

Investigation of novel pharmacotherapeutic approaches in an animal model of acute lung injury

Doctoral (PhD) thesis



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2026

1. INTRODUCTION, BACKGROUND

1.1 Acute lung injury (ALI) and acute respiratory distress syndrome (ARDS)

Acute lung injury (ALI) and its more severe form, acute respiratory distress syndrome (ARDS), are characterized by diffuse structural and functional damage to the lungs. Clinically, they are primarily manifested by acute hypoxemia, bilateral pulmonary infiltrates, intrapulmonary shunting, and non-cardiogenic pulmonary edema, ultimately leading to severe respiratory failure (1,2). A central feature of ALI/ARDS pathophysiology is diffuse alveolar damage resulting from the progressive disruption of the endothelial–epithelial barrier (3).

ARDS is associated with a high mortality rate of approximately 39–40% (4). Current management is mainly based on supportive care, including adequate oxygenation and lung-protective mechanical ventilation (5,6). Pharmacological treatment options remain limited, as no approved targeted therapy is currently available (7). Although glucocorticoids are widely recommended and used in clinical practice (6,8,9), their efficacy remains controversial. This may be attributed to the heterogeneity of patient populations, differences in treatment regimens and duration, the occurrence of adverse effects such as secondary infections, hyperglycemia, and myopathy, as well as the incompletely understood complexity of the inflammatory pathways underlying the disease (9–11).

Consequently, ALI/ARDS continues to represent a major healthcare challenge, particularly in glucocorticoid-resistant cases where effective therapeutic options remain limited (9). Therefore, elucidating the key pathomechanisms and molecular pathways involved in disease progression, as well as identifying novel therapeutic targets and potential pharmacological agents, remains essential for the development of more effective treatment strategies.

1.2 Animal models of acute lung injury: LPS-induced ALI model

Experimental animal models constitute a fundamental tool in preclinical research to identify the molecular pathways underlying disease pathophysiology, discover novel biomarkers and therapeutic targets, and evaluate the efficacy of potential drug candidates *in vivo*. Experimental models of ALI/ARDS reproduce distinct components of the complex and heterogeneous pathophysiology of the disease, depending on the intra- or extrapulmonary predisposing or triggering factors employed (7).

Among intrapulmonary models, the lipopolysaccharide (LPS)-induced acute interstitial pneumonitis model is the most widely used experimental model of ALI in preclinical research owing to its relative simplicity, broad applicability, and high reproducibility (7). LPS, an endotoxin located in the outer membrane of Gram-negative bacteria, induces a macrophage-mediated, neutrophil-dominant inflammatory response through activation of the innate immune system via the toll-like receptor 4 (TLR4) signaling pathway (12). Subsequent activation of downstream signaling cascades promotes the production of pro-inflammatory cytokines and chemokines, including TNF- α , IL-1 β , IL-6, IL-18, MCP-1, and interferon- γ (IFN- γ), thereby amplifying the inflammatory response (12–14).

A major limitation of LPS-induced ALI models used for evaluating anti-inflammatory therapies is the considerable heterogeneity of preclinical study designs. Substantial variability exists regarding the LPS dose (0.25–15 mg/kg), serotype, route of administration, mouse strain, and experimental endpoints, while many studies are conducted without the inclusion of an appropriate positive control reference compound (15–29).

1.3 Sigma-1 receptor as a potential therapeutic target

The sigma-1 receptor (S1R) is a highly conserved ligand-regulated transmembrane chaperone protein predominantly localized at the mitochondria-associated endoplasmic reticulum membrane (MAM) (30). Although it is highly expressed in the central nervous system, S1R is also present in peripheral tissues, including the lungs (31,32). Beyond its structural and stabilizing functions, S1R interacts with a wide range of proteins, including G protein-coupled receptors, ion channels, and lipids. Furthermore, it regulates intracellular calcium homeostasis, modulates endoplasmic reticulum stress signaling pathways, and contributes to cell survival (30,33–35).

