Tropical Journal of Pharmaceutical Research February 2023; 22 (2): 335-342
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v22i2.16

Original Research Article

Effect of amifostine on pulmonary contusion from blunt chest trauma in rats

Ahmet Acıpayam[1]*, Nadire Eser[2], Aslı Eryılmaz Yaylalı[3], Kerim Tülüce[4], Atilla Yoldaş[5], Fatma İnanç Tolun[6]

[1]Kahramanmaras Sutcu Imam University Faculty of Medicine, Department of Thoracic Surgery, [2]Kahramanmaras Sutcu Imam University Faculty of Medicine, Department of Pharmacology, [3]Kahramanmaras Sutcu Imam University Faculty of Medicine, Department of Histology, [4]Recep Tayyip Erdoğan Unıversity Faculty of Medicine, Department of Thoracic Surgery, [5]Kahramanmaras Sutcu Imam University Faculty of Medicine, Department of Anatomy, [6]Kahramanmaras Sutcu Imam University Faculty of Medicine, Department of Biochemistry, Kahramanmaras, Turkey

For correspondence:* ***Email:* *ahmetacipayam@hotmail.com;* ***Tel:*** *+90-5072870857*

Sent for review: 25 July 2022 *Revised accepted: 28 January 2023*

Abstract

Purpose: *To investigate the efficacy of different doses of amifostine (AMI) and dexamethasone (DXM) on bilateral pulmonary contusion in rats.*
Methods: *Forty-two Sprague Dawley rats were divided into six groups of seven animals each (control, pulmonary contusion (PC), PC + AMI 400 mg/kg, PC + AMI 200 mg/kg, PC + AMI 400 mg/kg + DXM, and PC + DXM-treated groups). RelAssay commercial kit was used to determine total oxidant status (TOS), total antioxidant status (TAS), and oxidative stress index (OSI). Nitric oxide (NO), inducible nitric oxide synthase (iNOS), interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) activity associated with blunt trauma induced pulmonary damage were determined by enzyme-linked immunosorbent assay (ELISA). Blood gas was determined using a blood gas analyzer, while histopathological examinations were performed on the tissues by hematoxylin and eosin (H & E) staining method.*
Results: *Significant improvement in biochemical parameters and histopathological findings were observed in the groups treated with dexamethasone and high-dose amifostine (400 mg/kg; $p < 0.05$). However, low-dose amifostine (200 mg/kg) and a combination of dexamethasone with amifostine (400 g/kg) was ineffective in balancing the biochemical changes due to trauma ($p > 0.05$).*
Conclusion: *High-dose amifostine combined with dexamethasone mitigates trauma-induced damage in rat by preventing the elevation of key inflammatory markers. Further preclinical and clinical studies are required to determine the efficacy of amifostine in humans and to compare its activity with other agents.*

Keywords: *Pulmonary contusion, Amifostine, Dexamethasone, Inflammatory markers, Chest trauma*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, Web of Science, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Trauma is a leading cause of death before the age of 40. Approximately 20 - 25 % of these are associated with thoracic trauma. Pulmonary contusion (PC) is observed in 30 - 75 % of major thoracic injuries. Pulmonary contusion develops in a majority of patients exposed to severe

thoracic trauma and is the most common pathology among potentially fatal thoracic traumas. Two mechanisms are thought to be responsible for the development of PC. The first one is that the traumatic agent directly compresses the parenchyma and causes injury, and the other one is the severe substitution of the lung, mediastinum, and tracheobronchial tree. Several changes occur in the pulmonary parenchyma following contusion. These include impairment of ventilation-perfusion ratio, hemorrhage resulting in decreased compliance resulting in hypoventilation and hypoxia, edema, and consolidation [1].

Interstitial edema and hemorrhage in the alveoli develop in some areas of the parenchyma in mild injuries. In the event of severe injuries, interstitial edema and hemorrhage in the alveoli occur in more extensive areas of the parenchyma. Increased capillary passage results in the filling of the bronchi with blood and fluid. Consolidation and atelectasis may develop in neighbouring regions of the lung as a result [2]. Secondary damage occurs in the early period of injury following the primary damage. Oxidative stress and the inflammatory response lead to secondary damage [3]. Since no broadly recognized and standardized pharmacological therapeutic approaches have been established for PC associated with blunt chest trauma, therapeutic options are based on empirical observations and clinical judgment [4]. The therapeutic options for such patients are often restricted but contain basic support therapies, including complementary oxygen, analgesia, pulmonary hygiene, and cardiopulmonary monitoring.

