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JZL184 modulates lung injury via occludin and TNF-α in two distinct ards models

JZL184 iki farklı ards modelinde okludin ve TNF-α yoluyla akciğer hasarını modüle etmektedir

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ABSTRACT

Aim: Acute respiratory distress syndrome (ARDS) is a severe condition with high morbidity and mortality, currently treated with limited therapeutic options. Recent studies indicate that cannabinoids could play a protective role in preventing tissue damage, particularly in pulmonary disorders. While their benefits in lung pathologies are established, the specific role of cannabinoids in ARDS mechanisms remains underexplored. This study aims to investigate the protective effects of cannabinoids, particularly through occludin and TNF-α regulation, in two ARDS models with distinct pathophysiological mechanisms.

Materials and Methods: Rats were treated with LPS for the direct ARDS model or ANTU for the indirect ARDS model. The inhibitor of MAGL, the enzyme that degrades the endocannabinoid 2-AG, JZL184, was administered 30 minutes prior to the administration of LPS or ANTU. Lung tissue samples were collected 24 hours post-LPS and 4 hours post-ANTU administration. Biochemical analyses, including MDA, SOD and catalase, as well as immunohistochemical evaluations of occludin and TNF-α, were conducted on lung tissue samples.

Results: Biochemical analysis showed that MDA levels decreased in the ANTU and LPS groups, but increased in the ANTU+JZL184 group and decreased in the LPS+JZL184 group, reflecting paradoxical effects. SOD and catalase levels were higher in the ANTU group and lower in the ANTU+JZL184 group. Immunohistochemical analysis demonstrated the restoration of occludin expression in both endothelial and bronchiolar cells and a reduction in TNF-α levels following JZL184 treatment.

Conclusion: JZL184 attenuates inflammation and restores occludin expression in both ARDS models, indicating its possible use as a therapeutic strategy for ARDS.

Keywords: ANTU, JZL184, LPS, occludin, TNF-α

ÖZ

Amaç: *Akut solunum sıkıntısı sendromu (ARDS), yüksek morbidite ve mortaliteye sahip ciddi bir durum olup, şu anda sınırlı terapötik seçenekler ile tedavi edilmektedir. Son veriler, kannabinoidlerin akciğer hastalıkları da dahil olmak üzere doku hasarına karşı koruyucu etkiler sunabileceğini göstermektedir.*

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Akciğer patolojilerindeki faydaları kanıtlanmış olsa da kannabinoidlerin ARDS mekanizmalarındaki özgül rolü henüz yeterince araştırılmamıştır. Bu çalışma, farklı patofizyolojik mekanizmalara sahip iki ARDS modelinde, özellikle okludin ve TNF-α yoluyla kannabinoidlerin koruyucu etkilerini incelemeyi amaçlamaktadır.

Gereç ve Yöntem: *Sıçanlar, doğrudan ARDS modeli için LPS ile veya dolaylı ARDS modeli için ANTU ile muamele edilmiştir. Endokannabinoid 2-AG'yi parçalayan MAGL enziminin inhibitörü olan JZL184, LPS veya ANTU verilmeden 30 dakika önce uygulanmıştır. Akciğer doku örnekleri, LPS uygulamasından 24 saat ve ANTU uygulamasından 4 saat sonra toplanmıştır. Akciğer doku örneklerinde MDA, SOD ve katalaz olmak üzere biyokimyasal analizler ile okludin ve TNF-α'nın immünohistokimyasal değerlendirmeleri yapılmıştır.*

Bulgular: *Biyokimyasal analizler, MDA seviyelerinin ANTU ve LPS gruplarında azaldığını, ancak ANTU+JZL184 grubunda arttığını ve LPS+JZL184 grubunda azaldığını göstermiştir, bu da paradoksal etkileri yansıtmaktadır. SOD ve katalaz seviyeleri, ANTU grubunda daha yüksek ve ANTU+JZL184 grubunda daha düşük bulunmuştur. İmmünohistokimyasal analizler ise JZL184 tedavisi sonrasında, endotel ve bronşiolar hücrelerin her ikisinde okludin ekspresyonunun düzeldiğini ve TNF-α seviyelerinin azaldığını göstermiştir.*