The therapeutic potential of S1R agonists has been demonstrated in several disorders of the central nervous system, partly through their anti-inflammatory properties (33). However, the role of S1R in peripheral inflammatory diseases has not yet been comprehensively investigated. In recent years, our collaborators from the Diabetes research group of the Pediatric Center at Semmelweis University demonstrated the protective effects of S1R activation in experimental models of acute kidney injury and transplantation (36–38). These beneficial effects were closely associated with a marked reduction in the expression of pro-inflammatory cytokines and decreased immune cell infiltration, suggesting that S1R may represent a promising anti-inflammatory therapeutic target. Fluvoxamine (FLU), a widely used and well-tolerated

selective serotonin reuptake inhibitor (SSRI), binds to S1R with high affinity and has recently emerged as a promising candidate for drug repurposing because of its potential anti-inflammatory effects (39–41). However, the efficacy of FLU as a repurposed drug or adjunctive therapy for the early treatment of COVID-19 remains uncertain.

Given the central role of inflammation in the pathogenesis of ALI/ARDS, activation of S1R may exert protective effects in the lung and therefore represents a promising therapeutic target.

1.4 SzV-1287 as a novel multi-target anti-inflammatory drug candidate

SzV-1287 is a novel multi-target anti-inflammatory and analgesic drug candidate patented by our research group (WO2015159112A1), for which Phase Ia clinical testing has been successfully completed. It was originally developed for the treatment of neuropathic pain (42). SzV-1287 irreversibly inhibits the copper-containing amine oxidase 3 (AOC3) enzyme, which is abundantly expressed in several tissues, including the lungs (42,43). AOC3 catalyzes the oxidative deamination of primary amines, generating tissue-irritating metabolites and reactive oxygen species. Its products, including formaldehyde, methylglyoxal, acrolein, hydrogen peroxide, and ammonia, are known activators of the transient receptor potential ankyrin 1 (TRPA1) non-selective cation channel, which plays an important role in the pathogenesis of inflammatory airway diseases (44,45). Our research group demonstrated that, in addition to its indirect inhibitory effect on TRPA1 through AOC3 inhibition, SzV-1287 also directly antagonizes TRPA1 and transient receptor potential vanilloid 1 (TRPV1) channels (46). In recent years, AOC3 inhibition has attracted considerable research interest. Previous studies have demonstrated that pharmacological blockade of AOC3 reduces inflammatory cell infiltration and the production of pro-inflammatory cytokines, including TNF- α and IL-6, in mouse models of chronic obstructive pulmonary disease (COPD) and acute lung injury (47,48).

2. PRIMARY AIMS

The primary aim of my research was to develop a reliable and comprehensively characterized LPS-induced mouse model of acute lung injury suitable for the evaluation of potential drug candidates and the identification of novel therapeutic targets.

- 1) Optimization, characterization, and pharmacological validation of a preclinical model of acute lung injury induced by intratracheal LPS administration in C57BL/6J and NMRI mice, using dexamethasone as a widely applied gold-standard reference compound.

- 2) Investigation of the potential repurposing of fluvoxamine, traditionally used as an antidepressant, for a novel therapeutic indication, and thereby exploration of the role of the sigma-1 receptor in the optimized and validated mouse model of LPS-induced acute lung injury.
- 3) Investigation of a novel multi-target anti-inflammatory and analgesic drug candidate (SzV-1287) patented by our research group, in the optimized and validated mouse model of LPS-induced acute lung injury.

3. EXPERIMENTAL MODELS AND INVESTIGATIONAL METHODS

3.1 Animals and research ethics

A total of 109 female C57BL/6J mice (8–10 weeks old, 20 ± 3 g) and 44 female Crl(Han) (NMRI) mice (8–10 weeks old, 30 ± 2 g) were used for the optimization and validation of the LPS-induced ALI model. The investigation of the sigma-1 receptor agonist fluvoxamine involved 51 female C57BL/6J and 61 sigma-1 receptor-deficient (*S1r^{-/-}*) mice, also aged 8–10 weeks. The SzV-1287 study included 23 female C57BL/6J mice aged 8–10 weeks. Studies were approved and license was given by the Ethics Committee on Animal Research of the University of Pécs, Pécs, Hungary, according to the Ethical Codex of Animal Experiments (licence No.: BA02/2000-38/2023, BA/73/00607-6/2021, BA02/2000-16/2023).

