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## The Effects of Ketamine, Midazolam and Ketamine/Xylazine on Acute Lung Injury Induced by $\alpha$ -Naphthylthiourea in Rats\*

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article; G – other

### Abstract

**Objectives.** Ketamine is a drug used in human and veterinary medicine, primarily for the induction and maintenance of general anesthesia, analgesia (particularly in emergency medicine), and treatment of bronchospasm. Midazolam is the preferred drug in intensive care units for sedation and anesthesia. Ketamine/xylazine combination is used as an anesthetic agent in veterinary medicine and experimental animals. Aside from anaesthetic properties, these agents can cause physiologic and metabolic alterations and modulate and improve the inflammatory responses. The objective of the present study was to investigate the effects of ketamine, midazolam, and veterinary and experimentally used ketamine/xylazine combination in acute lung injury induced by  $\alpha$ -naphthylthiourea (ANTU).

**Material and Methods.** ANTU was injected intraperitoneally (*i.p.*) in rats at the dose of 10 mg/kg. Ketamine (15, and 50 mg/kg, *i.p.*), midazolam (2 and 4 mg/kg, *i.p.*), and ketamine/xylazine (50/10 mg/kg, *i.p.*) administered to rats 30 min prior to ANTU. Four hours later, the lung weight/body weight (LW/BW) ratio and pleural effusion (PE) were measured. Histopathological changes were documented in each lung tissue, including intra-alveolar hemorrhage, alveolar edema and inflammation. The severity of the lung injury was scored (0–3).

**Results.** Ketamine, midazolam and ketamine/xylazine had a significant prophylactic effect on pleural effusion formation at all doses and significantly reduced pleural effusion. Ketamine caused a significant reduction of inflammation, hemorrhage and edema scoring and midazolam (2 mg/kg) and ketamine/xylazine caused a significant reduction of inflammation and edema scoring.

**Conclusions.** It can be concluded that ketamine and midazolam may attenuate lung injuries induced by ANTU. In addition to their anesthetic or sedative properties, the prophylactic effects of these agents on lung tissue damage will contribute to the treatment of intensive care unit diseases including acute lung injury. Similarly, the effects of these agents on lung pathophysiology should be considered in experimental applications (*Adv Clin Exp Med* 2014, 23, 3, 343–351).

**Key words:** acute lung injury, pulmonary edema, pleural effusion, anaesthetic agents,  $\alpha$ -naphthylthiourea.

Acute lung injury (ALI) and its more severe form, acute respiratory distress syndrome (ARDS), are both defined by acute onset of respiratory failure due to a variety of direct and indirect injuries of the lung. ALI/ARDS is a major cause of death and cost in intensive care units (ICU). However, pharmacological interventions have revealed little benefit in those patients, with the mortality rate remaining

as high as 40–50% [1]. An increase in capillary permeability and pulmonary edema play a critical role in the development of ALI/ARDS. Pulmonary inflammation and protein rich alveolar edema leads to acute respiratory failure in these patients [1–4]. However, the exact mechanisms of lung injury, time courses, inflammatory pathways and cell repair processes are not well-understood [1].

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Many anaesthetic agents modulate the lung inflammatory responses [5–7]. Aside from its anaesthetic properties, these agents have been shown to induce physiological changes that suggest a powerful anti-inflammatory effect [6, 8, 9]. Ketamine and midazolam are preferred drugs in intensive care units for sedation and anesthesia. The anti-inflammatory effects of these agents have been demonstrated in previous experimental animal models [8–10]; however, there are no findings about the effects of these agents on ANTU induced pulmonary edema and pleural effusion. The rodenticide ANTU causes acute pulmonary vascular injury in animals [11]. Gross manifestations of this injury include increased lung weight due to pulmonary edema and pleural effusion [12], resulting from injuries to the endothelial cells and pneumocytes in the lung [13]. Because the effects of ANTU are rather specifically directed at the lungs, use of this agent has become a popular method of investigating the physiological changes of ALI. In this study the effects of the clinically used intravenous anesthetics ketamine and midazolam, and veterinary and experimentally used ketamine/xylazine tested on ALI model induced by ANTU in rats. We hypothesized that ketamine, midazolam and ketamine/xylazine could attenuate pulmonary tissue damage/pleural effusion and ketamine and midazolam usage in the ICU may prevent/decrease the development of ALI/ARDS.

