

## Vitamin E modulates lung oxidative stress, serum copper, zinc, and iron levels in rats with pulmonary contusion

Mehmet SIRMALI<sup>1\*</sup>, Okan SOLAK<sup>2</sup>, Talip ÇEVİK<sup>3</sup>, Rana SIRMALI<sup>4</sup>, Bünyamin ÖZAYDIN<sup>5</sup>,  
Zeynep GİNİŞ<sup>4</sup>, Yetkin AĞAÇKIRAN<sup>6</sup>, Namık DELİBAŞ<sup>7</sup>

<sup>1</sup>Department of Surgery, School of Medicine, University of Alabama at Birmingham, Birmingham, AL, USA

<sup>2</sup>Department of Thoracic Surgery, Faculty of Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey

<sup>3</sup>Department of Emergency Medicine, Faculty of Medicine, Afyon Kocatepe University, Afyonkarahisar, Turkey

<sup>4</sup>Department of Biochemistry, Dışkapı Yıldırım Beyazıt Research Hospital, Ankara, Turkey

<sup>5</sup>Department of Anesthesiology, Faculty of Medicine, University of Alabama at Birmingham, Birmingham, AL, USA

<sup>6</sup>Department of Pathology, Atatürk Chest Diseases and Thoracic Surgery Research Hospital, Ankara, Turkey

<sup>7</sup>Department of Biochemistry, Faculty of Medicine, Bozok University, Yozgat, Turkey

Received: 27.11.2013 • Accepted: 29.03.2014 • Published Online: 01.04.2015 • Printed: 30.04.2015

**Background/aim:** To evaluate the effects of oxidant/antioxidant mechanisms and levels of trace elements on trauma-stimulated moderate pulmonary contusions after vitamin E administration.

**Materials and methods:** Sixty-three male Sprague Dawley rats were used. Animals were studied in 4 groups. Vitamin E (150 mg/kg) was injected intraperitoneally 30 min after trauma and on the first and second days. Blood samples were obtained for nitric oxide (NO) levels and superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities. Zinc (Zn<sup>2+</sup>), copper (Cu<sup>2+</sup>), and iron (Fe<sup>3+</sup>) were measured in serum.

**Results:** Lung contusion increased serum and tissue NO levels and SOD activities and decreased GSH-Px activities (P < 0.05). Vitamin E significantly (P < 0.05) decreased NO levels and SOD activities and increased GSH-Px. Serum Zn<sup>2+</sup>, Cu<sup>2+</sup>, and Fe<sup>3+</sup> levels were statistically significantly influenced by the administration of vitamin E (P < 0.05). Group 4 had lower scores compared to Group 3 (P < 0.05) and no difference compared to Group 1 (P > 0.05).

**Conclusion:** These results suggest that treatment with vitamin E reduces lung oxidative stress and related mechanisms in isolated lung contusion as demonstrated by an experimental rat model.

**Key words:** Pulmonary contusion, vitamin E, oxidative stress, copper, zinc, iron

### 1. Introduction

Some of the important fat-soluble components of vitamin E are tocotrienols and tocopherols (1). Tocopherols have antioxidant features and they have been demonstrated to be effective in treatment of chronic diseases associated with oxidative stress (2). Because of its antioxidant activity, the role of vitamin E in prevention of chronic diseases, particularly those thought to have an oxidative stress component such as atherosclerosis and cardiovascular diseases, has been widely studied.

Some epidemiological studies reported that high vitamin E intake is associated with decreased risk of cardiovascular diseases, but intakes of other dietary antioxidants (such as  $\beta$ -carotene and vitamin C) are not. Therefore, it has been argued that the effect of vitamin E

is beyond its antioxidant functions (3,4). Vitamin E has also been reported to have other effects on enzymatic properties, neurological functions, and gene expression. Some even argued that the most important effect of vitamin E is to be a signaling molecule (5). Since it is an antioxidant, vitamin E behaves as a peroxy radical scavenger and inhibits the propagation of free radicals into tissues by forming a tocopheryl radical with them. It is then oxidized by a hydrogen donor and returned to its reduced state. As a result, it gets into the cell membranes and protects the whole system from oxidative damage (1,5).

Systemic inflammatory response can be instigated by ischemia, inflammation, trauma, infection, or a combination of several insults. Despite many studies

\* Correspondence: mehmet@uab.edu

published on this subject, the role of lung contusions within systemic inflammatory response is still not clear. Pathophysiological changes of pulmonary contusion include higher alveolocapillary permeability and intrapulmonary shunting, ventilation/perfusion mismatching, a loss of compliance, inflammation, and pulmonary edema (6). Acute lung injury models in rats are described as an intense inflammatory response in the pulmonary parenchyma. The main inflammatory response is initiated by either direct or indirect injuries to the lung and the inflammation involves increase of blood leukocytes, activation of tissue macrophages, and production of various mediators such as chemokines, oxygen radicals, cytokines, coagulation cascades, arachidonic acid metabolites, components of the complement, and coagulation cascades (7). Inflammatory lung injuries are related to cell-based actions and soluble mediators such as chemokines and cytokines mediate their interactions (8).