Sonuç: *JZL184, her iki ARDS modelinde inflamasyonu hafifletmekte ve okludin ekspresyonunu düzeltmektedir, bu da ARDS için terapötik bir yaklaşım olarak potansiyelini ortaya koymaktadır.*

Anahtar Sözcükler: ANTU, JZL184, LPS, okludin, TNF-α

INTRODUCTION

Acute Respiratory Distress Syndrome (ARDS) represents a severe pulmonary disorder characterized by widespread alveolar damage, hypoxemia, and inflammation, often leading to respiratory failure. It is triggered by a variety of etiological factors, both direct, such as pneumonia and aspiration, and indirect, including sepsis and trauma (1,2). Despite its high incidence and substantial mortality rate, ARDS treatment remains limited to supportive care, with no effective pharmacological treatments yet established in clinical practice (3,4). This highlights the critical need for exploring novel therapeutic strategies to improve outcomes in ARDS patients.

The role of the endocannabinoid system in a wide range of diseases has positioned it as a promising therapeutic target. The discovery of CB1 and CB2 receptors, and their key ligands, anandamide and 2-arachidonoylglycerol (2-AG), as well as associated enzymes, has enhanced comprehension of this system. Given its widespread presence in mammals and its regulatory influence on essential physiological processes such as immune response and inflammation, it holds considerable pharmacological significance (5).

The two main ARDS models commonly used in experimental research are the lipopolysaccharide (LPS)-induced direct injury model and the alpha-naphthylthiourea (ANTU)-induced indirect injury model. LPS, a potent endotoxin from Gram-negative bacteria, triggers a strong inflammatory response and lung damage when administered intratracheally, mimicking the pathophysiology of direct lung injury in ARDS (6). In contrast, ANTU, a rodenticide, induces pulmonary edema and pleural effusion through indirect mechanisms, such as endothelial damage, leading to lung injury that is less directly associated with inflammation of the alveolar epithelium (7-12). These models provide valuable insights into the different mechanisms of ARDS and are essential for evaluating potential therapeutic agents.

The integrity of the alveolar barrier is crucial in ARDS pathogenesis. Tight junction proteins, such as occludin, play a pivotal role in maintaining this barrier. Studies have demonstrated that occludin expression is reduced in ARDS patients, leading to compromised alveolar barrier function (13). Therefore, interventions that preserve or restore occludin expression may offer therapeutic benefits.

This study aimed to investigate the effects of JZL184 in two distinct ARDS models, focusing on

its potential to attenuate pathological lung damage. Additionally, it seeks to uncover the underlying mechanisms by which cannabinoids may offer therapeutic benefits in ARDS by exploring the roles of occludin, a key protein essential for maintaining the integrity of epithelial and endothelial barriers, and tumor necrosis factor-alpha (TNF-α), a multifunctional cytokine involved in inflammation.

MATERIALS AND METHODS

Animals

This study utilized 50 male Wistar albino rats, weighing 200–250 g and aged 3–4 months. The animals were housed under standard laboratory conditions, including a controlled temperature of 22 ± 2 °C, a 12-hour light/dark cycle, and unrestricted access to water and a 21% protein pellet diet.

Experimental Groups

The rats were randomly divided into five distinct experimental groups, with each group comprising 10 animals (n = 10) (Table-1).

Table-1. Experimental groups.

	Groups	Dose / Route of Application
1	Control	-
2	ANTU	10 mg/kg / i.p.
3	LPS	5 mg/kg / intratracheal
4	ANTU+JZL184	10 mg/kg + 10 mg/kg / i.p./i.p.
5	LPS+JZL184	5 mg/kg + 10 mg/kg / intratracheal/i.p.