## Material and Methods

### Experimental Animals and Materials

The experiment was carried out on Wistar albino male rats weighing 200–240 g obtained from the Animal Laboratory of Bulent Ecevit University. They were housed under standard laboratory conditions with a 12-h light/dark cycle and were allowed free access to food and water. The procedures and protocols of the study were in accord with our institutional guideline, which is accordance to the “Guide for the Care and Use of Laboratory Animals (US National Institutes of Health)”. Approval for the experiments was obtained from the Bulent Ecevit University Animal Experiments Local Ethics Committee.

### Animal Model/Assessment of Acute Lung Injury

During the experiment, the animals were placed in separate cages and kept at room temperature (22°C). ANTU (suspended in olive oil at 4 mg/mL)

was injected intraperitoneally (*i.p.*) at a dose of 10 mg/kg. When injected into rats, ANTU produces pulmonary edema, as indicated by an increase in the lung weight/body weight ratio (LW/BW) and pleural effusion (PE), reaching a maximum within 4 h. The solvent control group received the same volume of olive oil alone. Four hours later, the animals were anesthetized with thiopental sodium (50 mg/kg *i.p.*) and exsanguinated by cutting the abdominal aorta. The thorax was opened, and any PE was collected carefully by suction and measured volumetrically. Care was also taken to eliminate blood contamination of the PE. The lungs were removed, all surrounding tissues were dissected and weighed with an analytical balance and used for histological scoring (0–3). The volume of PE (milliliters) and the LW/BW and pleural effusion/body weight (PE/BW) ratios were calculated.

## Experimental Protocol

The animals were divided into 8 groups. There were 8 animals in each group. The groups are described in Table 1. All of the drugs were prepared daily. ANTU (suspended in olive oil at 4 mg/mL) was injected intraperitoneally (*i.p.*) at a dose of 10 mg/kg. The solvent control group received the same volume of olive oil alone. Ketamine (15 and 50 mg/kg, *i.p.*), midazolam (2 and 4 mg/kg, *i.p.*), and ketamine/xylazine (50/10 mg/kg, *i.p.*) carried out to rats 30 min prior to ANTU. Four hours after ANTU injection, PE was collected and the lungs were removed.

**Table 1.** Study groups are listed below

Groups	Chemicals	
1	control	
2	olive oil	<i>i.p.</i>
3	ANTU (10 mg/kg)	<i>i.p.</i>
4	ANTU + Ketamine (15 mg/kg) <sup>a</sup>	<i>i.p./i.p.</i>
5	ANTU + Ketamine (50 mg/kg) <sup>a</sup>	<i>i.p./i.p.</i>
6	ANTU + Midazolam (2 mg/kg) <sup>a</sup>	<i>i.p./i.p.</i>
7	ANTU + Midazolam (4 mg/kg) <sup>a</sup>	<i>i.p./i.p.</i>
8	ANTU + Ketamine (50 mg/kg) <sup>a</sup> + xylazine (10 mg/kg) <sup>a</sup>	<i>i.p./i.p.</i>

<sup>a</sup> All these treatments were made 30 min before ANTU injection.

## Histological Examination

For the histopathological examination, the lung tissue samples were fixed in 10% formalin immediately after removal, dehydrated in graded

concentrations of ethanol, cleared in xylene and embedded in paraffin. All lung lobes were used for the histological examination. At least eight tissue sections, 10  $\mu\text{m}$  thick, were obtained and then stained with hematoxylin and eosin. A pathologist performed a blind examination of the sections under a light microscope. All histopathological changes were documented in each lung tissue, including intra-alveolar hemorrhage, alveolar edema and inflammation. Alveolar edema (swelling of the alveolar wall), and intra-alveolar hemorrhage were scored on a scale from 0 to 3, where 0 = absence of pathology (< 5% of maximum pathology), 1 = mild (< 10%), 2 = moderate (15–20%), and 3 = severe (20–25%) [14]. Leukocyte infiltration was evaluated to determinate the severity of alveolar inflammation. Each section was divided into 10 subsections, and leukocyte infiltration was examined in each of the subsections at a magnification of  $\times 400$  with the following scale: 0 = no extravascular leukocytes; 1 = < 10 leukocytes; 2 = 10–45 leukocytes; 3 = > 45 leukocytes. The average of the numbers was used for comparison [15].

