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Effect of glutamine and arginine treatment on the inflammatory response in a rat model of pulmonary contusion



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Abstract

Introduction: Pulmonary contusion leads to alveolar damage and inflammation, which can progress to acute lung injury and acute respiratory distress syndrome. Glutamine and L-arginine play critical roles in immune regulation and inflammation control, showing potential protective effects in lung injury models.

Aim: This study aimed to investigate the effects of glutamine and/or arginine, which play significant roles in the immuno-inflammatory response, on mitigating inflammation in an experimentally induced pulmonary contusion model.

Material and methods: Thirty male Wistar Albino rats from the same colony were randomly divided into five groups (n = 6 per group). Group I (sham) received no trauma or treatment. Bilateral pulmonary contusion was induced in Groups II–V. Group II received 0.9% saline; Group III received 200 mg/kg/day intraperitoneal glutamine; Group IV received 200 mg/kg/day intraperitoneal arginine; and Group V received both glutamine (200 mg/kg/day) and arginine (150 mg/kg/day). All treatments were administered for 3 consecutive days. Rats were sacrificed three days after the procedure, and histopathologic evaluation was performed afterwards. Three different parameters were scored: inflammation intensity, intraalveolar hemorrhage, and alveolar congestion. Statistical analysis was performed using the Mann-Whitney U test.

Results: Compared to the untreated trauma group, all treatment groups showed significant histopathological improvement ($p < 0.05$). The arginine-only group demonstrated greater effectiveness across all parameters compared to the combined treatment group ($p < 0.05$). No significant difference was observed between the glutamine-only and arginine-only groups ($p > 0.05$).

Conclusions: Both glutamine and arginine treatments were effective in reducing inflammation following pulmonary contusion. However, combined treatment was less effective than arginine alone.

Key words: glutamine, arginine, inflammation, pulmonary contusion.

Introduction

Pulmonary contusion (PC) is observed in approximately 24–34% of blunt thoracic traumas [1]. It is characterized by the rupture of alveoli, intraalveolar hemorrhage, interstitial edema, and damage to the alveolocapillary membrane. Severe cases can progress to acute lung injury (ALI) and acute respiratory distress syndrome (ARDS), which are associated with high morbidity and mortality. Due to the lack of targeted therapies, experimental and clinical research on lung contusion continues to the present time.

Glutamine is the most abundant amino acid in the body and plays a critical role in nitrogen transport, immune function, and pH regulation [2]. It has been shown to inhibit neutrophil migration and also total protein concentrations in bronchoalveolar lavage (BAL) fluid in a rat model of ALI [3].

L-arginine plays a significant role in the metabolic, immune and reparative response following trauma. As the sole substrate for nitric oxide synthase, it is essential for the production of nitric oxide (NO), a key regulator of cellular metabolism that exerts its effects via cyclic guanosine monophosphate (cGMP) [4]. Nitric oxide has several physiological effects such as vasodilation, inhibition of platelet aggregation, neutrophil adhesion and suppression of xanthine oxidase (XO) activity [5]. Under catabolic conditions

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such as hemolytic anemia, burns, sepsis, and trauma, endogenous arginine synthesis decreases [6, 7]. Therefore, arginine supplementation may be beneficial particularly during oxidative stress, as it helps maintain T-cell function and reduce the risk of infection [8].

Aim

This study aimed to evaluate the histopathological effects of early glutamine and/or arginine administration, which plays an important role in the immuno-inflammatory response for the reduction of inflammation following experimental pulmonary contusion.

Material and methods

This study was approved by the Animal Ethics Committee of Dokuz Eylul University (Protocol No: 23/2020). Thirty male Wistar Albino rats (mean weight 441 g; range 405–493 g) were housed under standard laboratory conditions (21–23°C, 12-hour light/dark cycle). The rat model was used due to its easy availability, reliable results, and high reproducibility.