ANTU-Induced Indirect ARDS Model

The indirect ARDS model was established using ANTU. ANTU was prepared as a suspension in olive oil at a concentration of 4 mg/ml and administered to the rats at a dose of 10 mg/kg via gavage. Four hours after ANTU administration, the rats were anesthetized with a combination of ketamine (75 mg/kg, intraperitoneal/i.p.) and xylazine (5 mg/kg, intramuscular). Following anesthesia, the animals were euthanized via exsanguination through the abdominal aorta. Once bleeding had ceased, the thoracic cavity was carefully opened, and pleural effusion was collected using syringes (7-12).

Intratracheal LPS-Induced Direct ARDS Model

The direct ARDS model was induced using LPS. Under ketamine (75 mg/kg, i.p.) and xylazine (5 mg/kg, intramuscular) anesthesia, the neck area of each animal was shaved, and the rats were placed in a supine position, and a 1 cm midline incision was made to expose the trachea. LPS, dissolved in saline, was administered intratracheally at a dose of 5 mg/kg. Subsequent to the procedure, the rats were carefully rotated to a vertical position to ensure the uniform spread of LPS throughout the lungs. To minimize the risk of respiratory suppression, the rats were positioned within their cages at a 45-degree incline and closely monitored until they had fully regained consciousness from anesthesia. 24 hours following LPS administration, the rats were anesthetized and then euthanized for the assessment of pulmonary effects. The thoracic cavity was exposed, and the lungs were carefully removed and cleaned for further examination.

Biochemical Analyses

MDA Measurement (nmol/L)

Malondialdehyde (MDA) levels were determined using a thiobarbituric acid (TBA)-based assay. The reaction involved MDA or MDA-like substances reacting with TBA at 100 °C and pH 2–3 for 15 minutes, producing a pink pigment with maximum absorbance at 532 nm. Lung tissue samples were mixed with 10% trichloroacetic acid to precipitate proteins, followed by centrifugation. An aliquot of the supernatant was reacted with 0.67% TBA in a boiling water bath for 15 minutes. Absorbance was measured at 532 nm after cooling. Commercial kits (Rel Assay Diagnostics, Gaziantep, Turkey) were used for this analysis.

SOD Activity (U/ml)

Superoxide dismutase (SOD) activity, which catalyzes the dismutation of superoxide radicals into hydrogen peroxide (H_2O_2) and oxygen, was measured using a xanthine/xanthine oxidase system to generate superoxide radicals. The inhibition of the reaction forming a red formazan dye was used to quantify SOD activity. Measurements were performed using a biochemistry analyzer (Mindray BS-400, Ohio, USA).

Catalase Activity (U/ml)

Catalase activity was assessed by its ability to decompose H_2O_2, with the reaction stopped using ammonium molybdate. Residual H_2O_2 reacted with ammonium molybdate to form a yellow complex, quantified by absorbance at 405 nm. Commercial kits (Rel Assay Diagnostics, Gaziantep, Turkey) were employed for this analysis.

Immunohistochemical Analyses

Lung tissue sections (5 µm) were placed on charged slides, deparaffinized, and subjected to antigen retrieval with citrate buffer in a microwave. After cooling and washing with phosphate-buffered saline (PBS), endogenous peroxidase activity was blocked with 3% hydrogen peroxide, followed by Ultra V block to reduce non-specific binding. Sections were incubated with anti-occludin polyclonal primary antibodies (Thermo Fisher Scientific, Massachusetts, USA) and anti TNF-α polyclonal primary antibodies (Thermo Fisher Scientific, Massachusetts, USA), then treated with a biotinylated secondary antibody and streptavidin-peroxidase. Staining was performed using diaminobenzidine (DAB) chromogen, with hematoxylin as the counterstain. To minimize background staining, all slides were processed in a humidity chamber. Stained sections were examined under a Zeiss Axio Lab.A1 photomicroscope, with images captured for further analysis.

The intensity of TNF-α staining was evaluated using the H-score method. This score was calculated by multiplying the percentage of positively stained cells by their staining intensity, following the formula H-score = $\sum i \times Pi$, where i represents the intensity score and Pi the percentage of cells. For statistical accuracy, scoring was performed at 40× magnification, with 20 fields analyzed per section.