## Statistical Analysis of Results

Results were expressed as mean  $\pm$  SEM. Comparisons between groups were made using One-Way ANOVA, followed by a Turkey test in case of significance.  $P < 0.05$  was accepted as significant. Statistical analysis was performed using SPSS for Windows 13.0 (SPSS Inc., Chicago, IL, USA).

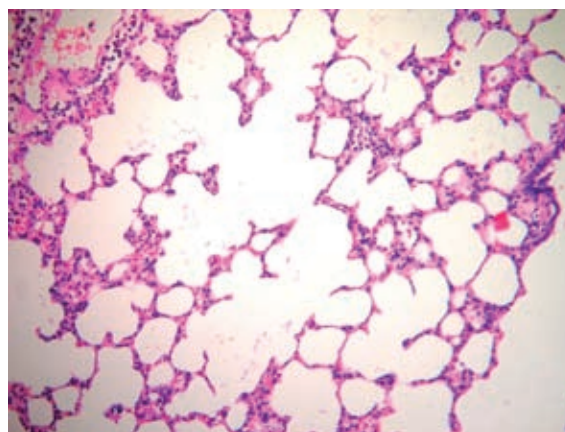
## Chemicals

The following chemicals were used in this study:  $\alpha$ -Naphthylthiourea (Interchim) was suspended in olive oil (4 mg/mL) and was a gift from Dr. E. Schillinger, Schering AG, Berlin, Germany. Olive oil was purchased from Sigma (St. Louis, MO, USA). Ketamine was purchased from Pfizer, midazolam was purchased from Roche, xylazine hydrochlorid was purchased from Alfasan International B.V. (3440 AB, Holland).

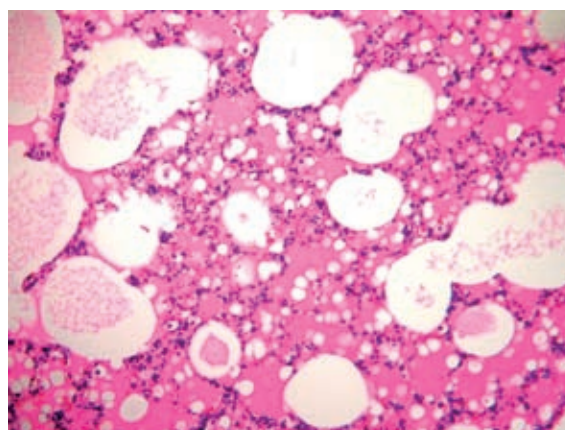
## Results

### Effect of ANTU on Pulmonary Tissue

In the histopathological examination, ANTU triggered a severe pulmonary injury mainly documented as an interstitial and intra-alveolar edema, alveolar wall destruction and interstitial inflammatory cell infiltration (Fig. 1B). There were no differences in histology and LW/BW ratio between



**Fig. 1A.** Normal histological appearance of olive oil-treated rat lungs (H&E  $\times 10$ )



**Fig. 1B.** Interstitial edema, erythrocyte extravasation, alveolar wall destruction and inflammatory cell infiltration after ANTU treatment (H & E  $\times 10$ )

control (Fig. 1A) and olive oil-treated (solvent control) rats.

