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Vitamin C as an antioxidant: evaluation of its role on pulmonary contusion experimental model

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Background/aim: To analyze the protective activity of vitamin C on the lungs by assessing biochemical and histopathological analysis after performing an experimental isolated lung contusion model.

Materials and methods: Fifty-four male Sprague-Dawley male rats were used. The rats were randomly separated into 4 groups. Vitamin C (200 mg/kg) was injected intraperitoneally 30 min after trauma. Blood samples were obtained for myeloperoxidase (MPO), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and catalase (CAT) activities and malondialdehyde (MDA) levels. Blood gas analysis and bronchoalveolar lavage was performed. The lung tissue was also extracted for histopathological examination.

Results: The lung contusion enhanced MDA, SOD, CAT, and MPO and diminished GSH-Px. Vitamin C administration after the pulmonary contusion was found to diminish the level of MDA and the activities of SOD, CAT, and MPO and to enhance the level of GSH-Px ($P < 0.05$). Contusion-induced disrupted gas analysis and leukocyte infiltration were both resolved by the vitamin C.

Conclusion: The present results indicate that vitamin C administration attenuated the oxidative damage and morphological changes induced by pulmonary contusion in an experimental rat study.

Key words: Vitamin C, antioxidant, lung injury, pulmonary contusion

1. Introduction

A pulmonary contusion is a widespread lung injury after blunt trauma to the chest. Pulmonary contusions will generally resolve in 3 to 5 days, provided that no secondary injury occurs. The most important complications of pulmonary contusions are acute respiratory distress syndrome (ARDS) and pneumonia. About 50% of patients with pulmonary contusions and 80% of patients with pulmonary contusions involving over 20% of lung volume experience ARDS. Direct lung trauma, alveolar hypoxia, and blood in the alveolar spaces are all grand activators of the inflammatory pathways that result from acute lung injury. Pulmonary contusion in traumatic cases with an accompanying acute inflammatory response is a widespread but poorly understood injury (1). Direct traumatic damage to the lungs generates an innate inflammatory reagent that includes the recruitment and activation of blood leukocytes, the activation of lung tissue macrophages, and the production of multiple mediators such as cytokines and chemokines. Neutrophils add significantly to the violence of inflammatory lung contusion injury. The inflammatory response from a lung contusion-induced tissue injury enhances epithelial cell apoptosis/necrosis and damages the barrier integrity of the alveolocapillary membrane (2).

Vitamin C is a free radical scavenger and displays antioxidant activity. We focused on the potential preservative effects of vitamin C after lung injury. Generally, lung injuries can result in the generation of reactive oxygen species (ROS) by pulmonary endothelial cells, which leads to autooxidative damage (3). In order to avoid undesired oxidative damage induced by reactive species, organisms are equipped with several lines of antioxidant mechanisms. Vitamin C is an aqueous phase antioxidant that has been studied for many decades and has several important aspects. It modulates the intracellular

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redox status through maintaining sulfhydryl compounds, including glutathione, in their reduced state. Activities of other enzymes related to glutathione metabolism, such as glutathione reductase, glutathione peroxidase, and glutathione-S-transferase, were also diminished. It could be supposed that increasing the concentration of vitamin C might interfere with antioxidant systems, thus lowering the level of reduced glutathione as well as the activities of glutathione metabolic enzymes.

We investigated the effects of vitamin C, an antioxidant and free radical scavenger, after isolated lung contusion created with a reproducible, sublethal model of isolated lung contusion in rats induced by focused external blunt chest trauma. Vitamin C is an electron donor, and this property accounts for all its known functions. As an electron donor, vitamin C is a potent water-soluble antioxidant in humans. Antioxidant effects of vitamin C have been displayed in many experiments in vitro at the same time (4).

Whether vitamin C functions as an antioxidant or prooxidant is specified by at least 3 factors: 1) the redox potential of the cellular surroundings, 2) the presence/absence of transition metals, and 3) the concentration of ascorbate (4). The last factor is particularly appropriate in treatments that depend on the antioxidant/prooxidant property of vitamin C, because it can be readily manipulated and controlled in vivo to acquire the desired effects.

The precise prooxidant and antioxidant status of vitamin C after an isolated lung contusion is still not clear. To add new insight to this question, changes in the lipid peroxidation product malondialdehyde (MDA) and the activities of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and myeloperoxidase (MPO) activity were evaluated by blood gas analysis, inflammatory cells in bronchoalveolar lavage, and lung tissue sampling. To our knowledge, this is the first study examining the effect of vitamin C as an antioxidant after isolated lung contusion model by assessing both biochemical tests and histopathological analysis.