Chemicals

The following chemicals and reagents were used in this study: JZL184 (Santa Cruz Biotechnology, Texas, USA); olive oil (Sigma-Aldrich, St. Louis and Burlington, MA, USA); ANTU (Sigma-Aldrich, St. Louis and Burlington, MA, USA); LPS (Sigma-Aldrich, St. Louis and Burlington, MA, USA); xylazine (Bioveta, Komenského, Czech Republic); ketamine (Pfizer, New York, USA); saline (Polifarma, Tekirdağ, Turkey).

Statistical Analysis of Data

Statistical analysis was conducted with Jamovi 2.3.21 (Computer Software, retrieved from https://www.jamovi.org, Sydney, Australia). Data are presented as mean ± standard deviation (SD). The normality of the data was assessed using the Shapiro-Wilk test. For variables that were not normally distributed, the Kruskal-Wallis test was performed, followed by Mann-Whitney U tests with Bonferroni correction for post-hoc analysis. A p-value of < 0.05 was considered statistically significant.

RESULTS

ELISA

MDA levels

Biochemical analysis revealed a statistically significant decrease in MDA levels in the lung tissue of the ANTU group compared to the control group. Although differences were observed in the LPS, ANTU+JZL184, and LPS+JZL184 groups compared to their respective controls, these changes were not statistically significant (Figure-1A).

SOD levels

In the ANTU group, SOD levels showed a significant increase compared to the control group. Additionally, SOD levels were significantly higher in the ANTU group than in the LPS group. In contrast, SOD levels in the LPS group did not differ significantly from the control group. Although variations in SOD levels were observed in the treatment groups (ANTU+JZL184 and LPS+JZL184) compared to their corresponding pathology groups, these changes were not statistically significant (Figure-1B).

CAT levels

Catalase levels exhibited variations in the ANTU group compared to the control group, with additional changes observed following JZL184 treatment. However, no statistically significant differences in catalase levels were observed between the groups (Figure-1C).

Immunohistochemistry

Occludin staining was observed in bronchiolar epithelial cells but not in endothelial cells in the ANTU group. In contrast, the ANTU+JZL184 group showed occludin staining in both

bronchiolar epithelial and endothelial cells. Similarly, in the LPS group, occludin staining was present in endothelial cells but absent in bronchiolar epithelial cells, whereas the LPS+JZL184 group exhibited staining in both cell types (Figure-2). Immunohistochemical analysis also revealed a significant increase in TNF-α levels in the alveolar septum and inflammatory cell cytoplasm infiltrating the expanded perivascular areas of the ANTU group. Similarly, the LPS group showed elevated TNF-α protein expression in the bronchiolar epithelium and in the inflammatory cell cytoplasm infiltrating the alveolar septum and bronchiolar adventitia. In contrast, the ANTU+JZL184 and LPS+JZL184 treatment groups showed a statistically significant decrease in TNF-α staining compared to their respective pathology groups, indicating a potential therapeutic effect (Figure-3).