3.2 Experimental protocols

3.2.1 Protocol I: Optimization and validation of the LPS-induced mouse model of acute lung injury

Acute lung injury was induced in C57BL/6J mice by the intratracheal (i.t.) administration of four different doses of LPS (0.25, 1, 2.5, and 5 mg/kg; *Escherichia coli* endotoxin O111:B4) dissolved in 60 μ L of phosphate-buffered saline (PBS). The animals were randomized into nine groups: (1) negative control (PBS); (2–5) 0.25, 1, 2.5, or 5 mg/kg LPS + intraperitoneal (i.p.) physiological saline (100 μ L/10 g; vehicle: Veh); (6–9) positive control groups treated with 0.25, 1, 2.5, or 5 mg/kg LPS + the reference compound dexamethasone (DEXA, 5 mg/kg i.p.; Dexamethasone 4 mg/mL; Teva Pharmaceuticals Ltd., Debrecen, Hungary) 30 min before induction. Respiratory function and abdominal temperature were measured in conscious animals 24 h after PBS/LPS treatment, followed by removal of the lungs for histological analysis and measurement of MPO activity. The most optimal LPS dose (0.25 mg/kg) was selected and used for validation of the model in the NMRI strain.

3.2.2 Protocol II: Investigation of the effect of fluvoxamine in the LPS-induced model of acute lung injury

In the validated LPS-induced ALI model, lung inflammation was induced by the i.t. administration of 0.25 mg/kg LPS (dissolved in 60 µL PBS). Wild-type (WT) and *Slr^{-/-}* animals were randomized into four and three groups, respectively: (i) negative control mice were treated with i.t. PBS and i.p. Veh (physiological saline, 100 µL/10 g) 30 min before and 12 h after induction (PBS + Veh); (ii) i.t. LPS treatment and i.p. Veh 30 min before and 12 h after induction (LPS + Veh); (iii) i.t. LPS treatment and i.p. fluvoxamine maleate (FLU) (20 mg/kg) 30 min before and 12 h after induction (LPS + FLU). WT LPS-treated animals were randomized into an additional positive control group in which dexamethasone (DEXA) (5 mg/kg i.p.) was administered 30 min prior to LPS treatment (LPS + DEXA). Respiratory function measurements were performed 24 h after PBS/LPS administration. Lung tissues were removed for qPCR measurements and histological analysis.

3.2.3 Protocol III: Investigation of the effect of SzV-1287 in the LPS-induced model of acute lung injury

ALI was induced by i.t. administration of LPS (0.25 mg/kg, dissolved in 60 µL PBS). The animals were randomized into the following four groups: (i) i.t. PBS + i.p. Veh (Kolliphor, 100 µL/10 g); (ii) i.t. LPS + i.p. Veh (Kolliphor); (iii) i.t. LPS + i.p. SzV-1287 (20 mg/kg); and (iv) i.t. LPS + i.p. LJP-1207, a selective AOC3 inhibitor reference compound (20 mg/kg). The i.p. treatments were administered 30 min before and 12 h after PBS/LPS administration. Following the respiratory function measurement at 24 h, lung tissue was removed for histological analysis.

3.3 Induction of acute pneumonitis by intratracheal administration of LPS

Acute lung injury was induced by i.t. administration of LPS at the dose specified in each experimental protocol. The procedure was performed under ketamine–xylazine anesthesia (100 and 5 mg/kg i.p., respectively).

3.4 Pulmonary function measurement by whole-body plethysmometry

Respiratory function was assessed 24 h after PBS/LPS administration in conscious, restrained animals using the DSI FinePointe Non-Invasive Airway Mechanics (NAM) whole-body plethysmography system. A 15-min acclimatization period preceded each measurement.

3.5 Investigation of core body temperature

The measurements were conducted in collaboration with the research group of the Department of Thermophysiology at the Institute for Translational Medicine. At the beginning of the experiment, C57BL/6J mice were anesthetized with ketamine–xylazine and received

intramuscular gentamicin (6 mg/kg). For continuous monitoring of abdominal temperature (T_{ab}), a miniature telemetry transmitter (G2 E-Mitter series; Mini Mitter, Bend, OR, USA) was implanted into the peritoneal cavity. Following recovery, T_{ab} was continuously recorded after i.t. treatment using a telemetric thermometry system with telemetry receivers (model ER-4000; Mini Mitter).