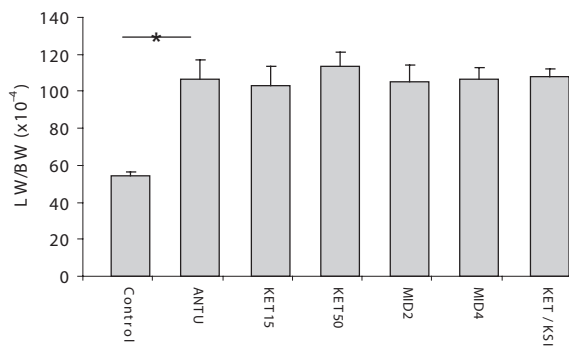
A significant lung edema was developed after 4 h *i.p.* injection of ANTU at the dose of 10 mg/kg as indicated by an increase in the LW/BW ratio and PE when compared with control rats. The LW/BW ratio was measured as  $106.9 \pm 9.9 \times 10^{-4}$  for ANTU-treated rats while it was found to be  $54.2 \pm 2.4 \times 10^{-4}$  for control rats ( $p < 0.05$ ) (Fig. 2A). PE was measured as  $3.2 \pm 0.2$  mL in ANTU-treated rats, while no PE was observed in control rats ( $p < 0.05$ ) (Fig. 2 BC).

### The Effects of Ketamine on ANTU-Induced ALI

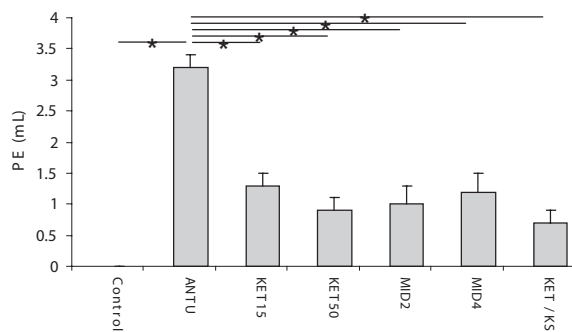
Ketamine alone has no deleterious effect on healthy lung parameters. On the other hand, ketamine had a prophylactic effect on ANTU-induced PE formation at all doses and significantly reduced PE and PE/BW ratio ( $p < 0.05$ ) (Fig. 2 BC).

Ketamine 15 and 50 mg/kg groups caused a significant reduction of inflammation, hemorrhage

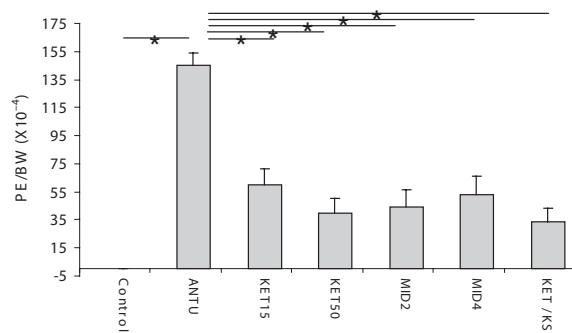




**Fig. 2A.** Figure represents the calculated results of acute lung injury induced by  $\alpha$ -naphthylthiourea and alterations by ketamine (KET) (15, and 50 mg/kg), midazolam (MID) (2 and 4 mg/kg), and ketamine/xylazine (KET/-KSI) (50/10 mg/kg), as evaluated by the changes of lung weight/body weight (LW/BW) ratio ( $\times 10^{-4}$ ), \*  $P < 0.05$ . (A), pleural effusion (PE) (mL)



**Fig. 2B.** Pleural effusion/body weight (PE/BW) ratio ( $\times 10^{-4}$ )



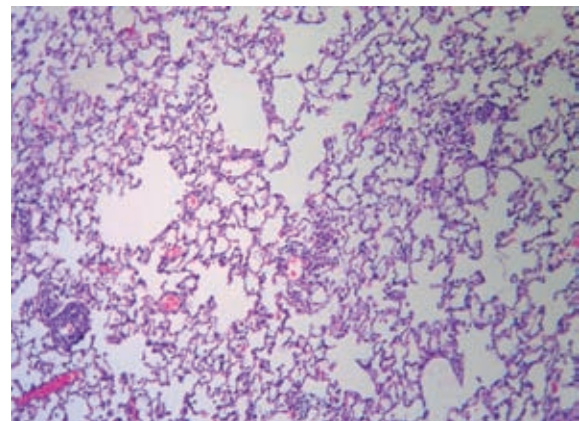
**Fig. 2C.** Each column shows the mean value of eight experiments, vertical bars on the columns represent SEM

and edema formation according to the histopathological scoring ( $p < 0.05$ ) (Fig. 3). Inflammation and hemorrhage results of 15 and 50 mg/kg groups were close to the control group. The best improvement in ANTU-induce lung damage was seen in ketamine 15 mg/kg group for inflammation, and ketamine 50 mg/kg group for edema and hemorrhage compared to all groups. Ketamine at the doses of 15 and 50 mg/kg demonstrated no significant prophylactic effect on LW/BW ratio (Fig. 2A).