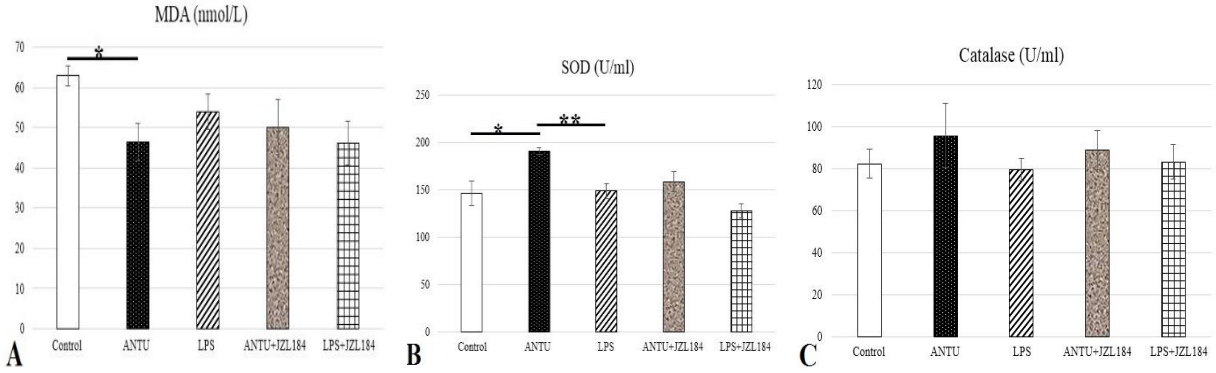

Figure-1. A-C: Biochemical parameters in ARDS models induced by ANTU and LPS, and the effects of JZL184 treatment. Data are shown as mean ± SD. ***** : Statistically significant difference between the ANTU and control groups ($p < 0.05$), ****** : Statistically significant difference between the ANTU and LPS groups ($p < 0.05$).

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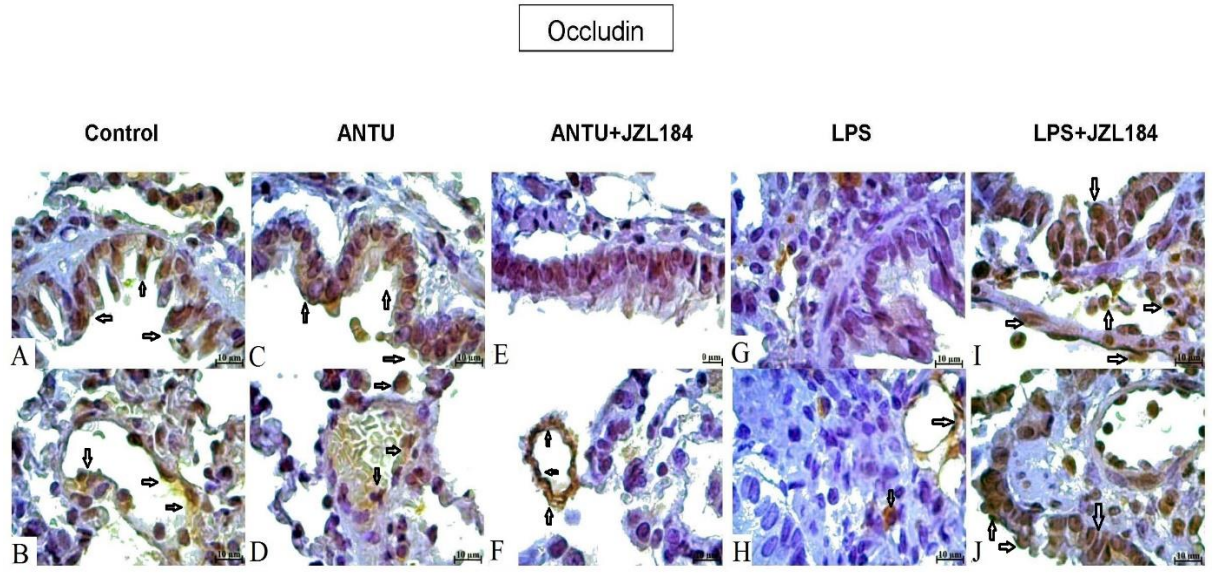

Figure-2. A-J. Immunohistochemical staining images to determine occludin staining intensity in all groups. Positive staining areas are indicated by arrows. Scale bar: 10 µm