2. Materials and methods

2.1. Animal care and lung contusion model

A total of 54 adult male Sprague-Dawley rats, weighing 298–321 g, were housed in a temperature controlled room (20–25 °C; humidity 55%–60%) with a 12-h light/dark cycle and were fed a standard rat pellet feed and water ad libitum. The experiments were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals (85 – 23, 1985). The approval of the ethics committee of the Ankara Numune Training and Research Center was acquired.

2.2. Group definitions

The rats were split randomly into 4 groups.

Group 1 (n = 6) included rats without any contusion or administered vitamin C.

Group 2 (n = 6) included rats treated with vitamin C (Redoxon; Roche, Basel, Switzerland) at 200 mg/kg administrated intraperitoneally without lung contusion.

Group 3 (n = 24) included rats with contusions without administered vitamin C. The group consisted of 4 subgroups that were generated according to analysis on days 0, 1, 2, and 3.

Group 4 (n = 18) included rats treated with vitamin C at 200 mg/kg administrated intraperitoneally 30 min after contusion. This group was divided into 3 subgroups according to analysis on days 1, 2, and 3. Vitamin C was administrated 200 mg/kg intraperitoneally on days 1 and 2.

2.3. Anesthesia and trauma model

Animals were anesthetized with an intramuscular ketamine/xylazine 100/10 mg/kg injection. In the trauma model, the rats were fasted for 12 h before the described process. A pipe system was designed to convey a specified weight via piston by free fall and a stand apparatus was used to prevent the impact of the weight to the head and abdominal space of the rat. In calculating the isolated impact force to the thoracic wall, a space was generated to preserve the sternum and heart. Impact energy (E; in joules, J) was computed by the E = mgh formula where m is mass (kg), g is gravitational acceleration (9.8 m/s^2), and h is height where the weight is left (meter friction force was ignored). Blunt thoracic trauma at 1.78 J was administered to the rats, except for those of groups 1 and 2 (5).

2.4. Biochemical analysis

Under semisterile conditions, midsternotomy was applied to all animals at the end of the procedure. This procedure was initiated 30 min after vitamin C administration in group 4. A 5-mL blood sample was withdrawn from the ascending aorta for biochemical analysis. Blood samples were centrifuged at 1500 × *g* for 15 min, and serum was separated in all samples. Serum samples were stored in a freezer at –20 °C and lung tissues were stored in a freezer at –80 °C until biochemical analysis. Samples were studied for 2 weeks. Determinations of CAT, SOD, and GSH-Px enzyme activities and MPO and MDA levels were performed on the samples of the right lungs of the rats from all experimental groups.

2.4.1. Determination of the myeloperoxidase level

The tissue was homogenized (QIAGEN, Switzerland) at 16,000 rpm on ice in 1 mL of PBS, pH 7.4. The homogenate was centrifuged at 5000 × *g* for 5 min at 4 °C. The supernatant was removed for analysis and was stored at –80 °C until the time of analysis. MPO levels were specified using a commercial ELISA kit (Cusabio Biotech

Co., China). MPO level was gauged in the supernatant and serum. Results were expressed as ng/mg protein wet tissue for lung tissue and ng/mL for serum.

This analysis employed the quantitative sandwich enzyme immunoassay technique. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution was added to the wells and color developed. After color progress was stopped, the intensity of the color was measured at 450 nm using a plate reader (EPOCH, USA)

2.4.2. Determination of the malondialdehyde level

The tissue was homogenized (QIAGEN) at 16,000 rpm on ice in 1 mL of PBS, pH 7.4. The homogenate was centrifuged at 5000 × *g* for 5 min at 4 °C. The supernatant was removed for assay, and it was stored at –80 °C until the time of analysis. MDA levels were identified using a commercial ELISA kit (Cusabio Biotech Co.). MDA level was gauged in the supernatant and serum. Results were expressed as pmol/mg protein wet tissue for lung tissue and pmol/mL for serum.

This analysis employed the quantitative sandwich enzyme immunoassay technique. The enzyme-substrate reaction was terminated by the addition of a sulfuric acid solution, and color change was measured at 450 nm using a plate reader (EPOCH).