In addition to the generally accepted therapeutic options for pulmonary contusion following blunt chest trauma, the use of agents to balance reactive oxygen species (ROS) and inflammatory activity is gaining attention [5]. In 1996, U.S. Food and Drug Administration approved Amifostine use since it reduces cumulative tissue toxicity caused by cisplatin in patients, who have advanced-stage cancer, except small cell lung cancer [6]. Preclinical and clinical studies have demonstrated that amifostine has an effect to prevent adverse effects such as nephrotoxicity, ototoxicity, and neurotoxicity. An organic thiophosphate, amifostine exhibits a protective effect against pulmonary contusion by restoring free oxygen radicals-induced DNA damage and by scavenging free radicals [7]. This study investigates the treatment efficacy of the combination of anti-inflammatory agent (amifostine) and immunosuppressive corticosteroid derivative (dexamethasone) in rats with bilateral pulmonary contusion.

EXPERIMENTAL

Animals

Forty-two male Sprague Dawley rats weighing 370 - 400 g were procured from Kahramanmaras Sutcu Imam University, Faculty of Medicine, Experimental Research Center, Kahramanmaras, Turkey. Rats were housed to acclimatize in a room for seven days at 21 - 22 °C in a 12 hours-light/dark cycle with *ad libitum* access. Experimental design and protocols were approved by the KSU Faculty of Medicine ethical committee (approval no. 01; dated 18.11.2019). Animal procedures were performed in compliance with the principles of the European Convention on Animal Welfare and the National Institutes of Health Guide for the Use and Care of Laboratory Animals [8].

Study design

Forty-two randomly selected animals were separated into six groups of seven animals each. They include the following:

(A) Control (C) group: This group received saline solution (0.9 % NaCl) intraperitoneally in a volume of 2.5 ml/kg.

(B) Pulmonary Contusion (PC) Group: This group was induced bilateral pulmonary contusion at a chest impact energy of 1.96 J.

(C) Pulmonary Contusion (PC) + AMI 400 Group: This group received 400 mg/kg Amifostine 45 min after PC.

(D) Pulmonary Contusion (PC) + AMI 200 Group: This group received 200 mg/kg Amifostine 45 min after PC.

(E) Pulmonary Contusion (PC) + AMI + DXM Group: This group received 400 mg/kg Amifostine combined with 10 mg/kg dexamethasone (DXM) 45 min after PC.

(F) Pulmonary Contusion (PC) + DXM Group: This group received 10 mg/kg DXM 45 min after PC.

The blunt chest trauma model

Bilateral pulmonary contusion was performed with the experimental model used in previous study [9]. Briefly, this relies on dropping a cylindrical mass of a specific weight (400 g) from

a specific height (50 cm) vertically onto the chest through a hollow, cylindrical pipe. The impact energy (E) created in this way is shown in Eq 1.

$E = mgh$ …… (1)

where g is gravity (9.8 m/s^2), h is the height from the platform (50 cm) and m is the mass of cylindrical weight (400 mg). The total energy transmitted to the rat chest was calculated as 1.96 J. All groups were kept in cages for 6 h after PC induction. Thereafter, the rats were treated to inhalation O_2 for 5 min prior to midsternotomy performed on all groups and subgroups for blood and lung tissue collection.

Arterial blood gas measurement

Six hours after blood trauma, blood specimens were collected during midsternotomy from the descending aorta using a heparinized injector. The blood samples were analyzed on the blood gas analyzer (Medica Easystat/USA) to evaluate the possible effects of AMI and DXM.

Biochemical assay

Lung tissue samples were washed with 0.9 % NaCl to eliminate hematoma and dried. Specimens were preserved at –20 °C in plastic bottles until biochemical analysis. Activities of IL-1β, IL-6, NO, iNOS, and TNF-α parameters were identified using the enzyme-linked immunosorbent assay (ELISA) method with a commercial kit (Shanghai Coon Koon Biotech) on the automated ELISA microplate reader (Thermo Scientific/Finland) and PC software (Skanltfor Multiscan FC 2.5).