Rats were randomly assigned to five groups (n = 6 each):

- Group I (sham): no trauma or treatment;
- Group II (trauma control): pulmonary contusion induced under anesthesia followed by intraperitoneal 0.9% saline (4 ml/day) for 3 days;
- Group III (glutamine): 200 mg/kg/day glutamine in 0.9% saline, intraperitoneally for 3 days [9];
- Group IV (arginine): 200 mg/kg/day arginine in 0.9% saline, intraperitoneally for 3 days [10];
- Group V (combined): glutamine (200 mg/kg/day) + arginine (150 mg/kg/day) in 0.9% saline, intraperitoneally for 3 days.

Anesthesia, trauma, and pulmonary contusion

All rats included in the study were weighed prior to experimental procedures. Anesthesia was administered intraperitoneally using 80 mg/kg ketamine hydrochloride and 8 mg/kg xylazine. Throughout the procedure, the rats remained under anesthesia with spontaneous respiration preserved.

Pulmonary contusion was induced by the free-fall impact of an aluminum cylindrical weight dropped through a vertically aligned stainless steel tube fixed on a platform (Figure 1). This was based on a modified version of the isolated bilateral pulmonary contusion model originally described by Raghavendran *et al*. [11]. The kinetic energy (E) imparted during impact was calculated using the standard equation $E = mgh$, where m represents mass (in kilograms), g is the gravitational acceleration (9.8 m/s²), and h denotes the height (in meters) from which the weight was dropped. Friction forces were considered negligible. With a mass of 180 g and a drop height of 110 cm, the calculated impact energy delivered to the chest wall was 1.94 joules.

Following trauma induction, oxygen therapy (Gez Oxyhome Oxygen Concentrator) was administered at a flow of rate 2 l/min (Figure 2). After trauma, all rats had unrestricted access to food and water, and their nutritional and hydration requirements were closely monitored. No limitations were imposed on feeding or fluid intake during the post-trauma period.

Histopathologic evaluation

Subjects in all groups were sacrificed on the third day after trauma. The right lung tissue was divided from the main bronchus and the tissues were placed in 10% formalin (Figure 3). The samples were kept in formalin for 48 hours and fixed. After fixation, they were processed with 70% alcohol, 95% alcohol, and 100% alcohol in the dehydration stage, xylene in the transparency stage, and paraffin in the infiltration stage. The samples were embedded in hard paraffin blocks. Rotary microtome (RM 2255, Leica) was used to obtain 5-µm thick sections. Each section was stained with hematoxylin-eosin stain to evaluate the general histomorphological features of the tissue (Figure 4).



Figure 1. Setup of free-fall trauma induction model using a vertically mounted aluminum cylinder

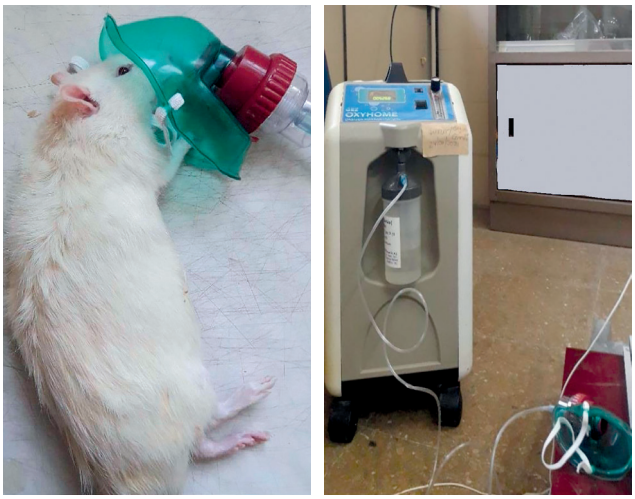


Figure 2. Oxygen support following trauma (2 l/min with Oxyhome concentrator)