The effects of oxidative stress in the pathogenesis of lung injury and the benefits or otherwise of antioxidants have been well documented (7,8). Regular cellular metabolism outputs high levels of reactive free radicals including hydrogen peroxide ( $H_2O_2$ ), superoxide anion ( $O_2^-$ ), and hydroxyl radical ( $OH^-$ ). These radicals are generally eliminated by antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) (9,10).

Vitamin E is an important membrane protectant to save cells from reactive oxygen species (ROS) and lipid peroxidation (LPO) (5). The positive effect of vitamin E, especially in trauma cases and chronic diseases, is suspected to be due to its specific molecular function, particularly its antioxidant function (1). This paper seeks to determine the ability of vitamin E to reverse free radical-mediated oxidative damage in isolated lung contusions in rats. To our knowledge, the levels of zinc ( $Zn^{+2}$ ), copper ( $Cu^{+2}$ ), iron ( $Fe^{+3}$ ), and antioxidant enzyme activities with lung contusion and their relationship with vitamin E administration were elucidated by this experimental model for the first time. Histopathologic evaluation of the lung tissue was also performed.

## 2. Materials and methods

### 2.1. Animal care and lung contusion model

Sixty-three adult male Sprague Dawley rats weighing 304–318 g were used. The experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals, DHEW Publication No. (NIH) 85–23, and approved by the ethics committee of Ankara Numune Training and Research Center (approval number 2011/13, date 07.02.2011).

### 2.2. Group definitions

The animals were divided into 4 groups:

Group 1 (n = 7): rats without lung contusion and no vitamin E administration.

Group 2 (n = 7): Vitamin E (Evigen; Aksu Farma, İstanbul, Turkey; 150 mg/kg) was injected intraperitoneally to rats without lung contusion.

Group 3 (n = 28): Rats with lung contusion and without vitamin E administration. This group was divided into 4 equal subgroups that were created according to analysis on days 0, 1, 2, and 3.

Group 4 (n = 21): Vitamin E (150 mg/kg) was injected intraperitoneally 30 min after injury to the rats with lung contusion. This group was divided into 3 equal subgroups according to analysis on days 1, 2, and 3. Vitamin E (150 mg/kg) was administered intraperitoneally both on days 1 and 2 of the injury.

### 2.3. Anesthesia and trauma model

Ketamine/xylazine 100/10 mg/kg injections were performed for all rats. At the beginning of the experiment the rats were fasted for 12 h. Blunt trauma was induced via an experimental pipe system through which a weight was transferred to a piston by free fall and a stand apparatus avoiding impact of weight to the head or the abdominal space of the rat. In order to protect the sternum and heart, the piston was used to transfer impact force to the thoracic wall only. Impact energy (E; in J) was computed by the formula  $E = mgh$ , where m = mass (kg), g = gravitational acceleration ( $9.8 \text{ m/s}^2$ ), h = height, and meter friction force was ignored. Blunt thoracic trauma was induced by 1.78 J force in rats, except Groups 1 and 2 (11).

### 2.4. Biochemical analysis

Midsternotomies were applied to all rats at the end of the procedure. This procedure was performed 30 min after vitamin E administration in Group 4. Blood samples were withdrawn via the ascending aorta for biochemical studies. The samples were centrifuged at  $1500 \times g$  for 15 min and serum was separated from blood. Serum samples were stored in a freezer at  $-20 \text{ }^\circ\text{C}$  and lung tissues were stored in a freezer at  $-80 \text{ }^\circ\text{C}$  until biochemical analysis. Samples were studied within 2 weeks. Determinations of NO, SOD, and GSH-Px enzyme activities were performed on samples of the right lungs of the rats in the 4 experimental groups.

#### 2.4.1. Nitric oxide

The samples were homogenized (QIAGEN, Switzerland) at 16,000 rpm on ice in 5–10 mL of PBS (pH 7.4) per gram of tissue. It was centrifuged at  $10,000 \times g$  for 20 min at  $4 \text{ }^\circ\text{C}$ . The supernatant was removed for assay and stored at  $-80 \text{ }^\circ\text{C}$  until analysis. Total NO product (nitrate + nitrite) levels were measured in the homogenate and serum by using a commercialized Nitrate/Nitrite Colorimetric Assay Kit (Cayman Chemical Co., USA). Results were expressed as  $\mu\text{M}/\text{mg}$  protein wet tissue for lung tissue and  $\mu\text{M}/\text{mL}$  for serum. The Nitrate/Nitrite Colorimetric Assay

Kit measures total nitrate/nitrite concentration in a simple 2-step process. The first step is the conversion of nitrate to nitrite utilizing nitrate reductase. The second step is the addition of Griess reagents, which convert nitrite into a deep purple azo compound. Photometric measurement of the absorbance at 540 nm, caused by this azo chromophore, accurately determines  $\text{NO}_2^-$  concentration (EPOCH, USA).