3.6 Determination of MPO activity from lung homogenates

MPO activity, reflecting the accumulation of activated neutrophils and macrophages and thus the extent of inflammation, was assessed in lung homogenate samples with spectrophotometry. Samples were homogenized (T50 Ultra-Turrax homogenizer) in 4 mL of 20 mM potassium phosphate buffer (pH 7.4) and centrifuged at $10,000 \times g$ for 10 min at 4 °C. The pellet was resuspended in 4 mL of 50 mM potassium phosphate buffer (pH 6.0) containing 0.5% hexadecyl-trimethyl-ammonium-bromide and centrifuged again. MPO enzyme activity in the pellets was compared with a human MPO standard (CAS No. 9003-99-0; Merck KGaA, Darmstadt, Germany) in 96-well microtiter plates using H_2O_2 -3,3',5,5'-tetramethyl-benzidine (TMB/ H_2O_2 ; Merck KGaA, Darmstadt, Germany). Optical density was measured at 620 nm using a microplate reader (Labsystems Multiskan RC 351).

3.7 Quantitative RT-PCR

Quantitative RT-PCR measurements were performed by our collaborators from the Diabetes research group of the Pediatric Center at Semmelweis University. Total RNA was isolated from lung homogenates using an RNeasy RNA Isolation Kit (Qiagen, Germantown, MD, USA), and its concentration was determined using a NanoDrop One spectrophotometer (Thermo Scientific, Waltham, MA, USA). Following reverse transcription using the Maxima First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA, USA), quantitative RT-PCR measurements were performed on a LightCycler 96 system (Roche Diagnostics, Mannheim, Germany) using SYBR Green I Master enzyme mix (Roche Diagnostics, Mannheim, Germany) and specific primers at a concentration of $10 \text{ pmol } \mu\text{L}^{-1}$ (IDT, Coralville, Iowa, USA). All data were normalized using the housekeeping gene *Rn18S*.

3.8 Quantitative and semiquantitative histopathological assessments

3.8.1 Histopathological assessment I: Optimization and validation of the LPS-induced mouse model of acute lung injury

Semiquantitative histopathological evaluation was performed on hematoxylin-eosin (HE)-stained 5 μm sections from three distinct depths and areas of the lung samples. Scores were determined by two independent pathologists in a blinded manner using the following criteria:

perivascular edema, peribronchial and perivascular neutrophil granulocyte accumulation, and eosinophil macrophage infiltration (0: none, 1: mild, 2: moderate, and 3: severe).

3.8.2 Histopathological assessment II: Investigation of the effect of fluvoxamine in the LPS-induced model of acute lung injury

The number of neutrophil granulocytes was assessed on 5 μm HE-stained sections. Ten lung septa were selected randomly from each section, and the number of neutrophils was counted in a blinded manner by a pathologist. CD68-immunopositive cells were counted in ten randomly selected parenchymal areas with 20 $\times$ magnification (Olympus BX51, Tokyo, Japan). Inflammatory cell counts were assessed using ImageJ software (version 1.54g) and expressed as the total number/ 10^6 mm^2 tissue.

3.8.3 Histopathological assessment III: Investigation of the effect of SzV-1287 in the LPS-induced model of acute lung injury

Perivascular edema formation was assessed by semiquantitative histopathological evaluation of 5 μm thick HE-stained sections, as described in Section 3.8.1. CD68-immunopositive cell quantification was performed according to the method described in Section 3.8.2.

3.9 Statistical analysis

Statistical analyses were performed using effect size analysis and GraphPad Prism v8 (GraphPad Software, San Diego, CA, USA). Values of all measurements are expressed as mean $\pm$ SEM. All data with a high effect size, indicated by Hedges' g value exceeding 0.8, were considered significant and marked on the figures. Further statistical analyses were performed using one-way ANOVA followed by Dunnett's post hoc test (lung index, respiratory function parameters, MPO activity, cytokine levels, and quantitative histopathological analysis), two-way ANOVA followed by Tukey's or Dunnett's post hoc test (body weight, body weight change, and T_{ab}), or the Kruskal–Wallis test followed by Dunnett's post hoc test (semiquantitative histopathological analysis).