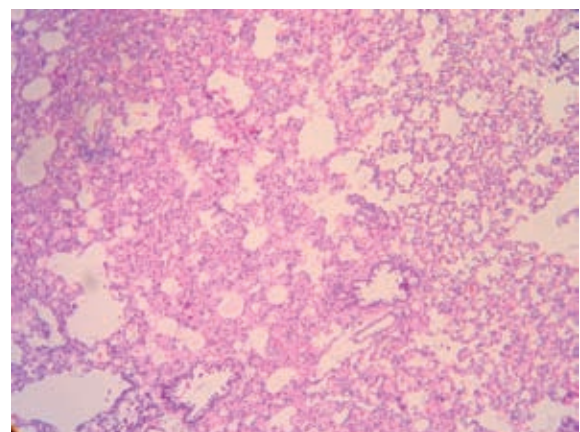
## The Effects of Midazolam on ANTU-Induced Acute Lung Injury

Midazolam alone has no deleterious effect on healthy lung parameters. Midazolam in doses of 2 and 4 mg/kg significantly reduced PE and PE/BW ratio and had a prophylactic effect on ANTU-induced PE formation in all doses ( $p < 0.05$ ) (Fig. 2 BC).

In the histopathological examination, midazolam 2 mg/kg group caused a significant reduction of inflammation and edema formation according to the histopathological scoring ( $p < 0.05$ ) (Fig. 4). Midazolam 4 mg/kg group caused a reduction of all scores but these are not significantly different from the ANTU group at the current dose and the lowest histopathological protection on ANTU-induce lung damage was seen in the midazolam group. Midazolam at the doses of 2 and 4 mg/kg demonstrated no significant prophylactic effect on LW/BW ratio (Fig. 2A).



**Fig. 3.** Histopathological examination after pre-treatment with ketamine 50 mg/kg (H & E  $\times 4$ )



**Fig. 4.** Histopathological examination after pre-treatment with midazolam 2 mg/kg (H & E  $\times 10$ )

**Table 2.** Histological scoring are listed below (0–3)

Groups	Inflammation	Edema	Hemorrhage
Control	0	0	0
ANTU (10 mg/kg)	2.4 ± 0.2 <sup>a</sup>	2.4 ± 0.2 <sup>a</sup>	1.3 ± 0.2 <sup>a</sup>
Ketamine (15 mg/kg)	0.4 ± 0.2 <sup>b</sup>	0.9 ± 0.1 <sup>b</sup>	0.4 ± 0.2 <sup>b</sup>
Ketamine (50 mg/kg)	0.5 ± 0.2 <sup>b</sup>	0.6 ± 0.2 <sup>b</sup>	0.3 ± 0.2 <sup>b</sup>
Midazolam (2 mg/kg)	1.5 ± 0.2 <sup>bcd</sup>	1.5 ± 0.2 <sup>bd</sup>	0.8 ± 0.2
Midazolam (4 mg/kg)	2 ± 0.3 <sup>cd</sup>	1.9 ± 0.1 <sup>cd</sup>	1.1 ± 0.2 <sup>cd</sup>
Ketamine + xylazine	0.9 ± 0.1 <sup>be</sup>	1 ± 0.3 <sup>be</sup>	0.9 ± 0.2

<sup>a</sup>  $p < 0.05$  vs. control; <sup>b</sup>  $p < 0.05$  vs. ANTU; <sup>c</sup>  $p < 0.05$  vs. Ketamine (15 mg/kg); <sup>d</sup>  $p < 0.05$  vs. Ketamine (50 mg/kg); <sup>e</sup>  $p < 0.05$  vs. Midazolam (4 mg/kg).