#### 2.4.2. Glutathione peroxidase

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  $10,000 \times g$  for 15 min at 4 °C. The supernatant was removed for assay and it was stored at –80 °C until analysis. GSH-Px activity was measured in the homogenate and serum by using a commercialized Glutathione Peroxidase Kit (Cayman Chemical Co.). Results were expressed as  $\text{nmol min}^{-1} \text{mg}^{-1}$  protein wet tissue for lung tissue and  $\text{nmol min}^{-1} \text{mL}^{-1}$  for serum. The Glutathione Peroxidase Kit measures GSH-Px activity indirectly by a coupled reaction with glutathione reductase. The oxidation of NADPH to  $\text{NADP}^+$  is accompanied by a decrease in absorbance at 340 nm using a plate reader (EPOCH).

#### 2.4.3. Superoxide dismutase

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  $1500 \times g$  for 5 min at 4 °C. The supernatant was removed for assay and stored at –80 °C until analysis. SOD activity was measured in the homogenate and serum by using a commercial SOD assay kit (Cayman Chemical Co.). Results were expressed as U/mg wet tissue for lung tissue and U/mL for serum. The SOD assay 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).

#### 2.4.4. Protein measurements

Protein evaluations were made in homogenate and supernatant by the method explained elsewhere by Lowry et al. (12).

#### 2.4.5. Zinc, copper, and iron levels

Blood samples were centrifuged at  $2465 \times g$  for 10 min and serum samples were aliquoted and stored at –20 °C for serum  $\text{Zn}^{+2}$  and  $\text{Cu}^{+2}$  measurements. Before the analyses, the stored samples were brought to room temperature and diluted with deionized water (1:10). The serum levels

of  $\text{Zn}^{+2}$  and  $\text{Cu}^{+2}$  were analyzed by atomic absorption spectrophotometer (Shimadzu-AA6501F, Japan) at 213.9 nm and 324.8 nm, respectively. Calibration was made using rat serum-based standards with known  $\text{Zn}^{+2}$  and  $\text{Cu}^{+2}$  concentrations. Certified reference serum was used as a quality control.  $\text{Fe}^{+3}$  levels were measured using the ferrozine method (Siemens Advia 2400, USA) in all groups.

#### 2.4.6. Blood gas analysis

Blood pH,  $\text{pO}_2$ ,  $\text{pCO}_2$ , and  $\text{HCO}_3^-$  values were determined using a blood gas analyzer (Siemens Advia Rapid Lab 1200).

#### 2.5. Histopathological analysis

Bronchoalveolar lavage (BAL) was performed for inflammatory cell counting. BAL was performed by administering 2 mL of isotonic (0.9% NaCl) fluid to the lung. The surface of the pulmonary membrane was irrigated with saline. The fluid was cytocentrifuged at 3500 rpm. Direct PAP stain was performed. Prepared smears were examined under light microscope by a pathologist. Cells were evaluated on the basis of BAL, inflammatory cell, and blood components and were scored as 0 when these were under 5%, 1 when they were between 5% and 50%, and 2 when they were over 50%.

Lung tissues were fixed in 10% formalin, dehydrated in graded concentrations of ethanol, cleared in xylene, and embedded in paraffin. Eight to 10 tissue sections of 5  $\mu\text{m}$  in thickness were prepared, stained with hematoxylin and eosin, and examined. Histopathological changes that were recorded included alveolar edema, intraalveolar hemorrhage, leukocyte infiltration and disruption, and congestion. Alveolar edema and congestion were scored on a scale from 0 to 3, where 0 = absence of pathology (<5% maximum pathology), 1 = mild (<10%), 2 = moderate (15%–20%), and 3 = severe (20%–25%). Leukocyte infiltration was examined to identify the severity of inflammation that resulted from contusion. Each section was divided into 10 subsections, and leukocytic infiltration was examined in each of subsections at a magnification of 400 $\times$  with the following scale: 0, no extravascular leukocytes; 1, <10 leukocytes; 2, 10–45 leukocytes; and 3, >45 leukocytes (13).

#### 2.6. Statistical analysis

Data were analyzed with SPSS 15.0. Distributions of the groups were evaluated with one-sample Kolmogorov–Smirnov tests. Biochemical results displayed normal distribution. One-way ANOVA was performed and post hoc multiple comparisons were done with the least significant difference test. Histopathological results were evaluated by Kruskal–Wallis test and Mann–Whitney U-test. Results were presented as means  $\pm$  SD. P-values of less than 0.05 were regarded as statistically significant.

### 3. Results

Isolated contusion-induced lung injury increased the level of NO and SOD activity and decreased GSH-Px activity. We were able to demonstrate that administration of vitamin E for 3 days significantly decreased SOD activity and NO level and increased GSH-Px activity ( $P < 0.05$ ) in Group 4 when compared to Group 3. The details of the measure of decline and increase according to the consecutive days when analysis was performed are given in Table 1. On day 1, both for serum and tissue, no difference was detected in NO levels and SOD and GSH-Px activity between Groups 3 and 4 ( $P > 0.05$ ). However, forthcoming days clearly showed improvement in NO levels and GSH-Px and SOD activity. NO levels and SOD activity decreased and GSH-Px activity increased on day 2 and results were statistically significantly different between Groups 4 and 3 ( $P < 0.05$ ). On day 3, no significant difference was detected in serum and tissue NO levels or GSH-Px and SOD activities between Groups 4 and 1 ( $P > 0.05$ ).