2.4.3. Determination of glutathione peroxidase activity

The tissue was homogenized (QIAGEN) at 16,000 rpm on ice in 5–10 mL of cold buffer (50 mM Tris HCl, pH 7.5; 5 mM EDTA; and 1 mM DTT) per gram of tissue. It was centrifuged at 10,000 × *g* for 15 min at 4 °C. The supernatant was removed for assay and was stored at –80 °C until the time of analysis. GSH-Px level was gauged in the homogenate and serum. GSH-Px activity was determined using a commercialized GSH-Px assay kit (Cayman Chemical Co., USA). Results were expressed as nmol min^{-1} mg $protein^{-1}$ wet tissue for lung tissue and nmol min^{-1} mL^{-1} for serum.

The glutathione peroxidase kit measures GSH-Px activity indirectly by measuring a coupled reaction with glutathione reductase. The oxidation of NADPH to $NADP^+$ is accompanied by a decrease in absorbance at 340 nm, using a plate reader (EPOCH).

2.4.4. Determination of the superoxide dismutase activity

The tissue was homogenized (QIAGEN) at 16,000 rpm on ice in 5–10 mL of cold buffer (20 mM HEPES buffer, pH 7.2, containing 1 mM EGTA, 210 mM mannitol, and 70 mm sucrose) per gram of tissue. It was centrifuged at 1500 × *g* for 5 min at 4 °C. The supernatant was subtracted for assay and was stored at –80 °C until the time of analysis. SOD activity was gauged in the homogenate and serum with a commercial SOD assay kit (Cayman Chemical Co.). The results were displayed as U/mg wet tissue for lung tissue and U/mL for serum.

The SOD analysis kit utilizes tetrazolium salt for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. The reactions were initiated by adding xanthine oxidase, incubating for 20 min at room temperature, and reading the absorbance at 440 nm using a plate reader (EPOCH).

2.4.5. Determination of the catalase activity

The tissue was homogenized (QIAGEN) at 16,000 rpm on ice in 5–10 mL of cold buffer (50 mM potassium phosphate, pH 7.0, containing 1 mM EDTA) per gram of tissue. It was centrifuged at 10,000 × *g* for 15 min at 4 °C. The supernatant was subtracted for assay. It was stored at –80 °C until the time of analysis. CAT activity was measured in the homogenate and the serum. CAT activity was identified using a commercialized catalase assay kit (Cayman Chemical Co.). Results were displayed as nmol min^{-1} mg^{-1} wet tissue for lung tissue and nmol min^{-1} mL^{-1} for the serum.

The method is based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H_2O_2. The formaldehyde produced is measured colorimetrically with 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald) as the chromogen. Purpald specifically forms a bicyclic heterocycle with aldehydes, which, upon oxidation, changes from colorless to purple. The catalase activity was gauged at 540 nm using a plate reader (EPOCH).

2.4.6. Protein measurements

Protein measurements were made in homogenate and supernatant using the method explained by Lowry et al. (6).

2.4.7. Blood gas analysis

Blood pH, pO_2, pCO_2, and HCO_3 values were evaluated with a Siemens Advia Rapid Lab 1200 blood gas analyzer.

2.5. Histopathological analysis

Bronchoalveolar lavage (BAL) was used for inflammatory cell counting. BAL was performed by administering isotonic (0.9% NaCl) fluid of 2 mL to the lungs through the trachea. The pulmonary surface was irrigated with normal saline. BAL fluid was cytocentrifuged at 3500 rpm. Staining was done with a direct PAP stain. Cell count was performed by examining prepared smears under a light microscope by a pathologist who was blind to the groups. When cells were examined on the basis of BAL, the inflammatory cells, blood components, and cells were scored as 0 if they fell under 5%, 1 if they were between 5% and 50%, or 2 if they were over 50%.

Lungs tissues were fixed in 10% formalin, dehydrated in graded concentrations of ethanol, cleared in xylene, and embedded in paraffin. At least 8 tissue sections of 5 μm in thickness were acquired; they were then stained with hematoxylin-eosin and examined by a pathologist

in a blinded manner. All histopathological changes were detailed in each lung tissue, including intraalveolar hemorrhage, alveolar edema, disruption and congestion, and leukocyte infiltration. Alveolar edema and congestion 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%). Leukocyte infiltration was examined to determine the severity of inflammation that resulted from contusion. Each section was split into 10 subsections, and leukocytic infiltration was examined in each of the subsections at a magnification of 400× with the following scale: 0, no extravascular leukocytes; 1, <10 leukocytes; 2, 10–45 leukocytes; or 3, >45 leukocytes. An average of the numbers was used for comparison (7).