Total antioxidant status (TAS)

Total antioxidant status (TAS) levels in liver tissue homogenates and serum were determined using a RelAssay commercial kit (RelAssay Kit Diagnostics, Turkey/Ref. No: RL0017, LOT No: JE 14042 A). The data were represented as mmol Trolox equiv/L. Total oxidant status (TOS) was also analysed using the RelAssay commercial kit (RelAssay Kit Diagnostics, Turkey/Ref. No: RL0024, LOT No: JE 14048Og). The results were represented as μmol H_2O_2 equiv/L [1]. The oxidative stress index (OSI) value was calculated as the ratio of the values of TOS to TAS, and the results were expressed as 'arbitrary units' (AU).

Histopathological examination of lungs

The dissection of lung tissue sample was performed by the common procedures for embedding the paraffin blocks, slicing, and also staining. A 5 μm Paraffin edges were acquired and stained by hematoxylin and eosin (H & E) to reveal the histological changes. For each animal, 10 sections were assessed by light microscope at x20 magnification (Olympus HB-2, Japan). The histopathological severity scoring of the tissues (0: none; + 0.5: very mild; + 1: mild; + 2: moderate; + 3: severe) was made by considering the tissue damage averages in each cross-sectional area stained with (H & E) [10].

Statistical analysis

Statistical analysis of data was performed by using GraphPad Prism-7-software version (Chicago, IL, USA). Data are presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was performed to identify changes among individual groups. Afterward, post hoc Turkey tests were applied. *P*-value < 0.05 was considered as statistically significant.

RESULTS

Rats with agenesis, rib fracture, pleural effusion, or pneumonic effusion at post-traumatic CT scanning were not included in the study. Pneumothorax was identified in one rat with a rib fracture, while atelectasis was in seven rats. Although the decrease in contusion and atelectasis at thoracic CT at the sixth hour after trauma, there was no macroscopic difference in the study groups (Figure 1).

No mortality occurred in rats exposed to blunt chest trauma. General evaluation of respiratory movements of contused rats throughout follow-up showed moderate bradypnea in the early post-traumatic period. Examination of the effects of blunt chest trauma on the lungs revealed contused areas in a heterogeneous pattern in the bilateral lungs. Arterial blood gas analysis revealed a significant decrease in PaO_2 values and increased PCO_2 values in rats exposed to contusion when compared with control group at sixth hour after blunt trauma ($p < 0.001$). PaO_2 increased significantly in the treatment groups except DXM + AMI and AMI 200 (Table 1). DXM, AMI + DXM, and low-dose amifostine were reduced PCO_2 values after trauma, but not statistically significant ($p > 0.05$). A comparison of the PC group with the control group revealed higher interstitial edema, interstitial hemorrhage and alveolar congestion ($p < 0.05$). The amifostine-treated groups showed a significant reduction of the following parameters as compared with the PC; interstitial edema, interstitial hemorrhage, and alveolar congestion (Figure 2).

Table 1: Blood gas data

Group	pH	PO_2	PCO_2
C	7.36±0.41	222.9±24.30	39.66±4.36
PC	7.34±0.39	80.08±5.18 ***	61.03±4.47***
PC+AMI 200	7.73±0.60	109.4± 16.02	55.88±3.32
PC+AMI 400	7.46±0.73	189.0±22,62^{+++}	42.32±4.65^{+++}
PC+DXM	7.58±0.82	170.8±20.17^{+++}	41.91±3.30^{+++}
PC+DXM+AMI	7.44±0.35	110.6±15.05	53.93±3.80^{+}

All data were expressed as mean ± SD (n = 7); *p < 0.05, **p < 0.01, ***p < 0.001 Significant differences compared pulmonary contusion (PC) with the control group (C) and ^{+}p < 0.05, ^{++}p < 0.05, ^{+++}p < 0.05 significant differences compared pulmonary contusion (PC) with the amifostine-treated groups (C: Control; PC: Pulmonary Contusion; AMI: Amifostine; DXM: Dexamethasone)