Table 2 shows detailed blood gas analysis in groups correlated with experimental days. Lung injury impairs

blood gas values, as seen in Group 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 differences were statistically significant ( $P < 0.05$ ). On day 1, no difference in blood gas values was found between Groups 3 and 4, but the pH,  $pO_2$ , and  $HCO_3^-$  levels were decreased and  $pCO_2$  was increased in Group 4 compared with Group 1 ( $P < 0.05$ ). The next day, there was a significant difference between Groups 3 and 4 in pH and  $pO_2$ , but  $HCO_3^-$  and  $pCO_2$  remained stable. On day 3, no difference was detected between Groups 4 and 1 ( $P > 0.05$ ).

Serum  $Zn^{+2}$ ,  $Cu^{+2}$ , and  $Fe^{+3}$  levels were stable between Groups 1 and 2. Contusion alone decreased  $Zn^{+2}$  and increased  $Cu^{+2}$  and  $Fe^{+3}$  serum levels. The stabilization and normalization of the serum  $Zn^{+2}$ ,  $Cu^{+2}$ , and  $Fe^{+3}$  levels are all illustrated in Table 3. Vitamin E administration after contusion statistically significantly restored  $Zn^{+2}$ ,  $Cu^{+2}$ , and  $Fe^{+3}$  levels when Group 3 was compared to Group 4 ( $P < 0.05$ ).

Both bronchoalveolar lavage inflammatory cell count and histopathological examination score revealed no

**Table 1.** Tissue and serum nitric oxide (NO) levels and superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities in pulmonary contusion injury in rats (n = 7 and mean  $\pm$  SD).

	SOD (tissue) (U/mg)	NO (tissue) ( $\mu$ M/mg)	GSH-Px (tissue) (nmol min <sup>-1</sup> mg <sup>-1</sup> )	SOD (serum) (U/mL)	NO (serum) ( $\mu$ M/mL)	GSH-Px (serum) (nmol min <sup>-1</sup> mL <sup>-1</sup> )
Group 1	152 $\pm$ 13	48 $\pm$ 11	52 $\pm$ 11	56 $\pm$ 14	22 $\pm$ 12	41 $\pm$ 13
Group 2	149 $\pm$ 16	45 $\pm$ 13	56 $\pm$ 13	53 $\pm$ 17	26 $\pm$ 14	44 $\pm$ 16
Group 3						
Day 0	386 $\pm$ 12	154 $\pm$ 22	22 $\pm$ 12	241 $\pm$ 13	61 $\pm$ 17	11 $\pm$ 12
Day 1	331 $\pm$ 14	151 $\pm$ 13	24 $\pm$ 11	231 $\pm$ 14	58 $\pm$ 14	18 $\pm$ 11
Day 2	320 $\pm$ 11	132 $\pm$ 17	29 $\pm$ 16	207 $\pm$ 23	51 $\pm$ 12	20 $\pm$ 14
Day 3	303 $\pm$ 18	120 $\pm$ 11	36 $\pm$ 14	194 $\pm$ 12	42 $\pm$ 19	21 $\pm$ 11
Group 4						
Day 1	312 $\pm$ 12	135 $\pm$ 14	27 $\pm$ 11	217 $\pm$ 21	54 $\pm$ 12	21 $\pm$ 19
Day 2	221 $\pm$ 19	92 $\pm$ 12	40 $\pm$ 13	153 $\pm$ 22	38 $\pm$ 11	30 $\pm$ 11
Day 3	161 $\pm$ 14	52 $\pm$ 17	48 $\pm$ 16	62 $\pm$ 11	26 $\pm$ 11	38 $\pm$ 11
P-values						
Day 0, Groups 1 & 2	ns	ns	ns	ns	ns	ns
Day 0, Groups 3 & 1	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
Day 1, Groups 3 & 4	ns	ns	ns	ns	ns	ns
Day 1, Groups 4 & 1	0.0001	0.0001	0.0001	0.002	0.003	0.003
Day 2, Groups 3 & 4	0.002	0.002	0.004	0.003	0.005	0.001
Day 2, Groups 4 & 1	0.005	0.004	0.003	0.004	0.001	0.003
Day 3, Groups 3 & 4	0.001	0.003	0.002	0.002	0.002	0.001
Day 3, Groups 4 & 1	ns	ns	ns	ns	ns	ns

**Table 2.** Mean values and standard deviations of blood pH, pO<sub>2</sub>, pCO<sub>2</sub>, and HCO<sub>3</sub><sup>-</sup> in pulmonary contusion injury in rats (n = 7 and mean ± SD).