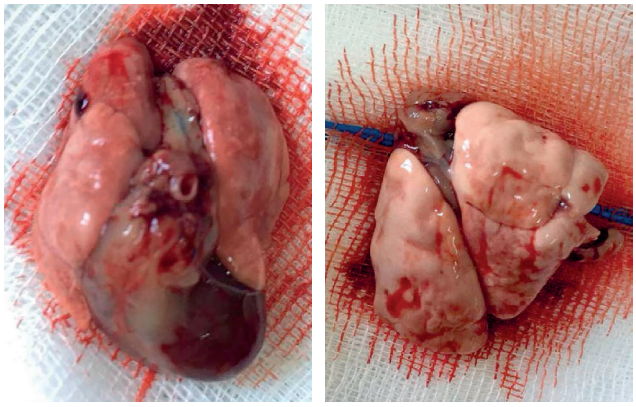


Figure 3. Lung tissue specimens placed in formalin for fixation after dissection

For each subject in the experimental groups, lung specimens were obtained from five regions of the right lung. Parenchymal inflammation, alveolar congestion, and alveolar hemorrhage were evaluated semiquantitatively according to intensity. Histologic damage was scored between 0 and 3 for each parameter (0: no change, 1: slight damage < 25%, 2: moderate damage 25–50% and 3: severe damage > 50%).

1. Parenchymal inflammation score: based on cell density. Grade 0 – no inflammation, Grade 1 – slight damage < 25%, Grade 2 – moderate damage 25–50%, Grade 3 – severe damage > 50%.
2. Intraalveolar hemorrhage score: Grade 0 – no hemorrhage, Grade 1 – focal hemorrhage, Grade 2 – patchy (foci smaller than 5 magnification fields, but multifocal), Grade 3 – diffuse (across 5 large magnification fields).
3. Alveolar congestion score: Grade 0 – no alveolar congestion, Grade 1 – focal alveolar congestion, Grade 2 – patchy (foci smaller than 5 magnification fields, but multifocal) Grade 3 – diffuse (across 5 large magnification fields).

Statistical analysis

Histopathological data were analyzed using the Mann-Whitney *U* test. A *p*-value < 0.05 was considered statistically significant.

Results

In the comparative histopathological evaluation, no inflammation, alveolar congestion, or intraalveolar hemorrhage was observed in the sham group (Group I) (Grade 0). In contrast, the trauma group (Group II) exhibited the most severe histopathologic changes across all parameters (Grade 3). The treatment groups – glutamine (Group III), arginine (Group IV), and combined glutamine + arginine (Group V) – showed notable reductions in histopathologic scores compared to the trauma group. All treatment groups exhibited significantly lower scores for inflammation, alveolar congestion, and hemorrhage ($p < 0.05$) (Table I).

No statistically significant difference was found between the glutamine-only and arginine-only groups ($p > 0.05$), suggesting similar efficacy. However, glutamine

