pharmacol*. 2020; 11:371. https://doi: 10.3389/fphar.2020.00371

Potere N, Del Buono MG, Caricchio R, Cremer PC, Vecchié A, Porreca E, Gasperina DD, Dentali F, Abbate A, Bonaventura A. Interleukin-1 and the NLRP3 inflammasome in COVID-19: pathogenetic and therapeutic implications. *EBioMedicine*. 2022; 85:104299. https://doi: 10.1016/j.ebiom.2022.104299

Qadir N, Bartz RR, Cooter ML, Hough CL, Lanspa MJ, Banner-Goodspeed VM, Chen JT, Giovanni S, Gomaa D, Sjoding MW, Hajizadeh N, Komisarow J, Duggal A, Khanna AK, Kashyap R, Khan A, Chang SY, Tonna JE, Anderson HL 3rd, Liebler JM, Mosier JM, Morris PE, Genthon A, Louh IK, Tidswell M, Stephens RS, Esper AM, Dries DJ, Martinez A, Schreyer KE, Bender W, Tiwari A, Guru PK, Hanna S, Gong MN, Park PK; Severe ARDS: Generating Evidence (SAGE) Study Investigators; Society of Critical Care Medicine's Discovery Network. Variation in Early Management Practices in Moderate-to-Severe ARDS in the United States: The Severe ARDS: Generating Evidence Study. Chest. 2021; 160(4):1304-1315. https://doi:10.1016/j.chest.2021.05.047

Rabaan AA, Al-Ahmed SH, Muhammad J, Khan A, Sule AA, Tirupathi R, Mutair AA, Alhumaid S, Al-Omari A, Dhawan M, Tiwari R, Sharun K, Mohapatra RK, Mitra S, Bilal M, Alyami SA, Emran TB, Moni MA, Dhama K. Role of Inflammatory Cytokines in COVID-19 Patients: A Review on Molecular Mechanisms, Immune Functions, Immunopathology and Immunomodulatory Drugs to Counter Cytokine Storm. Vaccines (Basel). 2021; 9(5):436. https://doi: 10.3390/vaccines9050436

Rafikov R, Dimitropoulou C, Aggarwal S, Kangath A, Gross C, Pardo D, Sharma S, Jezierska-Drutel A, Patel V, Snead C, Lucas R, Verin A, Fulton D, Catravas JD, Black

SM. Lipopolysaccharide-induced lung injury involves the nitration-mediated activation of RhoA. J Biol Chem. 2014; 289:4710-22. https://doi: 10.1074/jbc.M114.547596

Ragab D, Salah Eldin H, Taeimah M, Khattab R, Salem R. The COVID-19 Cytokine Storm; What We Know So Far. Front Immunol. 2020 Jun 16;11:1446. https://doi: 10.3389/fimmu.2020.01446

Raghavendran K, Napolitano LM. ALI and ARDS: challenges and advances. Crit Care Clin. 2011 Jul;27(3):xiii-xiv. https://doi: 10.1016/j.ccc.2011.05.012

Rahaman SO, Grove LM, Paruchuri S, Southern BD, Abraham S, Niese KA, Scheraga RG, Ghosh S, Thodeti CK, Zhang DX, Moran MM, Schilling WP, Tschumperlin DJ, Olman MA. TRPV4 mediates myofibroblast differentiation and pulmonary fibrosis in mice. *J Clin Invest*. 2014; 124(12):5225-38. https://doi: 10.1172/JCI75331

Ratnasingham M, Bradding P, Roach KM. The role of TRP channels in lung fibrosis: mechanisms and therapeutic potential. *Int J Biochem Cell Biol*. 2025; 180:106728. https://doi: 10.1016/j.biocel.2024.106728

Reilly JP, Calfee CS, Christie JD. Acute Respiratory Distress Syndrome Phenotypes. Semin Respir Crit Care Med. 2019 Feb;40(1):19-30. https://doi: 10.1055/s-0039-1684049

Rex DAB, Vaid N, Deepak K, Dagamajalu S, Prasad TSK. A comprehensive review on current understanding of bradykinin in COVID-19 and inflammatory diseases. Mol Biol Rep. 2022; 49:9915-27. https://doi: 10.1007/s11033-022-07539-2