Table 2: Histopathological severity score in all rat groups

Group	Hemorrhage	Edema	Congestion
C	0.0	0.0	0.0
PC	2.86±0.38***	2,57±0.79***	2.87±0.38***
PC+AMI 200	2.57±0.53	2.43±0.78	2.00±0.82
PC+AMI 400	1.86±0.69^{+}	1.28±0.49^{+}	1.43±0.53^{+++}
PC+DXM	2.0±0.82^{+}	1.29±0.76^{+}	1.57±0.53^{++}
PC+DXM+AMI	2.57±0.53	2.14±0.60	2.29±0.76

Results are expressed as mean ± SD (n = 7); *p < 0.05, **p < 0.01, ***p < 0.001 Significant differences when pulmonary contusion (PC) is compared with the control group (C) and ^{+}p < 0.05, ^{++}p < 0.05, ^{+++}p < 0.05 significant differences when pulmonary contusion (PC) is compared with the treatment groups (C: Control; PC: Pulmonary Contusion; AMI: Amifostine; DXM: Dexamethasone)

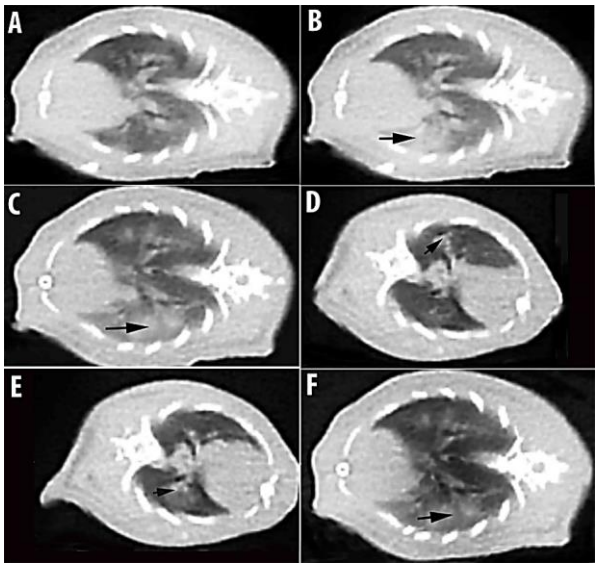

Figure 1: Tomography scans of rat groups; A: Control Group B: Pulmonary Contusion (PC) Group, C: PC+AMI 400 Group, D: PC+AMI 200 Group, E: PC+AMI+DXM Group, F: PC+DXM Group

However, there was no improvement in edema congestion and hemorrhage scores in lung tissues of PC + DXM + AMI, PC + AMI 200 groups (p > 0.05) (Table 2).

When PC group compared to the C group was produced higher TOS, OSI, and iNOS levels and lower TAS enzyme activities in lung tissue (Figure 3) and serum (Figure 5). When PC group was compared with the amifostine-treated groups (PC + DXM, PC + DXM + AMI, PC + AMI 200 and PC + AMI 400), the protective effects of amifostine-treated groups were significantly different (p < 0.05), except for TAS activities (p > 0.05) (Figure 3 and Figure 5).

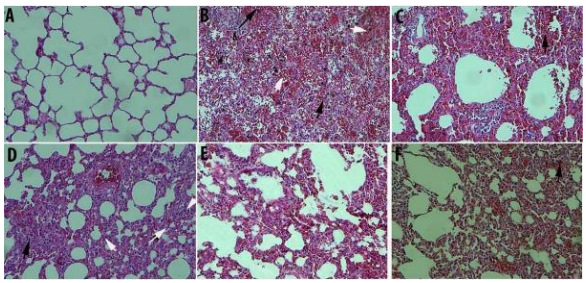

Figure 2: Microscopic images of lung inflammation in lung tissues stained with H & E (20X); A: Control Group B: Pulmonary Contusion (PC) Group, C: PC+AMI 400 Group, D: PC+AMI 200 Group, E: PC+AMI+DXM Group, F: PC+DXM Group. Black arrows; Hemorrhage, white arrows; Edema

When the PC group was compared with the C group in terms of inflammation mediators, there was a statistically significant increase in NO, IL-1β, TNF-α and IL-6 activities in tissue (Figure 4) and serum (Figure 6) of the PC group (p < 0.05). Meanwhile, inflammation parameters (NO, TNF-α, IL-6, and IL-1β) of both tissue and serum of all amifostine-treated groups were significantly lower when compared to the PC group (p < 0.05).