2.6. Statistical analysis

Data were evaluated with SPSS 15.0, which is a commercially available statistics software package. Distributions of the groups were evaluated with the one-sample Kolmogorov–Smirnov test. Biochemical results displayed normal distribution. A one-way ANOVA test was performed, and post hoc multiple comparisons were done with least significant differences. Histopathological results were evaluated by Kruskal–Wallis and Mann–Whitney U tests. Results were displayed as means ± standard deviations (SDs). P values of less than 0.05 were regarded as statistically significant.

3. Results

All enzyme activities are illustrated in Tables 1 and 2. Results displayed that the lung contusion alone created a statistically significant increase in the level of MDA and the activities of SOD, CAT, and MPO and a decrease in the level of GSH-Px in group 3 compared with group 1 ($P < 0.05$). During the first and the second day, the levels of MDA and GSH-Px and activities of SOD, CAT, and MPO showed no statistically significant difference between group 3 and group 4 ($P > 0.05$). However, we were able to demonstrate statistically significant difference in the levels of MDA and GSH-Px and activities of SOD, CAT, and MPO between group 3 and group 4 on the third day ($P < 0.05$). The vitamin C-treated contusion group showed a decreased level of MDA and activities of SOD, CAT, and MPO and an increased level of GSH-Px on the last experimental day. No significant difference was detected in serum or tissue levels of MDA and GSH-Px and activities of SOD, CAT, and MPO between group 4 and group 1 ($P > 0.05$).

Table 1. Tissue MDA and GSH-Px levels and SOD, CAT, and MPO activities in pulmonary contusion injury model in rats (n = 6; mean ± SD).

	MDA (tissue) (pmol/mg)	SOD (tissue) (U/mg)	CAT(tissue) (nmol min^{-1} mg^{-1})	GSH-Px (tissue) (nmol min^{-1} mg^{-1})	MPO (tissue) (ng/mg)
Group 1	198 ± 24	138 ± 18	82 ± 14	61 ± 13	892 ± 12
Group 2	187 ± 19	141 ± 26	85 ± 17	58 ± 17	908 ± 23
Group 3					
Day 0	541 ± 21	301 ± 21	212 ± 21	27 ± 13	1842 ± 31
Day 1	489 ± 19	286 ± 18	188 ± 19	32 ± 16	1729 ± 39
Day 2	422 ± 22	241 ± 23	152 ± 18	39 ± 11	1502 ± 27
Day 3	418 ± 25	214 ± 24	143 ± 18	38 ± 19	1417 ± 24
Group 4					
Day 1	477 ± 22	278 ± 26	169 ± 31	36 ± 19	1698 ± 29
Day 2	401 ± 19	219 ± 19	141 ± 19	41 ± 16	1456 ± 22
Day 3	221 ± 24	150 ± 22	94 ± 21	59 ± 13	931 ± 23
P-values					
Day 0, groups 1–2	n.s.	n.s.	n.s.	n.s.	n.s.
Day 0, groups 3–1	0.0001	0.0001	0.0001	0.0001	0.0001
Day 1, groups 3–4	n.s.	n.s.	n.s.	n.s.	n.s.
Day 1, groups 4–1	0.0001	0.0001	0.0001	0.0001	0.0001
Day 2, groups 3–4	n.s.	n.s.	n.s.	n.s.	n.s.
Day 2, groups 4–1	0.001	0.001	0.001	0.001	0.001
Day 3, groups 3–4	0.001	0.001	0.002	0.002	0.001
Day 3, groups 4–1	n.s.	n.s.	n.s.	n.s.	n.s.

Table 2. Serum MDA and GSH-Px levels and SOD, CAT, and MPO activities in pulmonary contusion injury model in rats (n = 6; mean ± SD).