Ricciardolo FLM, Folkerts G, Folino A, Mognetti B. Bradykinin in asthma: Modulation of airway inflammation and remodelling. Eur J Pharmacol. 2018; 827:181-188. https://doi: 10.1016/j.ejphar.2018.03.017

Rigoni M, Trevisani M, Gazzieri D, Nadaletto R, Tognetto M, Creminon C, Davis JB, Campi B, Amadesi S, Geppetti P, Harrison S. Neurogenic responses mediated by vanilloid receptor-1 (TRPV1) are blocked by the high affinity antagonist, iodo-resiniferatoxin. Br J Pharmacol. 2003; 138:977-85. https://doi: 10.1038/sj.bjp.0705110

Rocco PRM, Nieman GF. ARDS: what experimental models have taught us. Intensive Care Med. 2016 May;42(5):806-810. https://doi: 10.1007/s00134-016-4268-9

Roche JA, Roche R. A hypothesized role for dysregulated bradykinin signaling in COVID-19 respiratory complications. FASEB J. 2020 Jun;34(6):7265-7269. https://doi: 10.1096/fj.202000967

Saguil A, Fargo MV. Acute Respiratory Distress Syndrome: Diagnosis and Management. Am Fam Physician. 2020; 101:730-738

Schäfers M, Sorkin L. Effect of cytokines on neuronal excitability. Neurosci Lett. 2008; 437:188-193. https://doi: 10.1016/j.neulet.2008.03.052

Scheraga RG, Abraham S, Grove LM, Southern BD, Crish JF, Perelas A, McDonald C, Asosingh K, Hasday JD, Olman MA. TRPV4 protects the lung from bacterial pneumonia via MAPK molecular pathway switching. *J Immunol*. 2020; 204(5):1310–1321. https://doi: 10.4049/jimmunol.1901033

Scheraga RG, Abraham S, Niese KA, Southern BD, Grove LM, Hite RD, et al. TRPV4 mechanosensitive ion channel regulates lipopolysaccharide-stimulated macrophage phagocytosis. *J Immunol*. 2016; 196(1):428–36. https://doi: 10.4049/jimmunol.1501688

Scheraga RG, Southern BD, Grove LM, Olman MA. The Role of Transient Receptor Potential Vanilloid 4 in Pulmonary Inflammatory Diseases. Front Immunol. 2017; 8:503. https://doi: 10.3389/fimmu.2017.00503

Seger S, Stritt M, Vezzali E, Nayler O, Hess P, Groenen PMA, Stalder AK. A fully automated image analysis method to quantify lung fibrosis in the bleomycin-induced rat model. *PLoS One*. 2018; 13:193057. https://doi: 10.1371/journal.pone.0193057

Sert NP, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U, Emerson M, Garner P, Holgate ST, Howells DW, Hurst V, Karp NA, Lazic SE, Lidster K, MacCallum CJ, Macleod M, Pearl EJ, Petersen OH, Rawle F, Reynolds P, Rooney K, Sena ES, Silberberg SD, Steckler T, Würbel H. Reporting animal research: Explanation and elaboration for the ARRIVE guidelines 2.0. PLoS Biol. 2020;18:e3000411. https://doi: 10.1371/journal.pbio.3000411

Shih LJ, Yang CC, Liao MT, Lu KC, Hu WC, Lin CP. An important call: Suggestion of using IL-10 as therapeutic agent for COVID-19 with ARDS and other complications. Virulence. 2023; 14(1):2190650. https://doi: 10.1080/21505594.2023.2190650

Short KR, Kroeze EJBV, Fouchier RAM, Kuiken T. Pathogenesis of influenza-induced acute respiratory distress syndrome. Lancet Infect Dis. 2014; 14(1):57-69. https://doi: 10.1016/S1473-3099(13)70286-X

Simonsen U, Wandall-Frostholm C, Oliván-Viguera A, Köhler R. Emerging roles of calcium-activated K channels and TRPV4 channels in lung oedema and pulmonary circulatory collapse. Acta Physiol (Oxf). 2017; 219(1):176-187. https://doi: 10.1111/apha.12768