DISCUSSION

As previously reported by Raghaverden *et al* [9], energy was applied as the mechanism of creating pulmonary contusion. In this study, none of the rats exposed to trauma died. However, post-traumatic local and systemic inflammatory changes, as well as bradycardia, hypotension, and changes in reactive oxygen species (ROS) suggest that trauma is a serious process, as previously reported in humans [11].

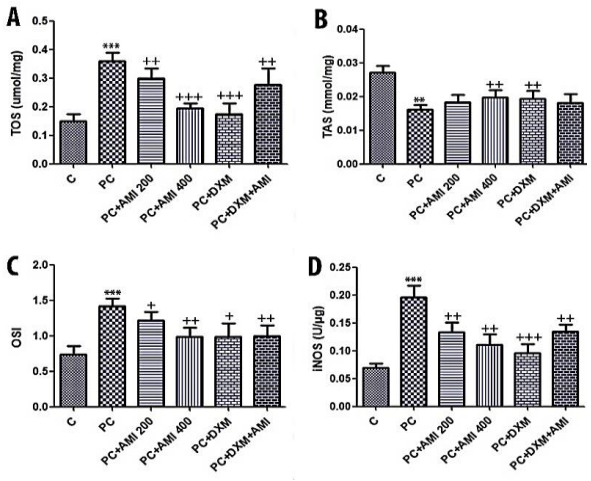

Figure 3: Effect of amifostine and dexamethasone on lung tissue oxidative mediators in blunt chest trauma. Results are mean ± SD (n = 7); ***p < 0.001 significant differences when pulmonary contusion (PC) is compared with the control group (C) and ^{+}p < 0.05, ^{++}p < 0.05, ^{+++}p < 0.05 significant differences when pulmonary contusion (PC) is compared with the amifostine-treated groups

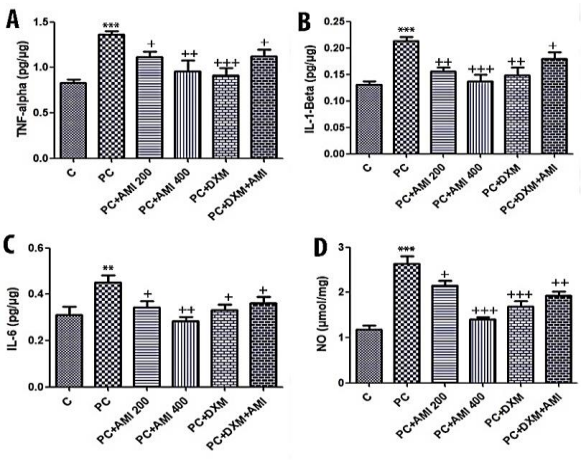

Figure 4: Effect of amifostine and dexamethasone on lung tissue inflammation mediators in blunt chest trauma. Results are mean ± SD (n = 7); **p < 0.01, ***p < 0.001 significant differences when pulmonary contusion (PC) is compared with the control group (C) and ^{+}p < 0.05, ^{++}p < 0.05, ^{+++}p < 0.05 significant differences when pulmonary contusion (PC) is compared to the amifostine-treated groups

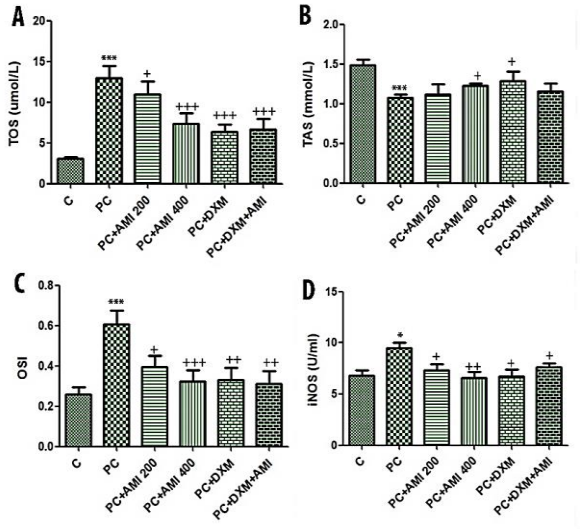

Figure 5: TOS, TAS, OSI, and iNOS activities in serum. Results are mean ± SD (n = 7); *p < 0.05, **p < 0.01, ***p < 0.001 significant differences when pulmonary contusion (PC) is compared with the control group (C) and ^{+}p < 0.05, ^{++}p < 0.05, ^{+++}p < 0.05 significant differences when pulmonary contusion (PC) is compared to the amifostine-treated groups