	MDA (serum) (pmol/mL)	SOD (serum) (U/mL)	CAT (serum) (nmol min^{-1} mL^{-1})	GSH-Px (serum) (nmol min^{-1} mL^{-1})	MPO (serum) (ng/mL)
Group 1	79 ± 18	49 ± 19	46 ± 12	36 ± 11	901 ± 21
Group 2	76 ± 16	47 ± 13	43 ± 16	34 ± 13	890 ± 27
Group 3					
Day 0	214 ± 19	186 ± 21	184 ± 22	19 ± 11	1728 ± 21
Day 1	208 ± 23	174 ± 14	175 ± 32	22 ± 16	1689 ± 29
Day 2	181 ± 25	152 ± 17	153 ± 24	22 ± 14	1566 ± 27
Day 3	170 ± 16	142 ± 19	142 ± 21	24 ± 17	1502 ± 31
Group 4					
Day 1	195 ± 17	179 ± 19	167 ± 21	24 ± 19	1627 ± 34
Day 2	173 ± 21	139 ± 25	129 ± 23	25 ± 16	1496 ± 47
Day 3	90 ± 23	61 ± 21	54 ± 19	34 ± 12	937 ± 29
P-values					
Day 0, groups 1–2	n.s.	n.s.	n.s.	n.s.	n.s.
Day 0, groups 3–1	0.0001	0.0001	0.0001	0.0001	0.0001
Day 1, groups 3–4	n.s.	n.s.	n.s.	n.s.	n.s.
Day 1, groups 4–1	0.0001	0.0001	0.0001	0.0001	0.0001
Day 2, groups 3–4	n.s.	n.s.	n.s.	n.s.	n.s.
Day 2, groups 4–1	0.001	0.001	0.001	0.002	0.002
Day 3, groups 3–4	0.001	0.002	0.002	0.001	0.001
Day 3, groups 4–1	n.s.	n.s.	n.s.	n.s.	n.s.

Table 3 demonstrates the values of pH, pO_2, pCO_2, and HCO_3. On day 0, pH, pO_2, and HCO_3 were decreased and pCO_2 was increased in group 3 when compared with group 1. The difference was statistically significant ($P < 0.05$). Similar to the results for enzyme activities, blood gas analysis resulted in a comparable outcome after vitamin C treatment. On the third day, the values of blood gases, except pCO_2, in the vitamin C-treated contused group (group 4) were similar to those of the noncontused group (group 1) ($P > 0.05$).

BAL scores in terms of inflammatory cell count and histopathological examination scores (Table 4) revealed no difference between group 1 and group 2 ($P > 0.05$). During the first and the second day, pathologic scores and BAL scores showed no statistically significant differences between group 3 and group 4 ($P > 0.05$). Both analyses resulted in statistically significant higher scores in group 4 compared with group 1 on days 1 and 2 ($P < 0.05$). On day 3, pathologic score and BAL score were significantly higher in group 3 compared with group 4 ($P < 0.05$), but no significant differences were seen in group 4 compared with group 1 ($P > 0.05$).

Microscopic findings in the lung specimens revealed normal lung parenchyma in group 1 (Figure 1). In contrast, the rats in group 3 showed disruption of normal alveolar structure with severe congestion and hemorrhage associated with infiltrating leukocytes (Figure 2). Furthermore, rats in group 4 had significantly less congestion, edema, hemorrhage, and leukocyte infiltration (Figure 3).

4. Discussion

Lung contusion is one of most important problems in the intensive care of trauma patients. Thoracic trauma is involved in nearly one-third of acute admissions of injured adults admitted to the hospital (8), and lung contusion is one of the most commonly diagnosed intrathoracic injuries after blunt trauma. Up to 25% of deaths can be seen after thoracic trauma cases (9). Acute lung injury is a very common clinical scenario seen in 30%–75% of blunt thoracic trauma cases. The pathophysiology of pulmonary contusion is not clearly understood, and only minimal advances have been made in the management of this entity over the past 2 decades (1). Lung injury after blunt trauma can cause leukocyte infiltration and the production of inflammatory mediators, free oxygen radicals that play an important role in pathogenesis. The cell has several ways to alleviate the effects of oxidative stress, by repairing the

Table 3. Mean values and standard deviations of blood pH, pO_2, pCO_2, and HCO_3 in a pulmonary contusion injury model in rats (n = 6; mean ± SD).

