International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 15s, 2025
https://www.theaspd.com/ijes.php

Protective Role of JAK Inhibitor in Rat Sepsis

Mohammed Merza[1],[4] Karez Abdulghany Omer[1],Taha Talal Abdulrahman[2], Halgurd Nadhim Mohammed[3]
[1]Clinical Analysis Department, College of Pharmacy, Hawler Medical University, Erbil, Kurdistan Region, Iraq.
[2]Department of Pharmacy, College of Pharmacy, Knowledge University, Erbil 44001, Iraq
[3]Kurdistan Technical Institute, Sulaymaniyah 46001, Iraq
[4]Nutrition and Dietetics Department, Tishk International University, Erbil, Iraq.

***Correspondence and request for reprints to:Mohammed Merza,**
MSc, Ph.D, Department of Clinical Analysis , College of Pharmacy, Hawler Medical University, Erbil, Kurdistan 100M street Erbil, mohammed.merza@hmu.edu.krd

Abstract
Acute lung injury (ALI) is a critical condition marked by inflammation-induced lung swelling, resulting in acute hypoxic respiratory failure. The JAK inhibitor tofacitinib has been implicated in the regulation of inflammatory responses. In this study, we hypothesized that the JAK inhibitor tofacitinib could modulate neutrophil infiltration in a lipopolysaccharide (LPS)-induced sepsis mouse model.
A total of 18 rats (8 weeks old, weight 220–250 grams) were randomly divided into three groups (n = 6 per group): Control group – received intraperitoneal normal saline. LPS group – received 30 mg/kg of LPS intraperitoneally. Tofacitinib + LPS group – received 30 mg/kg of the selective JAK inhibitor tofacitinib intraperitoneally, 15 minutes before LPS administration.
After 24 hours, the animals were euthanized. Inflammatory cell infiltration and levels of proinflammatory cytokines—TNF-α, IL-1β, IL-6, bacterial CFU and myeloperoxidase (MPO) activity—were assessed.
Compared to the LPS group, pretreatment with tofacitinib significantly reduced MPO activity, TNF-α, IL-1β, bacteria CFU in blood and IL-6 levels. Notably, inflammatory cell migration and infiltration were suppressed, as shown by decreased MPO activity in treatment group, $P<0.05$*. Similarly, IL-6, bacteria CFU concentrations in the treatment group were significantly lower than in the LPS group.*
These findings suggest that tofacitinib exerts protective and anti-inflammatory effects in LPS-induced ALI, potentially offering a therapeutic approach for managing sepsis-related lung injury.
Keywords: *JAK inhibitor tofacitinib, Chemokine, Sepsis,*

INTRODUCTION

Acute lung injury (ALI) is a serious complication of systemic inflammation and is associated with increased mortality in critically ill patients [1]. Disruption of the intestinal barrier and the subsequent translocation of bacteria and their toxins into the abdominal cavity trigger the local production of pro-inflammatory mediators, which then enter the systemic circulation. These mediators activate innate immune cells such as neutrophils and platelets, resulting in a systemic inflammatory response [2].

While white blood cells play a critical role in defending against bacterial infections, their excessive accumulation within the pulmonary microvasculature can impair gas exchange in the lungs. For example, neutrophil depletion has been shown to confer protection against lung injury in various sepsis models, suggesting that pulmonary leukocyte infiltration is a key step in the pathogenesis of ALI [3]. However, the precise spatial mechanisms governing leukocyte-endothelium interactions in the lung's narrow capillary network during systemic inflammation remain poorly understood, largely due to the technical challenges of studying these interactions in vivo [4].

In many organs—including the colon, liver, pancreas, brain, and skeletal muscle—leukocyte recruitment

follows a well-defined multi-step process: initial rolling contact, firm adhesion, and transendothelial migration. β2-integrins, such as CD11a/CD18 (lymphocyte function-associated antigen-1) and CD11b/CD18 (macrophage-1 antigen), have been shown to mediate leukocyte adhesion in these tissues. However, due to the technical difficulty of observing the lung microvasculature, it remains unclear whether the same mechanisms apply to the lungs [5,6].