4. RESULTS

4.1 Optimization, characterization and pharmacological validation of the endotoxin-induced acute pneumonitis mouse model

4.1.1 Dexamethasone can diminish LPS-induced body weight loss and increased lung index depending on the LPS dose

Endotoxin induced a significant body weight loss and increase in lung index (total lung weight/10 g body weight) in all LPS-treated groups at the 24-hour endpoint, which was counteracted by dexamethasone dose-dependently. The reference compound

attenuated/counteracted the LPS-induced 10–15 % decrease in body weight and lung weight increase in the 0.25, 1, and 2.5 mg/kg LPS-treated groups, whereas it had no effect on the highest 5 mg/kg LPS dose.

4.1.2 Dexamethasone counteracts endotoxin-induced respiratory function impairment depending on the LPS dose

Respiratory parameters, such as breathing frequency and peak expiratory flow rate increased, whereas tidal volume and minute ventilation decreased to the same extent in response to all applied LPS doses. These alterations were counteracted by dexamethasone in a dose-dependent manner. None of the respiratory parameters were effectively influenced by dexamethasone in the group treated with high-dose (5 mg/kg) LPS.

4.1.3 LPS induces hypothermia dose-dependently

The anaesthetic ketamine-xylazine markedly reduced the T_{ab} in all groups. In control animals, the temperature returned to baseline a few hours after administration, however in the LPS-treated groups 8–9 hours after induction T_{ab} began to converge with the control group in a dose-dependent manner. In the low-dose LPS group (0.25 mg/kg) T_{ab} recovered to control levels at approximately 17 h. In animals treated with intermediate LPS doses (1, 2.5 mg/kg) T_{ab} remained significantly lower compared to the PBS-treated control group by the end of the experiment with the lowest mean values recorded at the maximal LPS dose (5 mg/kg).

4.1.4 LPS-induced histopathological alterations are not dose-dependent

Histopathological inflammatory parameters such as neutrophil and macrophage infiltration as well as edema formation were elevated 24 hours after LPS administration based on the semiquantitative scores. Except for the eosinophil macrophage infiltration these parameters showed no dose-dependence. Dexamethasone only counteracted the 0.25 and 5 mg/kg LPS-induced edema formation as well as the 1 mg/kg LPS-evoked eosinophil macrophage infiltration. The composite inflammatory score was elevated in all LPS-treated groups, however, dexamethasone could effectively alleviate it only in the lowest 0.25 mg/kg LPS group.

4.1.5 LPS-induced MPO activity was not influenced by dexamethasone

MPO activity was significantly increased by LPS administration in all groups. Dexamethasone did not diminish this parameter significantly at this 24-hour timepoint. The regression analysis showed no dose-dependence by either LPS or dexamethasone treatment.

4.1.6 Dexamethasone counteracts LPS-induced increased total lung weight along with composite score and MPO activity in NMRI mice

Similarly to the results observed in C57BL/6J mice, 0.25 mg/kg LPS induced body weight loss, increased lung weight correlating with pulmonary edema formation, inflammatory histopathological changes represented by the composite score, as well as increased MPO activity. Dexamethasone diminished the LPS-induced alterations in the latter three parameters.

4.1.7 Dexamethasone diminishes LPS-induced respiratory function alterations in NMRI mice

The baseline frequency, tidal volume and minute ventilation of PBS-treated control animals showed a strain difference between C57BL/6J and NMRI mice. However, in NMRI mice, 0.25 mg/kg LPS induced a similar increase in breathing frequency and peak expiratory flow rate, along with a decrease in tidal volume and minute ventilation as observed in C57BL/6J mice. Dexamethasone also prevented the LPS-induced alterations in the latter three parameters in the NMRI strain.

4.2 Investigation of the effect of fluvoxamine in the optimized and validated LPS-induced model of acute lung injury

4.2.1 FLU rescues respiratory function in LPS-induced ALI

FLU counteracted the LPS-induced tidal volume, minute ventilation, peak expiratory, tidal mid-expiratory, and peak inspiratory flow decrement in the WT mice, similarly to the gold standard reference compound DEXA. However, it did not affect breathing frequency, inspiratory and expiratory time. Moreover, FLU further aggravated these latter parameters in the gene-deficient mice. Altogether, except for peak inspiratory flow, FLU showed no or aggravating impact in the *Slr*^{-/-} mice.

4.2.2 FLU ameliorates LPS-induced macrophage, but does not affect neutrophil granulocyte infiltration and edema formation

LPS induced substantial CD68⁺ macrophage infiltration in the lung tissue, in all groups, which was diminished by FLU in both wild-type and *Slr*^{-/-} animals. However, FLU did not influence neutrophil granulocyte count in either group.