	pH	pO <sub>2</sub>	pCO <sub>2</sub>	HCO <sub>3</sub> <sup>-</sup>
Group 1	7.3 ± 0.16	85.1 ± 2.4	31.9 ± 4.1	23.7 ± 1.6
Group 2	7.3 ± 0.11	86.7 ± 2.9	32.4 ± 3.7	24.2 ± 2.7
Group 3				
Day 0	6.8 ± 0.24	45.2 ± 1.7	53.2 ± 4.3	11.4 ± 1.2
Day 1	6.9 ± 0.29	51.9 ± 1.9	52.5 ± 2.1	14.3 ± 2.6
Day 2	7.0 ± 0.19	50.8 ± 2.4	50.7 ± 6.5	16.1 ± 3.4
Day 3	7.1 ± 0.28	53.9 ± 1.2	46.1 ± 9.7	17.2 ± 4.8
Group 4				
Day 1	7.0 ± 0.28	54.6 ± 1.3	51.3 ± 1.9	13.1 ± 2.4
Day 2	7.2 ± 0.28	67.7 ± 0.9	45.8 ± 3.8	18.2 ± 2.3
Day 3	7.3 ± 0.27	83.9 ± 0.9	35.9 ± 2.1	21.5 ± 3.6
P-values				
Day 0, Groups 1 & 2	ns	ns	ns	ns
Day 0, Groups 3 & 1	0.0001	0.0001	0.0001	0.0001
Day 1, Groups 3 & 4	ns	ns	ns	ns
Day 1, Groups 4 & 1	0.003	0.0001	0.0001	0.0001
Day 2, Groups 3 & 4	0.004	0.001	ns	ns
Day 2, Groups 4 & 1	0.003	0.004	0.005	0.002
Day 3, Groups 3 & 4	0.002	0.003	0.001	0.002
Day 3, Groups 4 & 1	ns	ns	ns	ns

**Table 3.** The effects of vitamin E administration on serum Cu<sup>+2</sup>, Zn<sup>+2</sup>, and Fe<sup>+3</sup> levels in pulmonary contusion injury in rats (n = 7 and mean ± SD).

	Zn <sup>+2</sup> (µg/dL)	Cu <sup>+2</sup> (µg/dL)	Fe <sup>+3</sup> (µg/dL)
Group 1	168.2 ± 2.1	78.4 ± 1.6	14.8 ± 1.8
Group 2	162.7 ± 1.6	79.7 ± 2.1	12.7 ± 1.6
Group 3			
Day 0	87.6 ± 4.6	196.3 ± 2.3	39.1 ± 2.1
Day 1	96.4 ± 2.1	150.7 ± 3.1	31 ± 3.4
Day 2	109.2 ± 3.1	139.2 ± 4.2	29 ± 1.9
Day 3	76.9 ± 4.2	130.9 ± 1.3	27 ± 3.1
Group 4			
Day 1	99.3 ± 3.2	143.7 ± 4.3	29 ± 2.9
Day 2	118.9 ± 2.3	99.1 ± 2.9	26 ± 3.4
Day 3	156.4 ± 2.8	86.2 ± 2.7	17 ± 2.7
P-values			
Day 0, Groups 1 & 2	ns	ns	ns
Day 0, Groups 3 & 1	0.0001	0.0001	0.0001
Day 1, Groups 3 & 4	ns	ns	ns
Day 1, Groups 4 & 1	0.001	0.002	0.004
Day 2, Groups 3 & 4	ns	ns	ns
Day 2, Groups 4 & 1	0.002	0.001	0.001
Day 3, Groups 3 & 4	0.001	0.002	0.003
Day 3, Groups 4 & 1	ns	ns	ns

difference between Groups 1 and 2 ( $P > 0.05$ ). However, pulmonary contusion statistically significantly increased the cell count and the score when compared to Groups 3 and 1 ( $P < 0.05$ ). Consecutive days showed statistically significantly reduced inflammation in the vitamin E group compared with the nontreated contused group ( $P < 0.05$ ). Moreover, on the last day, no difference was noted between Groups 4 and 1 ( $P > 0.05$ ) (Table 4). Representative photomicrographs from lungs of the 4 groups are shown in Figures 1A–1D.

#### 4. Discussion

Pulmonary contusion is to be expected in cases where lung tissue has been affected by blunt thoracic trauma. One of the most important consequences of pulmonary contusion is acute respiratory distress syndrome (ARDS). The rate of pulmonary contusion in blunt thoracic trauma cases is 82% and the ARDS rate in pulmonary contusion cases is 20% (6). Pathophysiological and physiological mechanisms in isolated lung contusion injury cases could significantly improve the diagnosis, prognosis, and treatment of this

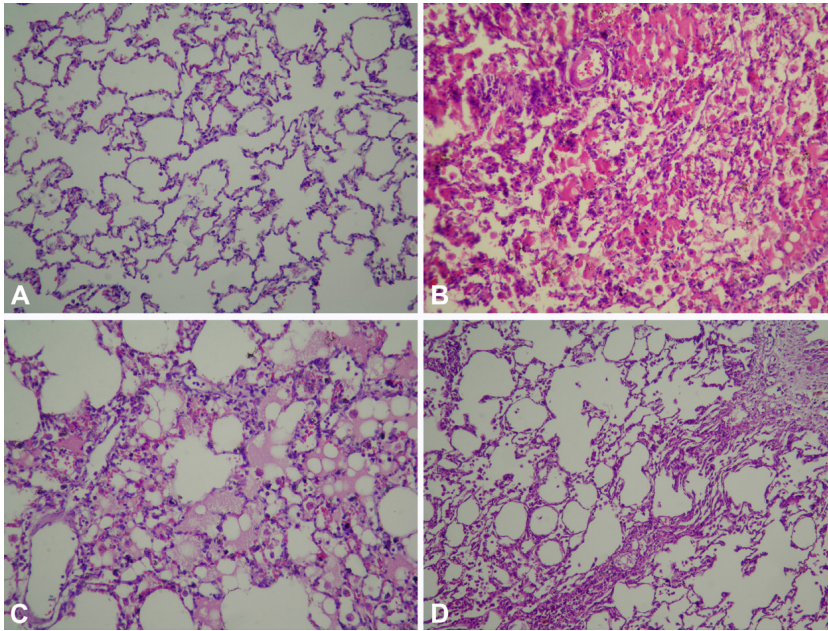
important condition. Some of the pathological changes in blunt thoracic trauma patient's lungs include increased alveolocapillary permeability and pulmonary edema, inflammation, increased intrapulmonary shunting, loss of compliance, and ventilation/perfusion mismatching.