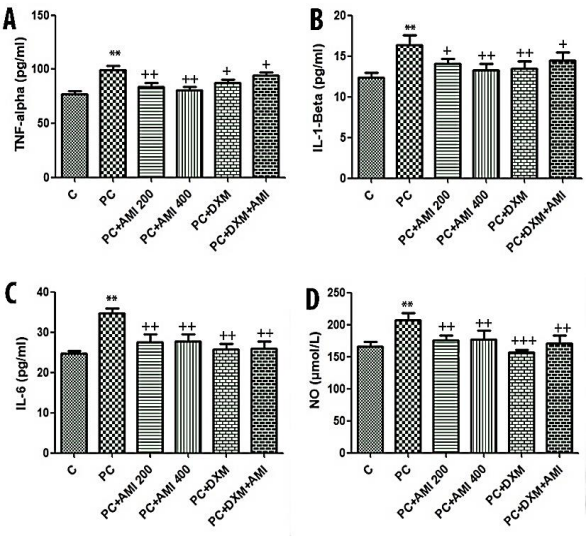

Figure 6: TNF-alpha, IL-1beta, IL-6, and NO activities in serum. Results are mean ± SD (n = 7); **p < 0.01, ***p < 0.001 significant differences when pulmonary contusion (PC) is compared with the control group (C) and ^{+}p < 0.05, ^{++}p < 0.05, ^{+++}p < 0.05 significant differences compared pulmonary contusion (PC) to the amifostine-treated groups

Several changes occur in the pulmonary parenchyma following contusion. These include hemorrhage, edema, and consolidation resulting in decreased compliance leading to an impaired ventilation/perfusion ratio, hypoventilation, and hypoxia [1]. Interstitial edema and hemorrhage in the alveoli are seen in localized areas in small-scale injuries. However, in more severe trauma cases, widespread hemorrhage and edema in

both alveolar and interstitial spaces, and regional bone fractures occur. The primary pathology in pulmonary contusion is an abnormal accumulation of fluid in the alveoli and interstitial spaces, and the formation of hypoxemia and hypercarbia with impaired gas exchange in the bronchioles and alveoli. Depending on the severity of the damage after trauma in lung contusions, findings can be obtained on posteroanterior x-rays for up to 4 h [2]. Intrapulmonary hemorrhage generally peaks 6 h after trauma. Computed tomography (CT) is therefore more useful in showing pulmonary contusion, particularly in threshold cases [1]. Widespread infiltration and patch-like consolidation are present at CT scanning. In a previous study, it was reported that with appropriate treatment, the radiological manifestation of pulmonary contusion began to improve within a few days (48 - 72 h) [5]. No statistically significant decreases in contusion and atelectasis were observed in this study as a result of radiological imaging performed immediately after the trauma and at 6 h.

Lung tissue was also subjected to histopathological examination. The findings of this study demonstrated that groups administrated DXM and AMI 400 mg/kg had a significant histopathological improvement in terms of interstitial edema, interstitial hemorrhage, alveolar congestion, and leukocyte infiltration compared with the control group. While therapeutic effects were observed histopathologically in rats treated with DXM alone or high-dose amifostine (400 mg/kg), the expected histopathological results were not seen with the drug combination (dexamethasone + amifostine) and low-dose amifostine (200 mg/kg). This may be due to the fact that the therapeutic effects in rats administered dexamethasone and amifostine may antagonize each other.