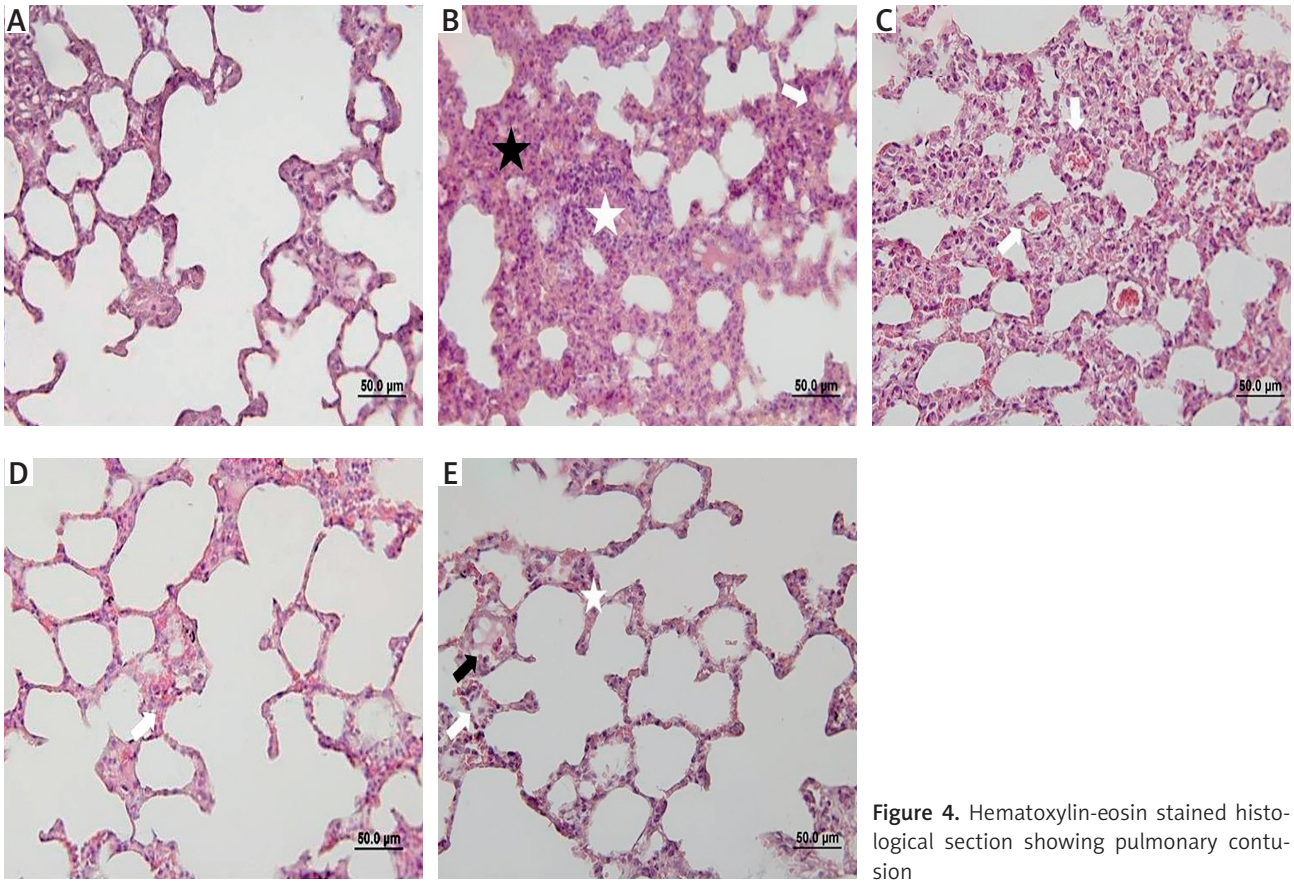


Figure 4. Hematoxylin-eosin stained histological section showing pulmonary contusion

Table I. Histopathological scores (mean ± SD, min.–max.). Statistically significant differences were observed between Group 2 and Groups 3, 4, and 5 for all parameters. Additional significant differences were observed between Groups 3 and 5 (parenchymal inflammation), and between Groups 4 and 5 (all parameters)

Groups	*N*	Parenchymal inflammation		Alveolar congestion		Intraalveolar hemorrhage	
		Mean ± SD	Min.–max.	Mean ± SD	Min.–max.	Mean ± SD	Min.–max.
Sham (Group 1)	6	0 ±0	0–0	0 ±0	0–0	0 ±0	0–0
Trauma (Group 2)	6	2.83 ±0.41	2–3	2.66 ±0.82	2–3	2.83 ±0.41	2–3
Glutamine (Group 3)	6	1.5 ±0.54	1–2	1.5 ±0.54	1–2	1.5 ±0.54	1–2
Arginine (Group 4)	6	1.17 ±.040	1–2	1.17 ±.040	1–2	1.17 ±.040	1–2
Glutamine + arginine (Group 5)	6	2.17 ±0.41	2–3	2 ±0.63	1–3	2 ±0.63	1–3

monotherapy was more effective than combined treatment in reducing parenchymal inflammation ($p < 0.05$) (Figure 5). Notably, arginine monotherapy was significantly superior to combined therapy in all three histopathologic parameters ($p < 0.05$) (Table II).

Discussion

This study investigated the therapeutic effects of glutamine and arginine on histopathological parameters in a rat model of pulmonary contusion induced by blunt thoracic trauma. Our findings confirmed that both agents, when administered individually, reduced inflammation, intra-alveolar hemorrhage, and alveolar congestion effectively.

Previous studies have demonstrated that pulmonary contusion triggers an acute inflammatory response within 24 hours, characterized by increased neutrophil infiltration. By 48 hours, subacute changes such as lymphocyte infiltration and alveolar edema become apparent [12].