Leukocyte recruitment in the pulmonary circulation is notably more complex than in other organs. Under physiological conditions, neutrophils—which are larger than the diameter of pulmonary capillaries—must deform to pass through the lung microcirculation. During systemic inflammation, activated neutrophils become stiffer, increasing their mechanical sequestration in the capillaries. Additionally, intercellular adhesion molecule-1 (ICAM-1), a β2-integrin ligand expressed on pulmonary endothelial cells, is upregulated during systemic inflammation and plays a critical role in facilitating leukocyte migration to the lungs [7,8].

Recent studies have demonstrated that Tofacitinib, can reduce severe inflammation, suggesting JAK kinase involvement in the inflammatory cascade. Moreover, inhibition of Tofacitinib, activity has been shown to reduce airway hyperresponsiveness and inflammation, particularly in smoking-related models [9].

Building on these findings, our study aims to investigate the role of Tofacitinib, in regulating leukocyte extravasation, as well as chemokine and cytokine expression in the lungs during systemic inflammation, with a specific focus on abdominal sepsis.

MATERIAL METHOD

Animals

Male albino Rat (6–8 weeks old, weighing 220–250 g) were obtained from the Laboratory Animal Facility of Hawler Medical University, College of Pharmacy (Erbil, Iraq). All animal procedures were approved by the Ethics Committee of Hawler Medical University's College of Pharmacy and were conducted in accordance with the "Regulations on the Administration of Laboratory Animals." The animals were housed under standard conditions with a 12-hour light/dark cycle, a constant temperature of 25 ± 2°C, and 70% relative humidity. Food and water were provided ad libitum.

Experimental Design and Induction of Sepsis

As previously described, mice were randomly assigned into five groups (n=6 per group) for the lipopolysaccharide (LPS)-induced septic shock model: Control group: received 0.9% NaCl (vehicle), LPS group: received 20 mg/kg LPS intraperitoneally (i.p.), JAK inhibitor tofacitinib + LPS (pre-treatment) group: received 30 mg/kg (i.p.) 10 minutes prior to LPS, LPS + JAK inhibitor tofacitinib (post-treatment) group: received 30 mg/kg 10 minutes after LPS, JAK inhibitor -only group: received 30 mg/kg JAK inhibitor without LPS. LPS was administered intraperitoneally at a dose of 20 mg/kg to induce systemic inflammation. JAK inhibitor, a selective JAK inhibitor, was administered at 30 mg/kg based on previous studies. Mice in the control and LPS groups received an equivalent volume of 0.9% NaCl. All animals were euthanized 24 hours post-injection for sample collection and analysis.

Enzyme-Linked Immunosorbent Assay (ELISA)

Blood samples and peritoneal lavage fluids were collected and centrifuged at 3500 rpm for 15 minutes. The levels of tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6) were quantified using commercially available ELISA kits (Neo Bioscience Technology, Shenzhen, China) according to the manufacturer's instructions.

Myeloperoxidase (MPO) Activity Assay

Lung tissues were homogenized in 1 mL of PBS containing aprotinin (10,000 KIE/mL; Trasylol®, Bayer HealthCare AG, Leverkusen, Germany) for 1 minute. The homogenates were centrifuged at 15,339 g for 10 minutes, and the supernatants were stored at –20°C. The resulting pellets were resuspended in 1 mL of 0.5% hexadecyltrimethylammonium bromide (HTAB), frozen for 24 hours, thawed, sonicated for 90 seconds, and incubated at 60°C for 2 hours in a water bath. MPO activity was then measured in the supernatants.

MPO enzyme activity was assessed spectrophotometrically by monitoring the change in absorbance at 450 nm (reference at 540 nm) at 25°C during the MPO-catalyzed redox reaction of H_2O_2. Results were expressed as MPO units per gram of lung tissue.

Bacterial Quantification

For bacterial load assessment, blood samples were collected from the inferior vena cava, and mesenteric lymph nodes (LNs) were harvested and immediately processed. Mesenteric LNs were weighed, homogenized, and cultured for bacterial growth. Blood and tissue samples were plated on blood agar and incubated at 37°C for 24 hours.