In this study, we present a rat model of lung contusion caused by blunt thoracic trauma; we investigated lung oxidative stress and serum  $\text{Cu}^{+2}$ ,  $\text{Zn}^{+2}$ , and  $\text{Fe}^{+3}$  levels after pulmonary contusion with and without vitamin E administration. The probability that vitamin E has an ameliorative effect in lung injury and disease has spurred interest in determining its specific molecular functions and whether these are related to its antioxidant effects. This study was performed to examine vitamin E's potential to reduce oxidative stress-mediated toxicity with isolated pulmonary contusion in rats.

ROS have one or more unpaired electrons. Antioxidant reserves help reduce oxidative stress. Scavenging antioxidants that remove ROS, thus preserving free radical chain reactions, are part of the antioxidant defense mechanism. They are composed of: 1) enzymes, e.g., SOD,

**Table 4.** The values of pathologic and bronchoalveolar lavage (BAL) scores in pulmonary contusion injury in rats ( $n = 7$  and mean  $\pm$  SD).

	Alveolar edema and congestion	Leukocyte infiltration	BAL score
Group 1	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
Group 2	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00
Group 3			
Day 0	2.46 $\pm$ 0.32	2.41 $\pm$ 0.25	1.86 $\pm$ 0.12
Day 1	2.34 $\pm$ 0.23	2.29 $\pm$ 0.18	1.69 $\pm$ 0.23
Day 2	2.23 $\pm$ 0.12	2.23 $\pm$ 0.19	1.62 $\pm$ 0.19
Day 3	2.19 $\pm$ 0.19	2.14 $\pm$ 0.32	1.57 $\pm$ 0.17
Group 4			
Day 1	1.86 $\pm$ 0.16	1.63 $\pm$ 0.23	1.12 $\pm$ 0.13
Day 2	1.21 $\pm$ 0.29	1.21 $\pm$ 0.21	0.51 $\pm$ 0.13
Day 3	0.29 $\pm$ 0.12	0.31 $\pm$ 0.11	0.18 $\pm$ 0.11
P-values			
Day 0, Groups 1 & 2	ns	ns	ns
Day 0, Groups 3 & 1	0.0001	0.0001	0.0001
Day 1, Groups 3 & 4	0.003	0.002	0.001
Day 1, Groups 4 & 1	0.003	0.003	0.002
Day 2, Groups 3 & 4	0.002	0.002	0.003
Day 2, Groups 4 & 1	0.004	0.001	0.001
Day 3, Groups 3 & 4	0.003	0.002	0.002
Day 3, Groups 4 & 1	ns	ns	ns



**Figure 1.** **a)** Histological examination of lung tissue of Group 1. Normal pulmonary histology was observed (H&E, 200×). **b)** Histological examination of lung tissue of Group 3 on day 0. Extensive congestion, edema, hemorrhage, and leukocyte infiltration are shown (H&E, 200×). **c)** Histological examination of lung tissue of Group 4 on day 1. A moderate reduction of the contusion-induced histopathological changes is seen in the rat lung (H&E, 200×). **d)** Histological examination of lung tissue of Group 4 on day 3. Congestion, edema, hemorrhage, and leukocyte infiltration are observed in a very reduced manner (H&E, 200×).

GSH-Px, and CAT (9,10); 2) lipophilic and hydrophilic low-molecular-weight antioxidants, e.g., glutathione (GSH), ascorbate (vitamin C),  $\alpha$ -tocopherol (vitamin E), bilirubin, uric acid, carotenoids (vitamin A), and flavonoids (14); 3) repair enzymes that repair or remove ROS-damaged biomolecules, which include DNA repair enzymes and methionine sulfoxide reductase. Among the lipid-soluble antioxidants, vitamin E ( $\alpha$ -tocopherol) seems to play the most important factor as it prevents LPO by donating hydrogen to superoxide radicals (15,16).

Generally, toxic oxygen metabolites are produced at a low level in lung cells by the transfer of a single electron in aerobic metabolism (17). ROS have an integral effect in the modulation of some physiological functions but can also be have a negative effect if produced in excessive amounts (18). On the other hand, reactive nitrogen species, including nitric oxide, nitrite, and peroxynitrite ( $\text{ONOO}^-$ ), are physiologically necessary and also potentially devastating. Isolated contusion-induced lung injuries increased the levels of NO and SOD activity but decreased GSH-Px activity. We were able to demonstrate that administration of vitamin E for 3 days significantly decreased SOD activity and NO level and increased GSH-Px activity ( $P < 0.05$ ) in Group 4 when compared to Group 3.