	pH	pO_2	pCO_2	HCO_3
Group 1	7.3 ± 0.31	86.9 ± 2.7	34.3 ± 4.8	23.2 ± 2.8
Group 2	7.3 ± 0.39	86.4 ± 3.9	34.8 ± 3.9	22.9 ± 1.9
Group 3				
Day 0	6.9 ± 0.36	51.3 ± 1.9	57.2 ± 3.2	14.1 ± 1.5
Day 1	6.9 ± 0.21	51.6 ± 2.1	54.5 ± 2.3	14.3 ± 1.9
Day 2	7.0 ± 0.28	54.1 ± 3.2	53.7 ± 2.4	15.2 ± 3.3
Day 3	7.0 ± 0.34	54.6 ± 0.7	52.1 ± 3.6	16.4 ± 3.8
Group 4				
Day 1	6.9 ± 0.38	53.8 ± 2.3	53.8 ± 0.9	15.2 ± 2.1
Day 2	7.0 ± 0.29	57.8 ± 2.9	48.9 ± 3.8	16.1 ± 4.3
Day 3	7.3 ± 0.11	82.8 ± 1.8	44.9 ± 2.1	21.3 ± 3.6
P-values				
Day 0, groups 1–2	n.s.	n.s.	n.s.	n.s.
Day 0, groups 3–1	0.0001	0.0001	0.0001	0.0001
Day 1, groups 3–4	n.s.	n.s.	n.s.	n.s.
Day 1, groups 4–1	0.0001	0.0001	0.0001	0.0001
Day 2, groups 3–4	n.s.	n.s.	n.s.	n.s.
Day 2, groups 4–1	0.002	0.001	0.002	0.001
Day 3, groups 3–4	0.001	0.002	0.001	0.001
Day 3, groups 4–1	n.s.	n.s.	0.001	n.s.

damage (damaged nucleotides and lipid peroxidation by-products) or by directly and indirectly decreasing the prooxidative state via enzymatic and nonenzymatic antioxidants. Enzymatic and nonenzymatic antioxidants have been shown to scavenge free radicals and ROS.

Several lines of evidence suggest that vitamin C is a powerful antioxidant in biological systems in vitro. However, its antioxidant role in humans is not supported by currently available clinical studies. Nonenzymatic antioxidants, such as vitamin C, act to recover from oxidative stress and are a part of the total antioxidant system in trauma cases. Vitamin C is hydrophilic and is one of the most important free radical scavengers in extracellular fluids, effective by trapping radicals in the aqueous phase and preserving biomembranes from peroxidative harm (10,11). In addition to its antioxidant effects, vitamin C is involved in the regeneration of tocopherol from tocopheroxyl radicals in the membrane.

Apart from their free radical-scavenging property, antioxidants are known to organize the expression of number of genes and signal regulatory pathways, and thereby may predict the incidence of cell death (10,11). ROS-induced harm has been accepted as an important contributor in postischemic cell injury. ROS, consisting of oxygen free radicals and associated entities, are natural products in the metabolism of cells. In pathological conditions, including ischemia–reperfusion injuries, ROS are overproduced while antioxidative enzymes are inactivated and antioxidants are overconsumed. When the presence of ROS overwhelms endogenous antioxidant defenses, excessive ROS would result in the self-perpetuating free radical reaction of lipid peroxidation, which could subsequently induce membrane damage, cell lysis, organelle dysfunction, and calcium dyshomeostasis. Since the pulmonary system contains a large lipid content and high oxygenation, it is especially susceptible to lipid peroxidation-related cellular damage (3). Vitamin C, which is a widely accepted pharmacological vitamin, confers protection through its ability to inhibit posttraumatic inflammatory responses.

MDA, the end product of lipid peroxidation, has also been measured to indicate the existence of free radicals and lipid peroxidation-induced toxicity. Other studies found that the increased activity of CAT is due to the adaptive response to the generated free radicals, indicating the failure of the total antioxidant defense mechanism to protect the tissues from mechanical damage caused by pesticides, as evidenced by lipid peroxidation (12–14).

Table 4. The values of pathologic score and bronchoalveolar lavage score in pulmonary contusion injury in rats (n = 6; mean ± SD).

	Alveolar edema and congestion	Leukocyte infiltration	Bronchoalveolar lavage score
Group 1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Group 2	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Group 3			
Day 0	2.42 ± 0.29	2.39 ± 0.19	1.81 ± 0.18
Day 1	2.34 ± 0.21	2.35 ± 0.11	1.75 ± 0.24
Day 2	2.19 ± 0.18	2.23 ± 0.21	1.68 ± 0.21
Day 3	2.19 ± 0.11	2.21 ± 0.31	1.61 ± 0.24
Group 4			
Day 1	2.31 ± 0.27	2.29 ± 0.45	1.69 ± 0.48
Day 2	2.09 ± 0.39	2.16 ± 0.31	1.64 ± 0.24
Day 3	0.31 ± 0.17	0.23 ± 0.11	0.18 ± 0.17
P-values			
Day 0, groups 1–2	n.s.	n.s.	n.s.
Day 0, groups 3–1	0.0001	0.0001	0.0001
Day 1, groups 3–4	n.s.	n.s.	n.s.
Day 1, groups 4–1	0.0001	0.0001	0.0001
Day 2, groups 3–4	n.s.	n.s.	n.s.
Day 2, groups 4–1	0.0001	0.0001	0.0001
Day 3, groups 3–4	0.0001	0.0001	0.0001
Day 3, groups 4–1	n.s.	n.s.	n.s.