Following incubation, bacterial colony counts were recorded. Results were expressed as colony-forming units per milliliter (CFU/mL) for blood samples and colony-forming units per gram (CFU/g) for mesenteric lymph node tissue.

Statistical Analysis

Data were presented as mean ± standard error of the mean (SEM). Statistical comparisons between groups were performed using non-parametric Mann-Whitney tests. A p-value < 0.05 was considered statistically significant. The symbol "n" represents the number of animals per group.

RESULT

JAK inhibitor tofacitinib Modulates Proinflammatory Cytokine Production

To assess the role of JAK inhibitor tofacitinib in acute lung injury, plasma levels of tumor necrosis factor-alpha (TNF-α) were measured as a key marker of systemic inflammation in adult male mice. Mice in the sham, JAK inhibitor tofacitinib alone, and JAK inhibitor tofacitinib treatment groups exhibited low plasma concentrations of TNF-α, indicating minimal inflammatory response. In contrast, the LPS-treated group demonstrated a significant elevation in TNF-α levels, confirming the induction of a robust inflammatory response.

Importantly, pre-treatment with the JAK inhibitor tofacitinib significantly reduced TNF-α concentrations compared to the LPS-only group, suggesting a potent anti-inflammatory effect. This reduction was statistically significant (P = 0.001) as shown in Table 1, indicating that JAK inhibitor tofacitinib effectively suppresses systemic proinflammatory cytokine production in LPS-induced acute lung injury.

JAK Inhibition Reduces LPS-Induced TNF-α and IL-1β Levels

To determine the role of JAK inhibitor in systemic inflammation, plasma TNF-α levels were measured 24 hours after LPS administration. Mice were divided into sham (normal saline), LPS-treated, vehicle + LPS, and JAK inhibitor, 30 mg/kg) + LPS groups. As shown in **Table 1**, LPS exposure resulted in a significant increase in plasma TNF-α levels compared to the sham group (*P < 0.05*). However, pretreatment with the JAK inhibitor markedly reduced TNF-α concentrations compared to the vehicle + LPS group (#P < 0.05). Data are presented as mean ± SEM (n = 5).

Table 1: TNF-α activity measured in plasma (pg/ml)

Parameters	**Mean**	**SE**	**P value**
Healthy	66	±8	<0.05
Vehicle + LPS	325*	±6	
JAK inhibitor + LPS	114#	±4	

Similarly, plasma IL-1β levels followed a trend comparable to that of TNF-α. The LPS-treated group exhibited a significant elevation in IL-1β levels compared to the sham group (**P = 0.001**). In contrast, mice pretreated with JAK demonstrated a substantial decrease in plasma IL-1β levels relative to the LPS group (**P = 0.001**), indicating that JAK inhibition effectively downregulates this key proinflammatory cytokine (**Table 2**).

Table 2: IL-1β activity measured in plasma (pg/ml).

Parameters	Mean	SE	P value
Healthy	62.5	±8	<0.05
Vehicle + LPS	314*	±4	
JAK inhibitor + LPS	86.2#	±6	

JAK Inhibition Attenuates Neutrophil Infiltration in the Lung

Myeloperoxidase (MPO) activity in lung tissue was measured as an indicator of neutrophil infiltration. As shown in Table 3, LPS administration resulted in a five-fold increase in lung MPO activity compared to the sham group (P = 0.003), reflecting a significant neutrophilic response during systemic inflammation.

Importantly, pretreatment with the JAK inhibitor significantly reduced LPS-induced MPO activity by approximately 30 % (P = 0.001), indicating that JAK inhibitor effectively limits neutrophil accumulation in the lungs.

These findings support the hypothesis that, during acute lung injury, activated neutrophils aggregate in the pulmonary microvasculature, and that JAK plays a key regulatory role in this process.

Table 3: MPO activity measured in lung tissue (U/g/Tissue)

Parameters	Mean	SE	P value
Healthy	3.0	±6	<0.05
Vehicle + LPS	8.4*	±6	
JAK inhibitor = LPS	3.2#	±4	

JAK Inhibition Reduces LPS-Induced IL-6 Production

Serum IL-6 levels were markedly elevated. The LPS group demonstrated the highest IL-6 concentrations (mean ± SEM: 242 ± 6 pg/mL) compared to the healthy group (mean ± SEM: 4.6 ± 4 pg/mL), with the difference reaching statistical significance (P = 0.003), as shown in Table 4.