### The Effects of Ketamine/ /Xylazine on ANTU-Induced Acute Lung Injury

Ketamine/xylazine (50/10 mg/kg) administration prior to ANTU significantly reduced PE and PE/BW ratio and had a prophylactic effect on ANTU-induced PE formation ( $p < 0.05$ ) (Fig. 2 BC). Maximum protective effect on PE formation was seen in ketamine/xylazine (50/10 mg/kg) group in all groups. On the other hand, xylazine increase the protective effect of ketamine (50 mg/kg) ( $0.9 \pm 0.2$  mL) on pleural effusion in the ketamine/xylazine group ( $0.7 \pm 0.2$  mL) but this is not significantly different at the current dose (Fig. 2 BC).

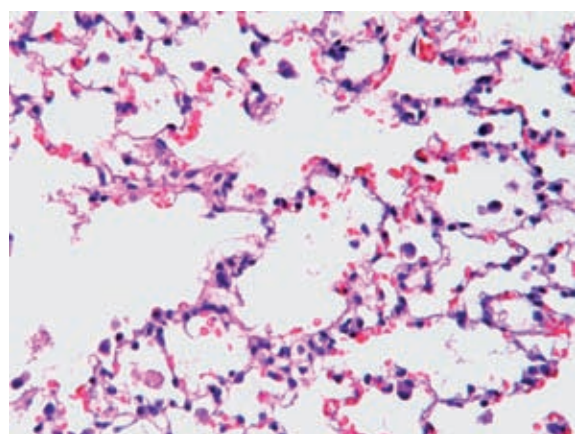
In the histopathological examination, ketamine/xylazine caused a significant reduction of inflammation and edema formation according to the histopathological scoring ( $p < 0.05$ ) (Fig. 5). Nevertheless, histopathological scores seemed to be lower but not significantly different in the ketamine (50 mg/kg) group than the ketamine/

/xylazine (50/10 mg/kg) group. Ketamine/xylazine demonstrated no significant prophylactic effect on LW/BW ratio (Fig. 2A).

### Discussion

Results of the present work have demonstrated for the first time that ketamine, midazolam and ketamine/xylazine attenuate the pulmonary tissue damage and pleural effusion induced by ANTU. All of these agents significantly prevent the development of pleural effusion. The maximum decrease in pleural effusion was observed in the ketamine/xylazine group. On the other hand, although these anesthetic agents caused a statistically significant reduction of histopathological scores, there was no significant lowering effect on LW/BW ratio at the current doses.

ANTU is a chemical agent that produces an inflammatory reaction leading to pulmonary edema, secondary to permeability changes in the lung microvasculature. The capillary endothelial cell damage in the lung is the primary reason for ANTU toxicity. Injury to the endothelium leads to an eventual loss of the endothelial barrier. This leads to interstitial and alveolar edema. Pulmonary edema and pleural exudate formation start approximately 1 h after administration of ANTU, reaching a maximum at 2–4 h, and then either resolve or cause death [6, 7, 11, 12, 16–19]. The mechanism of pleural effusion formation in the ANTU model is drainage of lung interstitial liquid, through the visceral pleura, into the pleural space. This occurs when the interstitial lung space is saturated with fluid [20]. Thus, the amount of pleural effusion is closely relevant to pulmonary edema development. It has been speculated that various vasoactive substances originating from pulmonary vascular bed



**Fig. 5.** Histopathological examination after pre-treatment with ketamine/xylazine (H & E  $\times 40$ )

and airways may contribute to the lung injury induced by ANTU [6, 16–18, 21, 22].

Glutamate is an essential amino acid and a transmitter in the mammalian nervous system. N-methyl-D-aspartate (NMDA),  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA), kainate and metabotropic receptors are activated by glutamate. In recent years the increasing number of investigators have demonstrated the involvement of glutamate and glutamate signaling in non-neuronal tissues, including bone osteoblasts and osteoclasts, keratinocytes, megakaryocytes, pancreatic islet cells, the lung, the liver, the heart, kidney cells, adrenal tissue, and taste buds [23]. Although the NMDA receptor subtypes have been characterized in terms of their functional roles in the central nervous system (CNS) and their relationship to neuronal excitotoxicity in neurological disorders, little or nothing is known about the physiologic and pathophysiologic significance of these receptors in the respiratory system. NMDA can induce excitotoxicity in the lung, in the form of an acute, high-permeability pulmonary edema, which was prevented by the non-competitive NMDA receptor antagonist MK-801, and that glutamate may be linked to the pathogenesis of bronchial asthma and airway inflammation [24].