Sodhi CP, Wohlford-Lenane C, Yamaguchi Y, Prindle T, Fulton WB, Wang S, McCray PB Jr, Chappell M, Hackam DJ, Jia H. Attenuation of pulmonary ACE2 activity impairs inactivation of des-Arg9 bradykinin/BKB1R axis and facilitates LPS-induced neutrophil infiltration. Am J Physiol Lung Cell Mol Physiol. 2018; 314:L17-31. https://doi: 10.1152/ajplung.00498.2016

Sweeney RM, McAuley DF. Acute respiratory distress syndrome. Lancet. 2016 Nov 12;388(10058):2416-2430. doi: 10.1016/S0140-6736(16)00578-X. Epub 2016 Apr 28. Erratum in: Lancet. 2016; 388(10058):2354. https://doi: 10.1016/S0140-6736(16)32136-5

Swenson KE, Swenson ER. Pathophysiology of Acute Respiratory Distress Syndrome and COVID-19 Lung Injury. Crit Care Clin. 2021; 37(4):749-776. https://doi: 10.1016/j.ccc.2021.05.003

Székács B, Várbíró S, Debreczeni L. High-dose ACEi might be harmful in COVID-19 patients with serious respiratory distress syndrome by leading to excessive bradykinin receptor activation. Physiol Int. 2021. https://doi: 10.1556/2060.2021.00007

Talbot S, Dias JP, Lahjouji K, Bogo MR, Campos MM, Gaudreau P, Couture R. Activation of TRPV1 by capsaicin induces functional kinin B(1) receptor in rat spinal cord microglia. J Neuroinflammation. 2012; 9:16. https://doi: 10.1186/1742-2094-9-16

Tao L, Cao F, Xu G, Xie H, Zhang M, Zhang C. Mogroside IIIE attenuates LPS-induced acute lung injury in mice partly through regulation of the TLR4/MAPK/NF-kB axis via AMPK activation. Phytother Res. 2017; 31:1097-106. https://doi: 10.1002/ptr.5833

Thomas KC, Roberts JK, Deering-Rice CE, Romero EG, Dull RO, Lee J, Yost GS, Reilly CA. Contributions of TRPV1, endovanilloids, and endoplasmic reticulum stress in lung cell death in vitro and lung injury. Am J Physiol Lung Cell Mol Physiol. 2012; 302:L111-9. https://doi: 10.1152/ajplung.00231.2011

Thorneloe KS, Cheung M, Bao W, Alsaid H, Lenhard S, Jian MY, Costell M, Maniscalco-Hauk K, Krawiec JA, Olzinski A, Gordon E, Lozinskaya I, Elefante L, Qin P, Matasic DS, James C, Tunstead J, Donovan B, Kallal L, Waszkiewicz A, Vaidya K, Davenport EA, Larkin J, Burgert M, Casillas LN, Marquis RW, Ye G, Eidam HS, Goodman KB, Toomey JR, Roethke TJ, Jucker BM, Schnackenberg CG, Townsley MI, Lepore JJ, Willette RN. An orally active TRPV4 channel blocker prevents and resolves pulmonary edema induced by heart failure. Sci Transl Med. 2012; 4(159):159ra148. https://doi: 10.1126/scitranslmed.3004276

Toumpanakis D, Chatzianastasiou A, Vassilakopoulou V, Mizi E, Dettoraki M, Perlikos F, Giatra G, Mikos N, Theocharis S, Vassilakopoulos T. TRPV4 inhibition exerts protective effects against resistive breathing induced lung injury. *Int J Chron Obstruct Pulmon Dis*. 2022; 17:343-353. https://doi: 10.2147/COPD.S336108

Van Hoecke L, Job ER, Saelens X, Roose K. Bronchoalveolar lavage of murine lungs to analyze inflammatory cell infiltration. *J Vis Exp*. 2017; 123:55398. https://doi: 10.3791/55398

Verma N, Hochhegger B, Mukhopadhyay S, Teixeira E Silva Torres PP, Mohammed TL. Acute lung injury. *J Thorac Imaging*. 2025; 40(3):0820. https://doi: 10.1097/RTI.0000000000000820