Our findings support these pathophysiological timelines. Nutrition-immunity interaction gained attention especially during the COVID-19 pandemic, highlighting the potential role of amino acid-based support [13]. The anti-inflammatory properties of glutamine have been demonstrated in several studies. Recent studies also confirm glutamine's protective effects on alveolar epithelial regeneration during lung injury [14]. For example, Oliveira *et al.* reported that intravenous glutamine (0.75 g/kg) significantly improved alveolocapillary membrane integrity and oxygenation while reducing neutrophilic infiltration and alveolar collapse [15]. Similarly, our study demonstrated significant reductions in inflammatory damage following intraperitoneal glutamine administration. Moreover, glutamine has been shown to reduce lung inflammation in LPS-induced injury models as well [16].

L-arginine, a precursor of nitric oxide, enhances immune function and improves microcirculatory dynamics under stress conditions. Additionally, arginine supplementation has been shown to support immune cell signaling and reduce inflammatory and septic responses [17, 18]. Dong *et al.* showed that arginine-treated rats had lower wet/dry lung ratios and better-preserved pulmonary histology after contusion [19]. In another study, L-arginine was found to prevent pulmonary neutrophil accumulation and preserve endothelial function following endotoxemia [20]. Our study also showed near-normal alveolar architecture and significantly improved histopathological scores in the arginine group, confirming its potent protective effect.

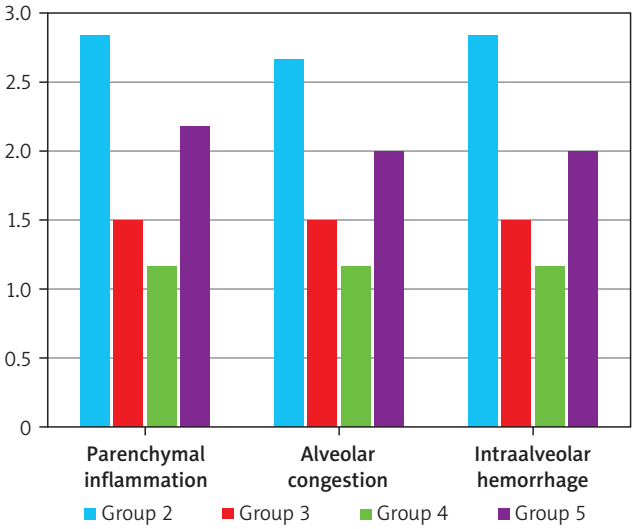


Figure 5. Bar graph showing histopathological scores of inflammation, congestion, and hemorrhage in each treatment group

*$P < 0.05$ vs. trauma group

Interestingly, the combined glutamine and arginine treatment was less effective than either agent used alone. This may be attributed to competitive or antagonistic metabolic pathways, as both amino acids influence similar immune and inflammatory cascades [21]. A similar finding was reported in a recent animal study, where combined L-arginine and L-glutamine supplementation improved immune response but did not enhance overall physiological outcomes [21]. These findings suggest that

Table II. Pairwise statistical comparisons (*p*-values)

Groups	Parenchymal inflammation *P*-value	Alveolar congestion *P*-value	Intraalveolar hemorrhage *P*-value
Group 1 – Other groups	< 0.05*	< 0.05*	< 0.05*
Group 2 – Groups 3, 4, 5	< 0.05*	< 0.05*	< 0.05*
Group 3 – Group 4	0.241	0.241	0.241
Group 3 – Group 5	0.043*	0.171	0.171
Group 4 – Group 5	0.006*	0.026*	0.026*

*$P < 0.05$ was considered statistically significant.

while each amino acid may have beneficial effects individually, their combination does not necessarily lead to additive outcomes and may, under certain physiological conditions, limit each other's efficacy. To our knowledge, such antagonistic behavior in the context of pulmonary contusion has not been previously reported and warrants further investigation.

Conclusions

Both glutamine and arginine treatments were effective in reducing histopathological damage in pulmonary contusion. However, their combined administration did not offer additional benefit and, in some respects, was less effective – particularly when compared with arginine monotherapy.

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Ethical approval

Approval number: 23/2020.

Disclosures

The authors report no conflict of interest.