The intravenous dissociative anesthetic, NMDA receptor antagonist ketamine has many pharmacological properties, including analgesic, anesthetic and sympathomimetic effects [25]. Ketamine is a drug used in human and veterinary medicine, primarily for the induction and maintenance of general anesthesia, usually in combination with a sedative. Other uses include sedation in intensive care, analgesia (particularly in emergency medicine), and treatment of bronchospasm [26]. Owing to its ability to induce relaxation of bronchial smooth muscle, ketamine is recommended as an optimizing anesthetic for asthmatic patients, and has been clinically used to treat bronchospasms, asthma exacerbation and status asthmaticus [27]. On the other hand, ketamine's role in neuroanesthesia is limited because of its association with increased intracranial pressure and other side effects such as psychotropic properties, tachycardia and hypertension [26]. Recent years studies have shown that ketamine plays a protective role against lung injury, via its anti-inflammatory properties [8–10]. Under ketamine anesthesia, neurogenic pulmonary edema is less pronounced in a rat model of spinal cord injury, and the mortality of severely burnt rats is reduced [28]. Yang et al. demonstrated that ketamine, only at a supra-anesthetic dosage, could inhibit endotoxin-induced pulmonary inflammation *in vivo* [9]. Ketamine has been shown to attenuate symptoms of endotoxemia in

a lipopolysaccharide-induced rat model of sepsis, by reducing nuclear factor kappa B (NF-kappa B) activity and tumor necrosis factor-alpha (TNF- $\alpha$ ) production [29], and decreasing the expression of iNOS in various rat tissues [30]. Furthermore, Yu et al. found that ketamine at sub-anesthetic doses also suppress the production of inflammatory cytokines on lung tissue such as TNF- $\alpha$  and interleukin-6 (IL-6), attenuate NF-kappa B activity, and inhibit toll-like receptor 2 (TLR2) and TLR4 expression in polymicrobial sepsis [8]. In addition, ketamine has weak suppressive effect on reactive oxygen species (ROS) production by neutrophils and modulate the stimulated adhesion molecule expression [31].

While there are numerous scientific publications on the influence of ketamine on the pulmonary system function, effects on pleural effusion are unknown. ANTU is a very important ALI model that also gives a chance to examine the effect of anesthetic agents on pleural effusion. In the present study we found for the first time that ketamine has a prophylactic effect in ANTU-induced PE. We can also suggest that acute inhibitory effects of ketamine on fluid accumulation were more effective in the pleural cavity (PE) than in the interstitial compartment (LW/BW ratio) in this experimental model. The exact mechanisms of the effects of ketamine on pleural effusion are still obscure. But these mechanisms might be related to the effects of ketamine on immune/inflammatory cells, ROS and iNOS expression [8, 9, 29–31]. These mechanisms are also known to play a role in ANTU-mediated damage [6, 16, 18]. On the other hand, we found that ketamine has also a lowering effect on inflammation, hemorrhage and edema formation. Inflammation and hemorrhage results of 15 and 50 mg/kg groups were close to the control group. The best improvement in ANTU-induced lung damage was seen in ketamine 15 mg/kg group for inflammation, and ketamine 50 mg/kg group for edema and hemorrhage compared to all groups. The protective effect of ketamine was more pronounced at a 50 mg/kg dose for all parameters. The data provided here indicates that NMDA receptor blockade shown an inhibitory effect on ANTU-induced lung damage. Recently, Gokcinar et al. demonstrated that ketamin, downregulated mediators of lung tissue inflammation and reduced the level of circulating cytokines and protected lung tissue against lipid peroxidation. They found that ketamine infusion decreased the level of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, NF- $\kappa$ B, and COX-2 mRNAs induced by LPS in lung tissue [32]. Because of ALI/ARDS defined as a syndrome of inflammation and increased permeability, we can suggest that NMDA receptors are involved in the inflammatory response in pulmonary



system. Our results may support earlier findings concerning the anti-inflammatory effects of ketamine on inflammation.