Inflammation is also oxygen-mediated. During inflammation, leukocytes, macrophages, and mast cells release mediators that are responsible for bronchoconstriction and edema after trauma. This study demonstrated that pulmonary contusion statistically significantly increased cell counts and scores when comparing Groups 3 and 1 ( $P < 0.05$ ). Consecutive days showed statistically significantly reduced inflammation in the vitamin E-administrated group.

Such antioxidants are said to be scavengers, as their acts are unavoidably suicidal. For instance, vitamin E ( $\alpha$ -tocopherol) is a membrane-bound antioxidant that works by terminating the chain reaction of lipid peroxidase by scavenging lipid peroxy radicals ( $\text{LOO}\cdot$ ) (14,19). Vitamin E becomes a radical in this reaction; however, it is much less reactive than  $\text{LOO}\cdot$ . On the other hand, the radical form of vitamin E may function as a prooxidant at high concentrations. The importance of vitamin E's prooxidant effect in vivo has not been shown yet. Vitamin E's prooxidant effect certainly diminishes in the presence of other coantioxidants, including ascorbic acid and ubiquinol, which is discussed in detail in the review by Upston et al. (20).

GSH is one of the main intracellular nonenzymatic antioxidants. It can detoxify toxic endogenous and exogenous substances, including free radicals and xenobiotics. Cyclophosphamide (CP) metabolism generates highly reactive electrophiles. Therefore, the decrease of GSH in a group treated by CP was probably caused by the electrophilic burden on the cells and the formation of acrolein that depletes GSH content. Vitamin E intake decreases the electrophilic burden and thereby increases GSH levels in the lungs. GST catalyzes conjugation of GSH with highly reactive electrophiles; therefore, it plays an important role in the detoxification of alkylating agents (21).

GSH-Px that contains selenium protects cells from ROS. In this study, GSH-Px activity increased significantly after vitamin E treatment. GSH has been shown to be an important component of the lung antioxidant defense system. GSH is not likely to function as a direct scavenger of  $H_2O_2$  but can be a cofactor for peroxidases such as the GSH-Px enzymes, because it reacts with peroxides (9,20,22,23). GSH-Px behaves in a manner opposite from other markers; after contusion, the GSH-Px level gradually decreases. However, vitamin E administration blocked the decrease starting from the second day of this experimental study.

SOD is present in all oxygen-metabolizing cells and its function is to provide a defense against the potentially damaging reactivities of superoxide and hydrogen peroxide. In this study, SOD activity was decreased significantly in the rats treated with vitamin E, but no significant changes were observed in the lung. Administration of vitamin E significantly elevated SOD activity, which suggests that it had the ability to restore the activities. NO is a highly reactive and short-lived free radical. NO is synthesized from L-arginine by the nitric oxide synthase enzyme. NO reacts spontaneously with

available superoxide radicals to form the more potent and versatile oxidant  $ONOO^-$  (24–26).

A relationship has been demonstrated between antioxidant defense mechanisms and some essential trace elements, which are needed in small concentrations as essential components of antioxidative enzymes. The cytoplasmic  $Cu^{+2}-Zn^{+2}$  SOD enzyme contains  $Cu^{+2}$  and  $Zn^{+2}$  metals as cofactors and the GSH-Px enzyme contains  $Se^{-2}$ . As transition metals, free  $Fe^{+3}$  and  $Cu^{+2}$  participate in the generation of free radicals by catalyzing the transformation of  $H_2O_2$  to the highly reactive hydroxyl radical ( $\cdot OH^-$ ) via Fenton and Haber–Weiss reactions (17).

Pulmonary contusion results in impaired oxygenation (6–8). In our study, blood gas analysis in groups correlated with experimental days. Lung injury impairs blood gas values, as seen in Group 3. On day 0, pH,  $pO_2$ , and  $HCO_3^-$  were decreased and  $pCO_2$  was increased in the contused group compared to the noncontused group and differences were statistically significant ( $P < 0.05$ ). On day 1, blood gas values were same between the vitamin E and nontreated contusion groups. However, the pH,  $pO_2$ , and  $HCO_3^-$  levels were decreased and  $pCO_2$  was increased in the vitamin E contusion group compared to Group 1 ( $P < 0.05$ ). Normalization in blood gases in the vitamin E group was achieved on day 3. On day 3, no difference was detected between Group 4 and Group 1 ( $P > 0.05$ ).

It was significant to determine the concentration of serum  $Zn^{+2}$ ,  $Cu^{+2}$ , and  $Fe^{+3}$  after lung injury. The data showed lung injury alone decreased serum  $Zn^{+2}$  and increased serum  $Cu^{+2}$  and  $Fe^{+3}$ . After contusion, vitamin E restored serum  $Zn^{+2}$ ,  $Cu^{+2}$ , and  $Fe^{+3}$  levels when Group 3 was compared to Group 4 ( $P < 0.05$ ).

Our study shows that elevation of antioxidant vitamin E to levels that can scavenge  $O_2$  may produce beneficial effects by regulating antioxidant enzyme systems in blunt thoracic trauma.