4.2.3 FLU does not influence LPS-induced body weight loss or pulmonary edema formation in either WT or *Slr*^{-/-} mice

LPS evoked a decrease in body weight and body weight change and an increase in lung index 24 hours after induction in WT and *Slr*^{-/-} mice. These alterations were not influenced by FLU treatment in either strain.

4.2.4 FLU alleviates inflammatory cytokine expression in WT, but not in *Slr*^{-/-} mice

FLU diminished the LPS-induced increase in the mRNA expression of the inflammatory cytokines TNF- α , IL-6, IL-1 α , IL-1 β , and MCP-1 in the WT but not in *Slr*^{-/-} mice; moreover, it aggravated the upregulated levels of IL-6 and TNF- α in the gene-deficient animals.

4.3 Investigation of the effect of SzV-1287 in the optimized and validated LPS-induced model of acute lung injury

4.3.1 SzV-1287 prevents LPS-induced body weight loss

SzV-1287 prevented LPS-induced body weight loss, in contrast to the selective AOC3 inhibitor reference compound LJP-1207.

4.3.2 SzV-1287 counteracts LPS-induced respiratory function impairment

SzV-1287 counteracted the LPS-induced reduction in tidal volume, peak inspiratory flow, minute ventilation, peak expiratory flow, mid-tidal expiratory flow, and functional residual capacity, whereas the reference compound LJP-1207 was effective only in the latter four parameters.

4.3.3 SzV-1287 mitigates LPS-induced macrophage infiltration and edema formation

LPS induced marked CD68⁺ macrophage infiltration in the lung tissue, which was reduced by SzV-1287, whereas the reference compound LJP-1207 showed no effect. Based on semiquantitative scoring, LPS-induced edema formation was prevented by both SzV-1287 and LJP-1207.

5. DISCUSSION

In recent years, ALI/ARDS have emerged as a major global health concern due to their substantial morbidity and mortality, attracting considerable scientific interest (49). Despite extensive investigation of numerous candidate compounds in preclinical and early-phase clinical studies, no specific pharmacological therapy is currently available for ALI/ARDS (50,51).

In preclinical research of airway inflammation, studies often apply different LPS doses, routes of administration, and serotypes, often without the inclusion of a positive control. Therefore, one of the key messages of the present research is that well-characterized, optimized, and pharmacologically validated ALI models are essential for the reliable identification of novel therapeutic targets and evaluation of potential drug candidates.

We demonstrated that very low LPS doses induce similar functional and morphological inflammatory pulmonary alterations to 20 times higher doses, but only the effect of 0.25 mg/kg LPS is the most sensitive to the gold standard glucocorticoid treatment. Therefore, this protocol is suggested to be used for controlled preclinical studies.

Most studies do not report on respiratory functions in the LPS-induced ALI model. The available data on lung functions are quite contradictory, reporting on different parameters and changes (25,28). The comparison and interpretation of respiratory parameters are also challenging due to the different measurement apparatuses and varying study endpoints (26,28,52,53). In our study we chose the 24 h-timepoint based on the maximum of the inflammatory response and related functional alterations, which decrease later by the 48 h- and 72 h-timepoints (20,25,54). The translational relevance of airway function might be debatable, since due to the differences in the anatomy and physiological functions in mice, as well as the various measurement techniques, the changes in certain parameters do not necessarily reflect human pathophysiology (7). Furthermore, respiratory functional alterations are not exclusively and directly influenced by the cellular inflammatory mechanisms, but more by the peribronchial/perivascular edema formation. Our results show that the LPS-induced decrease in minute ventilation, tidal volume and increase in peak expiratory flow rate was counteracted by dexamethasone, supported by its anti-edema action (55).

Dexamethasone did not influence LPS-induced increase in MPO activity in agreement with the lack of effect on the accumulation of these inflammatory cells based on the histopathological results. Our results highlight that LPS acutely induces distinct reproducible changes in mice, which are not strain-specific. Although baseline functional differences can be observed between the inbred C57BL/6J with more homogenous genetic background and the outbred NMRI mice, these are most likely due to the difference in their initial body weight (20 ± 3 g and 30 ± 2 g, respectively) (56). Therefore, both strains are appropriate and applicable, validating the model for preclinical testing.