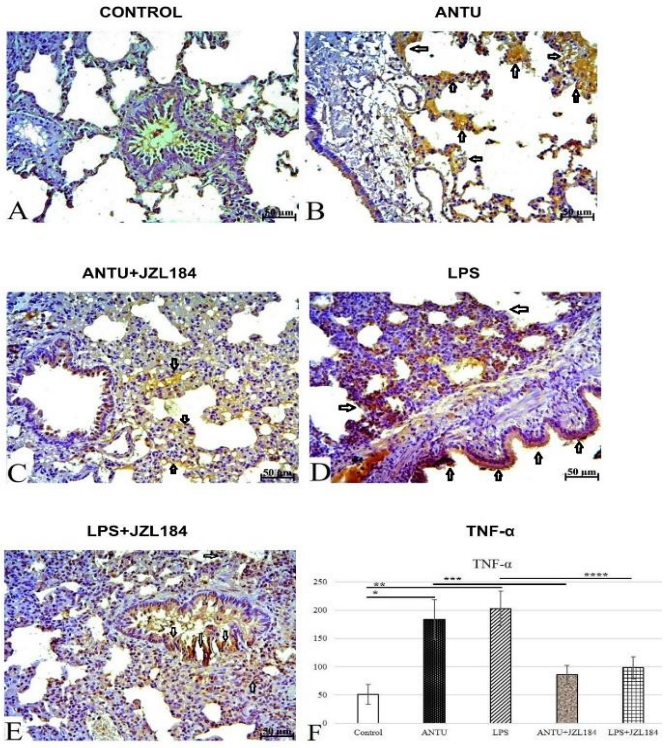

Figure-3. A-E. Immunohistochemical staining images to determine TNF-α expression in all groups.
A: Control, B: ANTU, C: ANTU+JZL184, D: LPS, E: LPS+JZL184. Positive staining areas are indicated by arrows. Scale bar: 50μm. **F:** Statistical analysis of TNF-α levels across the groups. Data are shown as mean ± SD.
***** : Statistically significant difference between the ANTU and control groups ($p < 0.05$),
****** : Statistically significant difference between the LPS and control groups ($p < 0.05$),
******* : Statistically significant difference between the ANTU and ANTU+JZL184 groups ($p < 0.05$),
******** : Statistically significant difference between the LPS and LPS+JZL184 groups ($p < 0.05$).

DISCUSSION

This is the first experimental study to demonstrate the efficacy of JZL184, which enhances endocannabinoid activity, in two distinct ARDS models by exerting anti-inflammatory and barrier-protective effects through the modulation of TNF-α and occludin expression. The findings indicate that JZL184 administration influences biochemical markers and immunohistochemical characteristics in these ARDS models, suggesting its potential therapeutic role in lung injury.

The endocannabinoid system (ECS), which plays a key role in regulating various physiological processes, has emerged as a promising target for treating inflammatory diseases, including lung injury. Cannabinoids, the active compounds derived from cannabis, have shown protective effects in conditions such as cardiovascular, autoimmune, and neurodegenerative disorders (14-19). ECS, which includes cannabinoid receptors and a variety of metabolites, plays a role in regulating immune cell activity. Cannabinoid receptors are widely expressed in human lung tissue (20). Evidence has demonstrated the presence of CB1 and CB2 receptors within lung tissues, and a variety of cells release endocannabinoids in response to inflammatory stimuli. However, the exact effects of these endocannabinoids on lung health and diseases remain unclear. Research has indicated that the CB2 receptor agonist JWH133 has the potential to alleviate lung-associated complications in pathologies like RSV infection in both human and mouse models (21), can exert a protective effect against pulmonary fibrosis (22), can reduce the damage in brain, lung, liver and heart (23). These findings suggest that

cannabinoids could be promising agents for treating lung diseases.

Endocannabinoids such as 2-AG and anandamide are metabolized by the enzymes monoacylglycerol lipase (MAGL) and FAAH, respectively (24). JZL184, an inhibitor of MAGL, enhances 2-AG levels and exerts notable anti-inflammatory effects (25). Preclinical models have shown that JZL184 can also protect tissues from oxidative damage and exert anti-apoptotic effects (26,27).

In the current study, biochemical analysis showed a significant decrease in MDA levels in the ANTU and LPS groups compared to the control. In the treatment groups, MDA levels were altered but not significantly. SOD levels were higher in the ANTU group but reduced with JZL184 treatment. Catalase levels were elevated in the ANTU group but decreased with JZL184 treatment, with no significant differences between groups.