## References

1. Brigelius FR, Traber MG. Vitamin E: function and metabolism. *FASEB J* 1999; 13: 1145–1155.
2. Bunyan J, McHale D, Green J, Marcinkiewicz S. Biological potencies of  $\epsilon$ - and  $\zeta$ 1-tocopherol and 5-methyltolcol. *Br J Nutr* 1961; 15: 253–257.
3. Stampfer MJ, Hennekens CH, Manson JE, Colditz GA, Rosner B, Willett WC. Vitamin E consumption and the risk of coronary disease in women. *N Engl J Med* 1993; 328: 1444–1449.
4. Rimm EB, Stampfer MJ, Ascherio A, Giovannucci E, Colditz GA, Willett WC. Vitamin E consumption and the risk of coronary heart disease in men. *N Engl J Med* 1993; 328: 1450–1456.
5. Azzi A. Molecular mechanism of alpha-tocopherol action. *Free Radic Biol Med* 2007; 43: 16–21.
6. Cohn SM, Zieg PM. Experimental pulmonary contusion: review of the literature and description of a new porcine model. *J Trauma* 1996; 41: 565–571.
7. Raghavendran K, Davidson BA, Helinski JD, Marshcke CJ, Manderscheid P, Woytash JA, Notter RH, Knight PR. A rat model for isolated bilateral lung contusion from blunt chest trauma. *Anesth Analg* 2005; 101: 1482–1489.
8. Ward PA. Acute lung injury: how the lung inflammatory response works. *Eur Respir J Suppl* 2003; 44: 22s–23s.
9. Fridovich I. The biology of oxygen radicals. *Science* 1978; 201: 875–880.
10. Sies H. Oxidative stress: from basic research to clinical application. *Am J Med* 1991; 91: 31–38.



11. 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. *Anesth Analg* 2005; 101: 1482–1489.
12. Lowry OH, Rosebrough NJ, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem* 1951; 182: 265–268.
13. Calikoglu M, Tamer L, Sucu N, Coskun B, Ercan B, Gul A, Calikoglu I, Kanik A. The effects of caffeic acid phenethyl ester on tissue damage in lung after hindlimb ischemia-reperfusion. *Pharmacol Res* 2003; 48: 397–403.
14. Sharma MK, Buettner GR. Interaction of vitamin C and vitamin E during free radical stress in plasma: an ESR study. *Free Radic Biol Med* 1993; 14: 649–653.
15. Özkaya D, Naziroğlu M, Armağan A, Demirel A, Köroglu BK, Çolakoğlu N, Kükner A, Sönmez TT. Dietary vitamin C and E modulates oxidative stress induced-kidney and lens injury in diabetic aged male rats through modulating glucose homeostasis and antioxidant systems. *Cell Biochem Funct* 2011; 29: 287–293.
16. Kayan M, Naziroğlu M, Celik O, Yalman K, Köylü H. Vitamin C and E combination modulates oxidative stress induced by X-ray in blood of smoker and nonsmoker radiology technicians. *Cell Biochem Funct* 2009; 27: 424–429.
17. Kocyigit A, Erel O, Gur S. Effects of tobacco smoking on plasma selenium, zinc, copper and iron concentrations and related antioxidative enzyme activities. *Clin Biochem* 2001; 34: 629–633.
18. Gutteridge JM. Lipid peroxidation and antioxidant as biomarkers of tissue damage. *Clin Chem* 1995; 41: 1819–1828.
19. Radi R, Beckman JS, Bush KM, Freeman BA. Peroxynitrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. *Arch Biochem Biophys* 1991; 288: 481–487.
20. Upston JM, Terentis AC, Stocker R. Tocopherol-mediated peroxidation of lipoproteins: implications for vitamin E as a potential antiatherogenic supplement. *FASEB J* 1999; 13: 977–994.
21. Misso NL, Powers KA, Gillon RL, Stewart GA, Thompson PJ. Reduced platelet glutathione peroxidase activity and plasma selenium concentration in atopic asthmatic patients. *Clin Exp Allergy* 1996; 26: 838–847.
22. Touliaatos JS, Neitzel L, Whitworth C, Rybak LP, Malafa M. Effect of cisplatin on the expression of glutathione-S-transferase in the cochlea of the rat. *Eur Arch Otorhinolaryngol* 2000; 25: 6–9.
23. Ceylan BG, Naziroğlu M, Uğuz AC, Barak C, Erdem B, Yavuz L. Effects of vitamin C and E combination on element and oxidative stress levels in the blood of operative patients under desflurane anesthesia. *Biol Trace Elem Res* 2011; 141: 16–25.
24. Tassiopoulos AK, Carlin RE, Gao Y, Pedoto A, Finck CM, Landas SK, Tice DG, Marx W, Hakim TS, McGraw DJ. Role of nitric oxide and tumor necrosis factor on lung injury caused by ischemia/reperfusion of the lower extremities. *J Vasc Surg* 1997; 26: 647–656.
25. Barnes PJ, Belvisi MG. Nitric oxide and lung disease. *Thorax* 1993; 48: 1034–1043.
26. Kelm M. Nitric oxide metabolism and breakdown. *Biochim Biophys Acta* 1999; 1411: 273–289.