References

1. Moloney JT, Fowler SJ, Chang W. Anesthetic management of thoracic trauma. Curr Opin Anaesthesiol 2008; 21: 41-46.
2. Cruzat V, Rogero MM, Keane KN, Curi R, Newsholme P. Glutamine: metabolism and immune function, supplementation, and clinical translation. Nutrients 2018; 10: 1564.
3. Zhang F, Wang X, Pan L, Wang W, Li N, Li J. Glutamine attenuates lipopolysaccharide-induced acute lung injury. Nutrition 2009; 25: 692-698.
4. Ignarro LJ, Buga GM, Wood KS, Byrns RE, Chaudhuri G. Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. Proc Natl Acad Sci USA 1987; 84: 9265-9269.
5. Lin W, Yang S, Chen K, Huang C. Protective effects of L-arginine on pulmonary oxidative stress during exhaustive exercise in rats. Nutrition 2005; 21: 992-999.
6. Morris CR, Poljakovic M, Lavrisha L, Machado L, Kuypers, FA, Morris Jr SM. Decreased arginine bioavailability and increased serum arginase activity in asthma. Am J Respir Crit Care Med 2004; 170: 148-153.
7. Bernard AC, Mistry SK, Morris SM, O'Brien WE, Tsuei BJ, Maley ME, Shirley LA, Kearney PA, Boulanger BR, Ochoa JB. Alterations in arginine metabolic enzymes in trauma. Shock 2001; 15: 215-219.
8. Jones NE, Heyland DK. Pharmaconutrition: a new emerging paradigm. Curr Opin Gastroenterol 2008; 24: 215-222.
9. Sanli A, Onen A, Sarioglu S, Sis B, Guneli E, Gokcen B, Karapolat S, Acikel U. Glutamine administration enhances the healing of lung parenchymal injuries in rats. Tohoku J Exp Med 2006; 210: 239-245.
10. Berikol GB, Ayrik C, Kose A, Babus SB, Gumus LT, Arpaci RB, Dag A, Ayan E, Gorur A. Antioxidant and neuroprotective effects of L-arginine administration after traumatic brain injury in rats. Turk Neurosurg 2023; 33: 379-385.
11. Raghavendran K, Davidson BA, Helinski JD, Marschke CJ. A rat model for isolated bilateral lung contusion. Anesth Analg 2005; 101: 1482-1489.
12. Ulugun FI, Sanli A, Guneli E, Gurel D. The effect of ozone treatment in lung contusion. J Basic Clin Health Sci 2018; 2: 68-75.
13. Wu D, Lewis ED, Pae M, Meydani SN. Nutritional modulation of immune function: lessons from COVID-19. Nutrients 2021; 13: 768.
14. Wang S, Li X, Ma Q, Wang Q, Wu J, Yu H, Li K, Li Y, Wang J, Zhang Q, Wang Y, Wu Q, Chen H. Glutamine metabolism is required for alveolar regeneration during lung injury. Biomolecules 2022; 12: 728.
15. Oliveira GP, Kitoko JZ, Lima-Gomes PS, Rochael NC. Glutamine reduces inflammation and extracellular trap release in ARDS. Nutrients 2019; 11: 831.
16. Chen CM, Cheng KC, Li CF, Zhang H. The protective effects of glutamine in a rat model of ventilator-induced lung injury. J Thorac Dis 2023; 15: 350-358.
17. Gurbuz AT, Kunzelman J, Ratzer EE. Supplemental arginine accelerates mucosal regeneration. J Surg Res 1998; 74: 149-156.
18. Gianotti L, Alexander JW, Pyles T, Fukushima R. Arginine-supplemented diets improve survival in sepsis. Ann Surg 1993; 217: 644-653.
19. Dong Y, Ji T, Zhang X, Xu J. Experimental study of L-arginine on lung contusion. J Respir Crit Care 2011; 10: 3.
20. Sheridan BC, McIntyre RC, Meldrum DR, Fullerton DA. L-arginine prevents lung neutrophil accumulation. Am J Physiol 1998; 274: L337-L344.
21. Wellington MO, Hulshof TG, Ernst K, Balemans A, Page GI, Van Hees HMJ. Impact of L-Arginine and L-Glutamine supplementation on growth performance and immune status in weanling pigs challenged with Escherichia coli F4. J Anim Sci 2023; 101: skad138.