The changes in MDA levels across treatment groups reflect complex interactions between oxidative stress and JZL184's therapeutic effects. In the ANTU and LPS groups, MDA levels decreased. However, MDA levels increased in the ANTU+JZL184 group, suggesting a compensatory response or altered balance between oxidative stress and antioxidant defenses. In contrast, MDA levels decreased in the LPS+JZL184 group, indicating the potential anti-inflammatory and antioxidative properties of JZL184 in LPS-induced pathology. These differences may be attributed to the varying effects of JZL184 in ARDS models, which are based on different pathophysiological mechanisms.

Regarding SOD, the significant increase in the ANTU group reflects a compensatory response to oxidative stress, while the reduction in SOD levels in the ANTU+JZL184 group suggests that JZL184 modulates oxidative stress and inflammatory responses, reducing the need for excessive antioxidant production. In the LPS group, SOD levels remained unchanged, but the decrease in the LPS+JZL184 group, similar to the ANTU+JZL184 group, suggests that JZL184 modulates oxidative stress, reducing the need for excessive antioxidant production. Elevated catalase levels in the ANTU group indicate an adaptive response to oxidative stress. The decrease in catalase levels in the ANTU+JZL184 group suggests that JZL184 modulated oxidative stress, reducing the need for excessive antioxidant production, similar to its effect on SOD in the same group. Catalase levels in the LPS and LPS+JZL184 groups remained similar to the control, indicating that JZL184 had less effect on catalase activity in LPS-induced lung injury.

Occludin, a critical tight junction protein, plays a vital role in maintaining the integrity of the alveolar barrier. Reduced expression of occludin is associated with lung injury, and restoring its function has been shown to enhance barrier integrity and mitigate lung damage (28). Moreover, infections can further impair the alveolar epithelial barrier by decreasing the expression of occludin in lung tissues, exacerbating lung injury (29).

In the present study, occludin expression displayed distinct patterns in the two ARDS models. In the ANTU group, which primarily causes endothelial damage, occludin staining was present in bronchiolar epithelial cells but absent in endothelial cells, indicating a disruption in endothelial cell integrity. Conversely, in the LPS group, which induces direct epithelial injury, occludin staining was observed in endothelial cells but significantly reduced in bronchiolar epithelial cells, highlighting epithelial damage and compromised barrier function in this model.

JZL184 treatment demonstrated a therapeutic effect by restoring occludin expression in both cell types. In the ANTU+JZL184 group, occludin staining was observed in both bronchiolar epithelial and endothelial cells, suggesting that JZL184 mitigates the endothelial damage and preserves barrier integrity in the context of indirect lung injury. Similarly, in the LPS+JZL184 group, occludin expression was restored in both cell types, indicating the protective role of JZL184 in maintaining epithelial integrity in the direct lung injury model induced by LPS. These findings highlight that JZL184 treatment corresponds to the unique pathophysiological mechanisms of each model, offering potential therapeutic benefits by enhancing occludin expression and promoting the preservation of the tight junctions.

The current observation aligns with the findings of Wang et al., who reported that JZL184 enhances occludin expression in intestinal models, thereby improving epithelial barrier function under stress (30). Similarly, cannabidiol

treatment in the traumatic brain injury model has been shown to increase occludin expression and improve blood-brain barrier integrity, highlighting its potential for neuroprotection by restoring tight junction proteins (31). JWH133, a cannabinoid receptor 2 agonist, treatment in an intracerebral hemorrhage model has also been demonstrated to enhance occludin expression and protect the blood-brain barrier, suggesting that cannabinoid-based therapies may play a crucial role in maintaining barrier function across various injury models (32).

TNF-α is a versatile cytokine that plays a critical role in numerous physiological and pathological processes, including inflammation, cell death, and immune system regulation (33). Elevated levels of TNF-α have been implicated in the pathogenesis of various pulmonary inflammatory diseases, including asthma, chronic obstructive pulmonary disease, and ARDS, by inducing inflammation and tissue remodeling, and its inhibition has been shown to enhance lung function (34, 35).