Possible inflammatory mediators, such as Neutrophils and macrophages become activated following chest trauma. Proteolytic enzymes and cytokines, which are reactive-oxygen metabolites, are released by leukocytes and macrophages, and lead to increases in the permeability of the alveoli-capillary membrane and microvascular efflux with alveolar edema fluid, lipolytic and proteolytic enzymes, and ROS formation [12]. Free oxygen radicals, adhesion molecules, and cytokines determine the relationship between the endothelium and neutrophils in tissue and blood vessels. Necrosis may develop during an injury in tissues due to hypovolemia, arterial thrombosis, and direct arterial damage. Restoration of blood flow before necrosis may reverse this process. In addition, the formation of free oxygen radicals during reperfusion generally further exacerbates tissue damage. It is important to reduce these deleterious effects of free radicals in patients with pulmonary contusion.

The principal cytokines involved in the acute phase reaction appearing against trauma and infection are IL-1β, IL-6, and TNF-α. While IL-1 and IL-6 are essentially responsible for the synthesis of acute-phase proteins in the liver during trauma. Meanwhile, IL-6 levels have been linked to the severity of the trauma, multiple organ failure, acute respiratory distress syndrome, sepsis, and respiration. Proinflammatory cytokines, which include TNF-α, IL-1β, and IL-6 are expressed within a few hours following trauma. The initiation of preventive therapies before the onset of secondary damage after trauma is highly important in terms of reducing morbidity and mortality. Consistent with previous research IL-1β, IL-6 and TNF-α have an increment in PC group compared with C group in the present study [13]. Although reductions were observed in all groups compared with the PC group, AMI was more effective in regulating proinflammatory cytokines in the group, that received amifostine at the dose of 400 mg/kg.

A balance between oxidant capacity and antioxidant capacity at the cellular level is of vital importance in terms of cell functions. Several authors have employed the parameters of TOS and TAS to evaluate the severity of damage or therapeutic effectiveness [8]. The degree of oxidative damage is reduced by free radical scavenging systems. On the other hand, it had been reported that amifostine affects the production of ROS and signaling cascades, which is redox-sensitive, including mitogen-activated protein kinases, p38, extracellular signal-regulated kinase 1/2, and also the nuclear factor-kB pathway [14]. In the present study, total oxidant status (TOS) levels increased in both serum and lung tissue following blunt trauma. However, TOS levels decreased significantly in rats given AMI 400 mg/kg and DXM. This finding is compatible with previous studies, indicating that the application of AMI 400 mg/kg and DXM may have a reducing effect on inflamed lungs in rats.

One of the striking findings is that serum NO and iNOS levels increased during trauma, but these were regulated in the groups given AMI 400 mg/kg and DXM. Since Nitric Oxide is involved as both a protective and harmful molecule in acute inflammatory events, it is of great importance that it should be balanced. It is known that high concentrations of NO produced

with iNOS increase acute events (including trauma, stress, and acute inflammation). Supporting previous studies [14,15], amifostine provided strong evidence for mitigating the inflammatory effect of trauma by preventing the elevation of key inflammatory markers such as NO and iNOS.

One other interesting finding, which supports the histopathological and biochemical findings, is arterial blood gas analysis 6 h after blunt trauma. It revealed a significant decrease in PaO_2 values and an increase in PCO_2. Statistically significant increases in PO_2 values were observed in all groups apart from DXM + AMI and AMI 200 mg.

CONCLUSION

P pulmonary contusion he pathological and histopathological findings of this study reveal that cellular response is regulated in rats that received AMI 400 mg/kg and only DXM; furthermore, the clinical signs are significantly reduced. This, therefore, strengthens the opinion that AMI and DXM, as antioxidant-supportive therapy in pulmonary contusion accelerate the healing process. However, since the study involves experimental rats, it is unclear whether the same result can be obtained with humans. Therefore, further studies are required to investigate the efficacy of amifostine and its combination with other agents.

DECLARATIONS

Acknowledgements

The study was supported by the Kahramanmaraş Sütçü İmam University Research jkj Projects Coordination Unity under project no. 2020/3 M.

Funding

None provided.

Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

REFERENCES

1. *Bliss D, Silen M. Pediatric thoracic trauma. Crit Care Med 2002; 30: 409-415.*
2. *Yücel O, Sapmaz E, Çaylak H, Gozubuyuk A, Gurkok S, Dakak M, Celik B, Cubuk S, Kavaklı K, Isık H et al. Analysis of 748 patients with thoracic trauma requiring hospitalization. Gulhane Med J 2009; 51(2): 86-90.*
3. *Hoth JJ, Stitzel JD, Gayzik FS, Brownlee NA, Miller PR, Yoza BK, McCall CE, Meredith JW, Payne RM. The pathogenesis of pulmonary contusion: an open chest model in the rat. J Trauma 2006; 61: 32–44. doi: 10.1097/01.ta.0000224141.69216.aa*
4. *Desselle WJ, Greenhaw JJ, Trenthem LL, Fabian TC, Proctor KG. Macrophage cyclooxygenase expression, immunosuppression, and cardiopulmonary dysfunction after blunt chest trauma. J Trauma 2001; 51: 239–252. doi: 10.1097/00005373-200108000-00005*
5. *Kelly ME, Miller PR, Greenhaw JJ, Fabian TC, Proctor KG. Novel resuscitation strategy for pulmonary contusion after severe chest trauma. J Trauma 2003; 55: 94–105. doi: 10.1097/01.TA.0000029042.37577.A6*
6. *Wasserman TH, Brizel DM. The role of amifostine as a radioprotector. Oncol (Williston Park) 15: 1349 1354, 2001.*
7. *Mercantepe F, Mercantepe T, Topçu Yılmaz A, Tumkaya L. Protective effects of amifostine, curcumin, and melatonin against cisplatin-induced acute kidney injury Naunyn Schmiedebergs Arch Pharmacol 2018; 391(9): 915-931. doi: 10.1007/s00210-018-1514-4.*
8. *Sayhan MB, Oguz S, Salt O, Can N, Yalta OT. Sesamin ameliorates mucosal tissue injury of mesenteric ischemia and reperfusion in an experimental rat model. Arch Med Sci 2019; 15: 1582-1588.*
9. *Raghavendran K, Davidson BA, Helinski JD, Marschke CJ, Manderscheid P, Woytash JA, Notter RH, Knight PR. A rat model for isolated Bilateral lung contusion*

from blunt chest trauma. AnesthAnalg 2005; 101: 1482–1489. doi: 10.1213/01.ANE.0000180201.25746.1F

10. Eser N, Yoldas A, Turk A, Kalaycı YA, Yalcin A, Cicek M. Ameliorative effects of garlic oil on FNDC5 and irisin sensitivity in liver of streptozotocin-induced diabetic rats. J Pharm Pharmacol 2021; 73(6): 824-834.doi: 10.1093/jpp/rgab023.
11. Ateş G, Yaman F, Bakar B, Kısa U, Atasoy P, Buyukkocak U. Evaluation of the systemic anti-inflammatory effects of levosimendan in an experimental blunt thoracic trauma model. Ulus Travma Acil Cerrahi Derg 2017; 23(5): 368–376 doi: 10.5505/tjtes.2016.26786
12. Strohmaier W, Trupka A, Pfeiler C, Thurnher M, Khakpour Z, Gippner-Steppert C, Jochum M, Redl H. Bilateral lavage with diluted surfactant improves lung function after unilateral lung contusion in pigs. Crit Care Med 2005; 33: 2286–2293.
13. Schmidt OI, Heyde CE, Ertel W, Stahel PF. Closed head injury-an inflammatory disease? Brain Res Rev 2005; 483: 388-399. doi:10.1016/j.brainresrev.2004.12.0282
14. Fu P, Birukova AA, Xing J, Sammani S, Murley JS, Garcia JGN, Grdina DJ, Birukovet KG. Amifostine reduces lung vascular permeability via suppression of inflammatory signalling. Eur Respir J. 2009; 33(3): 612-624. doi: 10.1183/09031936.00014808.
15. Barbosa SCM, Pereira VBM, Wong DVT, Santana APM, Lucetti LT, Carvalho LL, Barbosa CRN, Callado RB, Silva CAA, Lopes CDH, et al. Amifostine reduces inflammation and protects against 5-fluorouracil-induced oral mucositis and hyposalivation. Braz J Med Biol Res 2019; 52(3) https://doi.org/10.1590/1414-431X20188251