Wallace, H., 2019. Airway Pathogenesis Is Linked to TRP Channels, in: Neurobiology of TRP Channels. https://doi.org/10.4324/9781315152837-13

Wang J, Yang X, Li Y, Huang JA, Jiang J, Su N. Specific cytokines in the inflammatory cytokine storm of patients with COVID-19-associated acute respiratory distress

syndrome and extrapulmonary multiple-organ dysfunction. Virol J. 2021; 18(1):117. https://doi: 10.1186/s12985-021-01588-y

Wang JH. Blocking of Kinin B1 Receptor: A Promising Way for the Treatment of Acute Lung Injury. Crit Care Med. 2015; 43(11):2520-2. https://doi: 10.1097/CCM.0000000000001317

Wang M, Zhang Y, Cai X, Yang S, Sun S, Zhou S, Lv W, Du N, Li Y, Ma C, Ren K, Liu M, Tang B, Wang A, Chen X, Li P, Lv K, Zheng Z. Exploration and structure-activity relationship research of benzenesulfonamide derivatives as potent TRPV4 inhibitors for treating acute lung injury. *Bioorg Chem*. 2024; 147:107396. https://doi: 10.1016/j.bioorg.2024.107396

Ware LB, Matthay MA. Clinical practice. Acute pulmonary edema. N Engl J Med. 2005; 353(26):2788-96. https://doi: 10.1056/NEJMcp052699

Weber J, Rajan S, Schremmer C, Chao YK, Krasteva-Christ G, Kannler M, Yildirim AÖ, Brosien M, Schredelseker J, Weissmann N, Grimm C, Gudermann T, Dietrich A. TRPV4 channels are essential for alveolar epithelial barrier function as protection from lung edema. *JCI Insight*. 2020; 5(20):134464. https://doi: 10.1172/jci.insight.134464

Weng J, Liu Q, Li C, Feng Y, Chang Q, Xie M, Wang X, Li M, Zhang H, Mao R, Zhang N, Yang X, Chung KF, Adcock IM, Huang Y, Li F. TRPA1-PI3K/Akt-OPA1-ferroptosis axis in ozone-induced bronchial epithelial cell and lung injury. *Sci Total Environ*. 2024; 918:170668. https://doi: 10.1016/j.scitotenv.2024.170668

White JP, Cibelli M, Urban L, Nilius B, McGeown JG, Nagy I. TRPV4: Molecular Conductor of a Diverse Orchestra. Physiol Rev. 2016; 96(3):911-73. https://doi: 10.1152/physrev.00016.2015

Wilczynski SA, Wenceslau CF, McCarthy CG, Webb RC. A cytokine/bradykinin storm comparison: what is the relationship between hypertension and COVID-19? *Am J Hypertens*. 2021; 34:304–306. https://doi: 10.1093/ajh/hpaa217

Wirth K, Hock FJ, Albus U, Linz W, Martorana PA, Scholkens BA, Stasch JP. Hoe 140 a new potent and long acting bradykinin-antagonist: in vitro studies. *Br J Pharmacol*. 1991; 102:774–777. https://doi: 10.1111/j.1476-5381.1991.tb12249.x

Wortley MA, Birrell MA, Belvisi MG. Drugs Affecting TRP Channels. Handb Exp Pharmacol. 2017; 237:213-41. https://doi: 10.1007/164_2016_63

Yang CL, Yang WK, He ZH, Guo JH, Yang XG, Li HB. Quietness of circular RNA circ_0054633 alleviates the inflammation and proliferation in lipopolysaccharides-induced acute lung injury model through NF-κB signaling pathway. Gene. 2021 Jan; 766:145153. https://doi: 10.1016/j.gene.2020.145153

Yang HH, Duan JX, Liu SK, Xiong JB, Guan XX, Zhong WJ, Sun CC, Zhang CY, Luo XQ, Zhang YF, Chen P, Hammock BD, Hwang SH, Jiang JX, Zhou Y, Guan CX. A COX-2/sEH dual inhibitor PTUPB alleviates lipopolysaccharide-induced acute lung

injury in mice by inhibiting NLRP3 inflammasome activation. *Theranostics*. 2020; 10(11):4749-4761. https://doi: 10.7150/thno.43108