To preserve against harm, cells contain a number of defense mechanisms, including well-documented endogenous antioxidant enzymes such as CAT, SOD, GSH-Px, glutathione reductase, and glutathione S-transferase and low-molecular-weight antioxidants such as glutathione (GSH) (4,5). Sulfhydryl groups (–SH) play an important role in producing a response to oxidative stress; GSH/glutaredoxin and thioredoxin systems are crucial components in maintaining redox homeostasis of the cell.

As mentioned above, SOD and CAT are antioxidant enzymes and MDA is a main end product of free radical

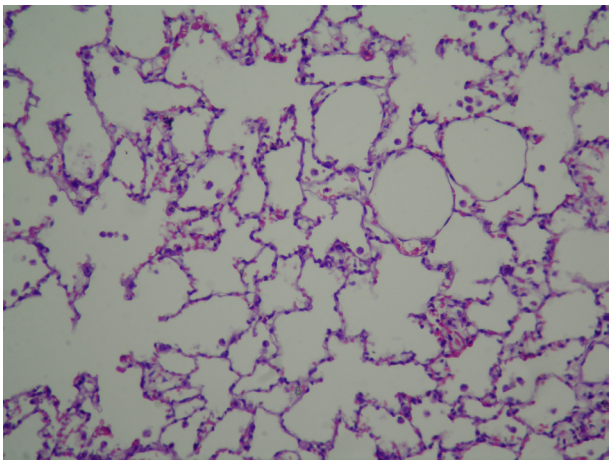

Figure 1. Representative photomicrograph from lungs of group 1 rats. Normal appearance of lung histology is shown (H&E; 200×).

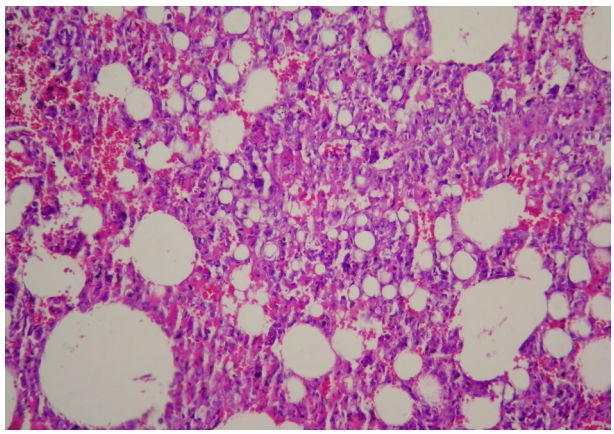

Figure 2. Representative photomicrograph from lungs of group 3 rats on day 0. Extensive congestion, edema, hemorrhage, and leukocyte infiltration are shown (H&E; 200×).

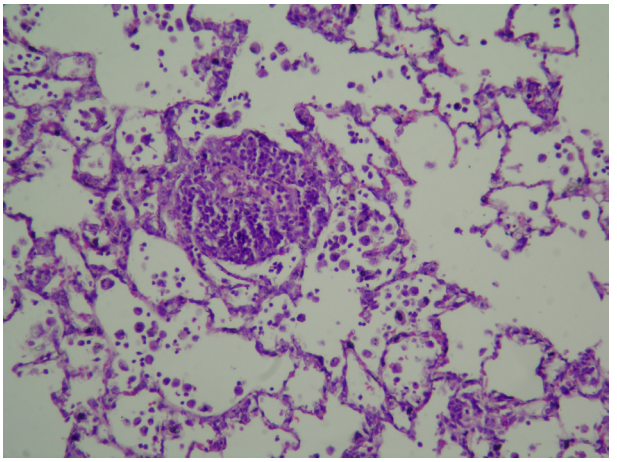

Figure 3. Representative photomicrograph from lungs of group 4 rats on day 3. Congestion, edema, hemorrhage, and leukocyte infiltration are observed in a very reduced state (H&E; 200×).

reaction. In the present study, a contusion-stimulated lung injury increased MPO, SOD, and CAT activities but diminished GSH-Px activity. However, we were only able to predict the quantity of the enzymes. Thus, while the amount of enzymes may have been foreseen, the activity of the enzymes was unpredictable. However, we could explain the diminished GSH-Px level by the depletion of reduced GSH molecules after the contusion-stimulated lung injury. The enhanced oxygen radicals deplete GSH, which correlates with the reduction of GSH-Px. The role of vitamin C during the antioxidant process might not only be seen in enzymatic activity, but it also mainly blocks the production of free oxygen radicals.