We demonstrate valuable data also regarding dose- and time-dependent LPS-induced thermoregulation changes. The initial hypothermia is mainly attributed to the well-known effect of general (ketamine-xylazine) anaesthesia (57,58), reaching its maximum 1–2 h after administration and returning to baseline within 4–5 h in PBS-treated control animals.

After optimization, characterization, and pharmacological validation of the LPS-induced ALI model using dexamethasone, we further investigated the effects of the S1R agonist FLU,

thereby exploring the role of S1R in this model. Furthermore, our collaborators have patented the therapeutic application of S1R agonists for the treatment of fibrosis in multiple organs, including the lung (PCT/HU2015/000014), and have demonstrated the anti-inflammatory effects of S1R activation in experimental models of both acute and chronic kidney injury (international patent: PCT/HU2017/050051) (36,59).

The LPS-evoked impairments in respiratory function such as tidal volume, minute ventilation, peak expiratory, tidal mid-expiratory, and peak inspiratory flow decrement were counteracted by FLU in the WT mice. However, except for peak inspiratory flow, FLU showed no or aggravating impact in the *S1r*^{-/-} mice. Our results also demonstrate that FLU alleviates LPS-induced pneumonitis by reducing TNF- α , IL-6, IL-1 α , IL-1 β , MCP-1 expression in the WT but not in the *S1r*^{-/-} mice. These findings are further supported by previous studies indicating that S1R activation plays a key role in suppressing cytokine production across various preclinical animal models, including mouse models of LPS-induced septic shock and ovalbumin-induced allergic asthma (35,60). Moreover, in the context of potential anti-SARS-CoV-2 therapy, it has been hypothesized that FLU may modulate the inositol-requiring enzyme 1 alpha (IRE1 α) upstream stress sensor pathways through S1R agonism, therefore downregulating inflammatory ER stress (35,61).

Our results showed that FLU reduced macrophage infiltration independently of S1R activation, indicating the involvement of distinct molecular pathways. Since the initial steps of inflammation, including recruitment and migration of immune cells, are mediated by cell adhesion molecules (62), FLU might inhibit CD68-positive cell accumulation via this mechanism. Despite alleviating cellular inflammatory components, FLU did not substantially decrease edema formation, suggesting its minor effect on venular plasma protein extravasation.

We further investigated the effects of SzV-1287 in the previously optimized and validated LPS-induced ALI model. SzV-1287, patented by our team (WO2015159112A1), is a novel multi-target anti-inflammatory and analgesic drug candidate molecule, which was originally developed for the treatment of neuropathic pain and has successfully completed Phase Ia clinical testing. Our research team has shown that in addition to its indirect TRPA1 inhibitory activity via AOC3 inhibition, SzV-1287 is a direct TRPA1 and TRPV1 antagonist, and its metabolite is a cyclooxygenase (COX) inhibitor (42,46). In recent years, increasing scientific attention has been directed toward TRPA1 expression in immune cells (63). TRPA1 has been

suggested to be an important chemosensor in the lung, activated by a variety of exogenous stimuli and environmental irritants (44,64,65).

Previous studies in gene-deficient mice demonstrated that TRPA1 complexly regulate airway inflammation contributing to chronic cigarette smoke exposure-evoked emphysema formation and respiratory deterioration (66).

Our results demonstrate that SzV-1287 counteracted the LPS-induced body weight loss and respiratory function impairment as well as the increased number of CD68+ cells and edema formation. In contrast, the selective AOC3 inhibitor LJP-1207 had no effect on the LPS-induced alteration regarding body weight, body weight change, tidal volume and peak inspiratory flow and CD68+ cell numbers.

The role of AOC3 in inflammatory airway diseases remains to be fully elucidated, however it may represent a promising therapeutic target. Previous studies have demonstrated that selective pharmacological inhibition of AOC3 attenuates inflammatory responses and airway hyperresponsiveness and reduces fibrotic alterations in mouse models of acute and chronic airway inflammation (47,48,67).

6. SUMMARY OF NEW RESULTS, CONCLUSIONS

1) Our study demonstrated that even low LPS doses induce characteristic functional and morphological inflammatory alterations in the lung, which do not worsen in response to even 20 times higher doses, but only the effect of 0.25 mg/kg LPS is the most sensitive to the gold standard DEXA treatment. Therefore, we suggest this protocol for testing novel anti-inflammatory agents.