The current analysis revealed distinct patterns of inflammation in the two ARDS models. In the ANTU group, elevated TNF-α levels in the alveolar septum and inflammatory cell cytoplasm infiltrating the expanded perivascular areas indicated enhanced endothelial damage, consistent with previous findings of TNF-α's role in endothelial dysfunction. Similarly, the LPS group showed increased TNF-α expression in the bronchiolar epithelium and in the inflammatory cell cytoplasm infiltrating the bronchiolar adventitia, highlighting epithelial damage and disruption of the epithelial barrier.

Notably, treatment with JZL184 resulted in a significant reduction in TNF-α staining in both the ANTU+JZL184 and LPS+JZL184 groups. This suggests that JZL184 may reduce TNF-α-mediated inflammation and protect against endothelial and epithelial damage in these lung injury models. These findings suggest that JZL184 holds therapeutic potential by modulating the inflammatory response and preserving barrier integrity in ARDS.

Several studies have demonstrated the anti-inflammatory effects of cannabinoids, particularly in reducing TNF-α levels. It has been reported that cannabinoids, including CBD, decrease TNF-α and modulate inflammation (36). Similarly, a systematic review showed that both CBD and CBD+THC combinations significantly lower TNF-α levels (37). Additionally, cannabinoids have been proposed to inhibit TNF-α and other inflammatory cytokines, providing potential therapeutic benefits in inflammatory diseases like SARS-CoV-2 infection (38). Furthermore, network-based pharmacology analysis identified key targets such as NF-κB and TNF receptors in the anti-inflammatory activity of cannabinoids, supporting the therapeutic potential of compounds like JZL184 (39).

Occludin's expression and function are influenced by inflammatory cytokines, particularly TNF-α, which disrupts tight junctions by reducing occludin expression or altering its cellular localization. This leads to increased permeability and contributes to the development of various inflammatory diseases, such as inflammatory bowel disease, neuroinflammation, and sepsis. Modulating the occludin-TNF-α interaction presents a potential therapeutic strategy to restore barrier integrity and mitigate disease progression. Recent studies have demonstrated the protective effects of baicalin and rhubarb extract through the modulation of occludin and TNF-α. In aging mice with periodontitis, baicalin upregulated occludin expression, restored intestinal permeability, and reduced TNF-α levels, thereby alleviating inflammation and preventing alveolar bone loss (40). Similarly, rhubarb extract improved intestinal barrier integrity by increasing occludin expression and decreasing TNF-α levels in both serum and the hippocampus, offering protection against cerebral ischaemia-reperfusion injury (CIRI) (41).

In the current study, in accordance with the results presented above, a decrease in occludin protein levels was observed in parallel with elevated TNF-α levels in the pathological groups. This change was reversed following treatment with JZL184, highlighting the therapeutic potential of targeting the occludin-TNF-α pathway for the treatment of ARDS.

Limitations

Due to budget constraints, a single dose of JZL184 was used in the present study, which limits the ability to assess its dose-dependent effects. Additionally, various parameters potentially involved in the distinct pathophysiological mechanisms underlying ARDS could not be investigated for the same reason. Further research is needed to fully

elucidate these mechanisms and the therapeutic potential of JZL184 in lung injury.

CONCLUSION

The results of this study demonstrate that JZL184 offers significant therapeutic potential in both direct and indirect ARDS models. By enhancing occludin expression and restoring tight junction integrity in both endothelial and epithelial cells, JZL184 mitigates tissue damage and supports the preservation of alveolar barrier function. Additionally, JZL184 reduces TNF-α expression, thereby attenuating inflammation and consequently contributing to the protection of lung tissue. The differential effects observed in the ANTU- and LPS-induced injury models underscore the importance of understanding the specific pathophysiological mechanisms underlying ARDS. Overall, these findings suggest that JZL184 could be a promising candidate for further exploration in the treatment of ARDS, offering a novel approach to managing this challenging and complex condition.

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Conflicts of interest: Authors declared no conflict of interest.

Ethical Approval

All experimental procedures described in this study were previously approved by the Ethical Committee of Experimental Animals of Zonguldak Bulent Ecevit University (Protocol No: 2025-04-20/02) and are in accordance with the "Guide for the Care and Use of Laboratory Animals" (US National Institute of Health, revised 1996).

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