In contrast, pretreatment with the JAK inhibitor significantly reduced plasma IL-6 levels in LPS-challenged mice (mean ± SEM: 142 ± 4 pg/mL) compared to the LPS-only group (P = 0.003). This reduction suggests that systemic JAK activation contributes to IL-6 overproduction during acute inflammation in this sepsis model.

Table 4: IL-6 activity measured in plasma (ng/ml)

Parameters	Mean	SE	P value
Health	88	± 6	<0.05
Vehicle + LPS	242*	± 4	
JAK + LPS	142. #	± 4	

JAK inhibits bacterial spread in sepsis

Challenge with Sepsis markedly increased the number of bacteria in the blood and mesenteric LNs. Pretreatment with JAK inhibitor significantly decreased the number of bacteria in the blood and mesenteric LNs of septic animals Table 5.

Table 5: The number of bacterial colonies is quantified in the blood

Parameters	**Mean (CFU of blood X10^2)/ml**	**SE**	**P value**
Health	0	0	<0.05
Vehicle + LPS	300*	± 6	
JAK + LPS	100 #	± 6	

DISCUSSION

The signaling pathways that govern pro-inflammatory responses in sepsis remain incompletely understood. This study provides the first experimental evidence demonstrating that JAK plays a critical role in modulating the pathophysiology of sepsis and acute lung injury (ALI) [10]. Our findings indicate that JAK activity contributes to the overexpression of Mac-1 on neutrophils, and its inhibition not only attenuates neutrophil infiltration in the lungs but also significantly reduces systemic and local levels of proinflammatory cytokines and chemokines.

We observed that JAK inhibition markedly suppressed myeloperoxidase (MPO) activity and neutrophil accumulation in the lungs, suggesting a pivotal role for JAK in regulating neutrophil recruitment during the systemic inflammatory response in ALI. These results are supported by previous studies that identified a role for JAK in regulating pro-inflammatory processes involving cell adhesion molecules and microglial activation in models of intracerebral hemorrhage and multiple sclerosis [11].

Using the selective JAK inhibitor, we showed that tissue injury in severe abdominal sepsis was significantly reduced. Treatment with JAK decreased circulating levels of TNF-α and IL-1β by approximately 30% and 55%, respectively, highlighting the broad anti-inflammatory potential of JAK inhibition in this model. To our knowledge, this is the first study to provide direct evidence that blocking JAK signaling significantly mitigates the inflammatory burden of sepsis, particularly in the lungs.

Although JAK has primarily been investigated in the context of cancer, particularly in targeting the JAK – ERK pathway in lung carcinoma, recent studies suggest it also regulates the expression of key chemokines [12]. Our current findings support this, revealing that JAK inhibition significantly reduced LPS-induced CFU bacteria colony. This suggests that JAK may regulate CFU colony.

Neutrophil infiltration is a well-established contributor to the pathogenesis of ALI. Several studies have demonstrated that depletion of neutrophils alleviates lung tissue injury [13,14]. Consistently, our study found that LPS exposure led to substantial increases in lung neutrophil infiltration and MPO activity. Notably, JAK treatment significantly reduced both MPO levels and neutrophil presence, indicating that JAK is a key modulator of neutrophil recruitment and activation during inflammatory lung injury.

Furthermore, IL-6, a major cytokine involved in pulmonary inflammation, was significantly elevated in LPS-treated mice. JAK inhibition led to a significant reduction in IL-6 levels, suggesting that JAK may also modulate systemic cytokine expression. This aligns with reports that IL-6 plays a critical role in the pathogenesis of both experimental and clinical pulmonary diseases.

Taken together, our findings demonstrate that JAK regulates both local and systemic inflammatory responses in sepsis-induced ALI, including neutrophil infiltration, cytokine release, and chemokine production. By targeting JAK it may be possible to disrupt the inflammatory cascade that drives lung injury in sepsis.