2) Investigation of the effects of fluvoxamine and thereby the role of S1R in the previously optimized and pharmacologically validated LPS model demonstrated for the first time that the S1R agonist FLU attenuates respiratory dysfunction and the inflammatory response in WT but not in *S1r*^{-/-} mice. Thus, targeting S1R may be an effective and safe alternative to glucocorticoids for the treatment of inflammatory lung injury.

3) SzV-1287 counteracted the LPS-induced alterations, whereas the selective AOC3 inhibitor reference compound LJP-1207 was ineffective in several parameters. Based on our findings, the protective anti-inflammatory effects of SzV-1287 may be attributed to its complex multi-target pharmacological profile involving indirect and direct TRPA1/TRPV1 as well as COX inhibition.

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8. LIST OF PUBLICATIONS

8.1 Publications related to the thesis:

Emese Ritter, Kitti Hohl, László Kereskai, Ágnes Kemény, Dóra Hargitai, Veronika Szombati, Anikó Perkecz, Eszter Pákai, András Garami, Ákos Zsembery, Zsuzsanna Helyes, Kata Csekő. Optimization, characterization and pharmacological validation of the endotoxin-induced acute pneumonitis mouse model. *Biomedicines*; 2025; 13(6):1498. doi: 10.3390/biomedicines13061498 **Q1**

IF: 3.9

Emese Ritter*, Kata Csekő*, Ádám Hosszú*, Ákos R. Tóth, Dóra Hargitai, László Kereskai, Andrea Fekete*, Zsuzsanna Helyes*. The sigma-1 receptor agonist fluvoxamine alleviates endotoxin-induced acute lung injury in mice. *Front. Pharmacol*; 2026; 17:1818198. doi: 10.3389/fphar.2026.1818198 **Q1**

- shared authorship, **IF: 5.4**

8.2 Publications not related to the thesis:

Petra Pallagi, Emese Tóth, Marietta Görög, Viktória Venglovecz, Tamara Madácsy, Árpád Varga, Tünde Molnár, Noémi Papp, Viktória Szabó, Enikő Kúthy-Sutus, Réka Molnár, Attila Ördög, Katalin Borka, Andrea Schnúr, Albert Kéri, Gyula Kajner, Kata Csekő, **Emese Ritter**, Dezső Csupor, Zsuzsanna Helyes, Gábor Galbács, Andrea Szentesi, László Czakó, Zoltán Rakonczay, Tamás Takács, József Maléth, Péter Hegyi. Heavy metals in cigarette smoke

strongly inhibit pancreatic ductal function and promote development of chronic pancreatitis. *Clinical and Translational Medicine*. 2024; 14(6):e1733. doi: 10.1002/ctm2.1733 **D1. IF: 10,6**

9. ACKNOWLEDGEMENTS

First and foremost, I would like to express my sincere gratitude to my supervisors, Dr. Kata Csekő and Prof. Dr. Zsuzsanna Helyes, for their professional support and encouragement throughout my PhD studies. Their scientific perspective, professional dedication, and continuous guidance have played a fundamental role in my doctoral work.

I am grateful to Prof. Dr. Erika Pintér, Head of the Department of Pharmacology and Pharmacotherapy and the Doctoral School, for providing me with the opportunity to conduct my research in a supportive and inspiring environment.

I would also like to thank our collaborators and co-authors, Prof. Dr. Andrea Fekete, Dr. Ádám Hosszú, Ákos Tóth, Prof. Dr. Ákos Zsembery, Dr. Dóra Hargitai, Dr. Eszter Pákai, Prof. Dr. András Garami, Dr. Ágnes Kemény, and Dr. László Kereskai, for their valuable scientific contributions and constructive feedback throughout this research.

I am thankful to Anikó Perkecz and Veronika Ordonicsné Szombati for their technical assistance during the experiments.

I would like to express my special gratitude to the Gedeon Richter Talentum Foundation and the New National Excellence Program of the Ministry for Innovation and Technology for their significant support in the completion of my PhD research.

Finally, I would like to thank my family, especially my fiancé and my parents, for their patience and unwavering support throughout this journey.