Yang Y, Xiao Z, Yang W, Sun Y, Sui X, Lin X, Yang X, Bao Z, Cui Z, Ma Y, Li W, Wang S, Yang J, Wang Y, Luo Y. Role of transient receptor potential ankyrin 1 in idiopathic pulmonary fibrosis: modulation of M2 macrophage polarization. *Cell Mol Life Sci*. 2024; 81(1):187. https://doi: 10.1007/s00018-024-05219-x

Yap JMG, Ueda T, Takeda N, Fukumitsu K, Fukuda S, Uemura T, Tajiri T, Ohkubo H, Maeno K, Ito Y, Kanemitsu Y, Niimi A. An inflammatory stimulus sensitizes TRPA1 channel to increase cytokine release in human lung fibroblasts. *Cytokine*. 2020; 129:155027. https://doi: 10.1016/j.cyto.2020.155027

Ye Q, Wang B, Mao J. The pathogenesis and treatment of the 'Cytokine Storm' in COVID-19. J Infect. 2020; 80:607-613. https://doi: 10.1016/j.jinf.2020.03.037

Yılmaz İ. Angiotensin-Converting Enzyme Inhibitors Induce Cough. Turk Thorac J. 2019; 20(1):36-42. https://doi: 10.5152/TurkThoracJ.2018.18014

Yu Y, Li X, Qu L, Chen Y, Dai Y, Wang M, Zou W. DXXK exerts anti-inflammatory effects by inhibiting the lipopolysaccharide-induced NF-κB/COX-2 signalling pathway and the expression of inflammatory mediators. *J Ethnopharmacol*. 2016; 178:199–208. https://doi: 10.1016/j.jep.2015.11.016

Zhang H, Wang C, Zhang K, Kamau PM, Luo A, Tian L, Lai R. The role of TRPA1 channels in thermosensation. *Cell Insight*. 2022; 1(6):100059. https://doi: 10.1016/j.cellin.2022.100059

Zhang J, Ge P, Liu J, Luo Y, Guo H, Zhang G, Xu C, Chen H. Glucocorticoid Treatment in Acute Respiratory Distress Syndrome: An Overview on Mechanistic Insights and Clinical Benefit. Int J Mol Sci. 2023; 24(15):12138. https://doi: 10.3390/ijms241512138

Zhang J, Guo Y, Mak M, Tao Z. Translational medicine for acute lung injury. *J Transl Med*. 2024; 22:25. https://doi: 10.1186/s12967-023-04828-7

Zhang L, Sun T, Liu L, Wang L. The research of the possible mechanism and the treatment for capsaicin-induced cough. Pulm Pharmacol Ther. 2018; 49:1-9. https://doi: 10.1016/j.pupt.2017.12.008

Zhang M, Ma Y, Ye X, Zhang N, Pan L, Wang B. TRP (transient receptor potential) ion channel family: structures, biological functions and therapeutic interventions for diseases. *Sig Transduct Target Ther*. 2023; 8:261. https://doi: 10.1038/s41392-023-01464-x

Zhang Y, Ding S, Li C, Wang Y, Chen Z, Wang Z. Effects of N-acetylcysteine treatment in acute respiratory distress syndrome: A meta-analysis. Exp Ther Med. 2017; 14(4):2863-2868. https://doi: 10.3892/etm.2017.4891

Zhou K, Lu J. Progress in cytokine research for ARDS: A comprehensive review. Open Med (Wars). 2024; 19(1):20241076. https://doi: 10.1515/med-2024-1076

Zhou W, Li H, Zhang Y, Huang M, He Q, Li P, Zhang F, Shi Y, Su X. Diagnostic value of galactomannan antigen test in serum and bronchoalveolar lavage fluid samples from patients with nonneutropenic invasive pulmonary aspergillosis. *J Clin Microbiol*. 2017; 55:2153-2161. https://doi: 10.1128/JCM.00345-17