REFERENCES

[1] Sun B, Lei M, Zhang J, Kang H, Liu H, Zhou F. Acute lung injury caused by sepsis: how does it happen? Front Med (Lausanne)2023;10:1289194.https://doi.org/10.3389/fmed.2023.1289194

[2] Merza M, Taufiq G, Othman BA, Ahmad KK.Interleukin-3 Inhibition Regulate Inflammation and Tissue Damage in Acute Pancreatitis. J Neonatal Surg2025; 14(11S):903-9. https://doi.org/10.52783/jns.v14.3073

[3] Mohammed Yousif Merza 1 HJS. Rac-1 Inhibitor Attenuates Neutrophil Infiltration and Tissue Damage in Severe Acute Pancreatitis. 5th Int Conf Biomed Health Sci CIC-BIOHS2024. DOI:http://doi.org/10.24086/biohs2024/paper.1359

[4] Hall S, Faridi S, Euodia I, Tanner S, Chojnacki AK, Patel KD, et al. Intravital Widefield Fluorescence Microscopy of Pulmonary Microcirculation in Experimental Acute Lung Injury Using a Vacuum-Stabilized Imaging System. J Vis Exp2022;(182). doi: 10.3791/63733.

[5] Tanaka T. Leukocyte Adhesion Molecules.encyclopedia-of-immunobiology2016;3. p. 505–11.https://doi.org/10.1016/B978-0-12-374279-7.07015-6

[6] Park I, Kim M, Choe K, Song E, Seo H, Hwang Y, et al. Neutrophils disturb pulmonary microcirculation in sepsis-induced acute lung injury. Eur Respir J 2019;53(3).https://doi.org/10.1183/13993003.00786-2018

[7] Haydinger CD, Ashander LM, Tan ACR, Smith JR. Intercellular Adhesion Molecule 1: More than a Leukocyte Adhesion Molecule. Biology 2023 ;12(5):743. doi: 10.3390/biology12050743.

[8] wang p, laster k, jia x, Dong z, Liu k. Targeting CRAF kinase in anti-cancer therapy: progress and opportunities. Mol Cancer 2023; 22:208. doi: 10.1186/s12943-023-01903-x

[9] Fang H, Wang X, Damarla M, Sun R, He Q, Li R, et al. Dimethyl Fumarate Protects against Lipopolysaccharide- (LPS-) Induced Sepsis through Inhibition of NF-κB Pathway in Mice. Mediators Inflamm2023;5133505. doi: 10.1155/2023/5133505.

[10] Hong Z, Li S, Liu X, Leng X, Miao Z, Kang X, et al. Blocking C-Raf alleviated high-dose small-volume radiation-induced epithelial mesenchymal transition in mice lung. Scientific reports 2020;10:11158.https://doi.org/10.1038/s41598-020-68175-z

[11] Huang B, Bau D, Chen T, Chuang I, Tsai C, Chang P. Pro-Inflammatory Stimuli Influence Expression of Intercellular Adhesion Molecule 1 in Human Anulus Fibrosus Cells through FAK/ERK/GSK3 and PKCδ Signaling Pathways. *Int. J. Mol. Sci*2019, *20*(1), 77. https://doi.org/10.3390/ijms20010077

[12] Yang G, Fan X, Mazhar M, Guo W, Zou Y, Dechsupa N, et al.Neuroinflammation of microglia polarization in intracerebral hemorrhage and its potential targets for intervention. Front Mol Neurosci 2022; 15:1013706. doi: 10.3389/fnmol.2022.1013706

[13] Merza M, Huda J, Zhian H, Kewan A,Roza Y. Physiological Modulation of Neutrophil Activation and Recruitment by Heat Shock Protein 90 in Acute Pancreatitis. Cuest.fisioter.2024.53(3):3392-3411. https://doi.org/10.48047/CU.

[14] Merza M, Azheen A, Maaroof M, Roza Y.Physiological Role of Targeting of the Receptor for Advanced Glycation End Products and Its Involvement in Severe Acute Pancreatitis. Cuest.fisioter.2024.53(3):3392-3411. https://doi.org/10.48047/CU.