Ketamine and xylazine are commonly used in combination as an anesthetic agent in experimental animal models. Ketamine has many known anti-inflammatory actions. In contrast to ketamine, xylazine, an  $\alpha_2$ -adrenergic agonist, has been shown to have proinflammatory effects. Xylazine has been shown to induce pulmonary edema and to increase LPS-induced mortality in rats. Furthermore, during endotoxemia, xylazine worsens mortality and decreases levels of the anti-inflammatory cytokine IL-10 [33, 34]. On the other hand, ketamine/xylazine attenuates lipopolysaccharide (LPS)-induced TNF- $\alpha$  protein levels and iNOS transcription [35] in a variety of tissues. We suppose that there is an interaction between several different pathways that work together to attenuate inflammation as shown in ketamine/xylazine group. In the present study we found that ketamine/xylazine caused a significant reduction of inflammation and edema formation but these scores seemed to be higher but not significantly different than the ketamine alone (50 mg/kg) group. These results suggest that xylazine did not show an additive effect on the protective effect of ketamine alone. On the contrary, xylazine reversed the protection effect of ketamine on hemorrhage. Moreover, these results concur with those obtained by other investigators which have demonstrated the negative effects of xylazine on lung tissue [33, 34].

In the present study it was found for the first time that ketamine/xylazine has a prophylactic effect in ANTU-induced PE. Maximum protective effect on PE formation was seen in ketamine/xylazine group in all groups; however, the amount of PE did not significantly differ between ketamine and ketamine/xylazine groups. Experimental results show that similar to other anesthetic agent, xylazine is not an inert agent and may change the data in animal experiments. Therefore, when using these agents in veterinary or experimental studies, these effects should be kept in mind.

Among the benzodiazepines, midazolam, water-soluble benzodiazepine is the most widely used anxiolytic and sedative drug for short procedures and in intensive care. Midazolam interferes the synthesis of NO and TNF- $\alpha$  generated by activated microglia cells, blood monocytes, and mast

cells, suggesting an inhibitory action on proinflammatory mediators. We have shown that midazolam at the doses of 2 and 4 mg/kg reduced inflammation and edema scoring and PE in ANTU induced ALI. The protective effect of midazolam was more pronounced and significant at 2 mg/kg dose ( $p < 0.05$ ). Our results concur with those obtained by other investigators which have demonstrated the modulatory effects of benzodiazepine receptors in immune/inflammatory process [36]. Recent evidence indicates that midazolam significantly inhibited TNF- $\alpha$  induced vascular cell adhesion molecule-1 and monocyte adhesion in human umbilical vein endothelial cells [37]. Meanwhile, midazolam not only modulates the production of cytokines, but also decreases the generation of NO, inhibits the function of neutrophils, suppresses the expression of LPS-stimulated iNOS, cyclooxygenase-2 and superoxide anion production [36, 38]. On the other hand, some earlier works have shown that midazolam does not have the protective effect on hemodynamics and organ dysfunction in the endotoxemic rats [39]. The controversy in literature may be partly due to the use of different inflammation models, doses and animals. These results demonstrate the need for the use of different tests and doses in the evaluation of the effects of midazolam in tissue injury as well as ALI.

The results of the present study indicate that ANTU induced ALI is reduced by *i.p.* injection of ketamine, midazolam and ketamine/xylazine. Acute inhibitory effects of these anesthetic agents on fluid accumulation were more effective in the pleural cavity than the interstitial compartment in this ALI model. We can conclude that anesthetic agents may affect and attenuate lung injury. The precise mechanisms underlying the inhibitory effect are still unknown but we can suggest that the effects of anesthetic agents on immune/inflammatory reactions might be important. Our results may corroborate and support earlier findings concerning the protective effects of ketamine and midazolam on tissue injury. Along with the literature, these results suggest that, aside from anesthetic properties, many anesthetic agents can cause physiologic and metabolic alterations and modulate and improve the lung inflammatory responses and these effects of anesthetic agents should be considered in clinical and experimental applications.

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