Some studies showed that superoxide radicals can inhibit GSH-Px and CAT activities, and singlet oxygen and peroxyl radicals can inhibit SOD and CAT activities (11–14). The increased H_2O_2 could cause SOD inhibition, such that superoxide radicals would increase. The increased superoxide radicals would inhibit both CAT and GSH-Px so that H_2O_2 would accumulate in the medium, causing SOD inhibition and enhanced superoxide radicals (15). Eventually, the inhibitions of SOD, CAT, and GSH-Px would gradually be enhanced. In the present study, the administration of vitamin C was somewhat effective in restoring the activities of SOD, CAT, and MPO and the level of MDA. Thus, free oxygen radical restoration to normal levels decreases the GSH-Px as demonstrated in the study.

Ince et al. (16) demonstrated that SOD and CAT activities were enhanced in an ischemia–reperfusion rat heart model. Vitamin C treatment prevented the enhancement of SOD enzyme activities and enhanced the level of glutathione. Akturk et al. (12) studied the effects of diazinon on lipid peroxidation and antioxidant enzymes in rat heart and the ameliorating roles of vitamin E and vitamin C. Their study found that vitamin E and C treatments led to diminished SOD and CAT activities in diazinon-induced damage of rat heart.

The critical role of neutrophils in the etiology of pulmonary endothelial harm is convincing. Morphological data reveal deposition and aggregation of neutrophils in the pulmonary vasculature in animals with acute lung injury. Clinical data are partially based on BAL fluid from patients with ARDS. Neutrophil elastase and neutrophil-derived oxidants in the lavage fluid have been displayed (17). Both the release of elastase and the generation of oxidants by neutrophils can lead to severe pulmonary injury in such cases. BAL and histopathological leukocyte scores showed a significant reduction in inflammation after vitamin C in the lung contusion group. The reduction in inflammation was prominent in BAL inflammatory cell counts. The histopathological score was better maintained after vitamin C was administered for the lung contusion. On the third day, no statistically significant difference was calculated in histopathological score between the vitamin C-treated contusion group and the noncontused group. The same conclusions were reached in other studies for the scores of leukocytic infiltration after blunt injury (18). As indicated, oxidative mechanisms in lung injury and pharmacological intervention in the inflammatory oxidative-induced lung injury can be performed by the downregulation of proinflammatory cytokine, blocking leukocyte infiltration.

Extensive lung inflammation and the abnormal accumulation of fluid in the lung tissue results in noncardiogenic pulmonary edema, pulmonary contusion, respiratory distress, and inadequate oxygenation of the blood in trauma cases (19,20). This then leads to ventilation–perfusion mismatch and worsening of gas exchange, as shown by Türüt et al. (21) in a rat traumatic contusion model. Regarding the blood gas analysis in our study, the values of blood gases, except pCO_2, in the vitamin C-treated contused group (group 4) were similar to those of the noncontused group (group 1) on day 3. Arterial oxygenation improved over time; the levels persisted within the range of acute lung injury at 4–24 h following contusion (22). Regarding the blood gas analysis in our study, all pO_2 values were decreased and all pCO_2 values were increased significantly soon after lung injury. No difference was detected on day 1 between the vitamin C-treated contusion group and the nontreated contusion group. Significant improvement in pH, pO_2, and HCO_3 in the vitamin C-treated contusion group compared to the nontreated contusion group was detected on day 3. Only the pCO_2 levels remained unchanged when comparing the vitamin C-treated contusion group (group 4) and the nontreated contusion group (group 3) on the last day.

Administration of vitamin C treatments significantly decreased lung injury according to our biochemical and histological findings. The preventive effect of vitamin C after a contusion-induced lung injury to decrease the level of MDA and the activities of SOD, CAT, and MPO and increase the GSH-Px activity possibly results from its antioxidant activity.

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