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Alcohol-Induced Lung Damage and Increased Oxidative Stress

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Key Words
Acute respiratory distress syndrome · Alcohol · Lung injury · Oxidative stress

Abstract
Background: Alcohol-induced lung damage may be associated with increased oxidative stress. ***Objective:*** Our aim was to investigate alcohol-induced changes in the biochemistry and histopathology of the lung. ***Methods:*** Rats were divided into two groups, a control group and an ethanol group. The ethanol group received 2 g/kg ethanol (total: 3 ml) intraperitoneally. The controls were given the same amount of saline via the same route. Three hours later, the rats were sacrificed, and blood and lung tissue samples were obtained. Oxidative stress was assessed by measuring the levels of erythrocyte reduced glutathione (GSH), tissue malondialdehyde (MDA), myeloperoxidase (MPO) and Na^+-K^+ ATPase. Histopathologic evaluation of the lung tissues was also performed. ***Results:*** In the ethanol group, serum and tissue MDA levels and MPO activities were increased ($p = 0.007$, $p = 0.001$ and $p = 0.000$), and lung tissue Na^+-K^+ ATPase activities and erythrocyte GSH were decreased ($p = 0.001$ and $p = 0.000$) compared to the controls. Histopathologic examination demonstrated alveolocapillary thickening, alveolar degeneration, leukocyte infiltration and erythrocyte extravasation in the lungs of the ethanol group ($p < 0.05$). ***Conclusion:*** These results suggest that high-dose acute alcohol administration aggravates systemic and local oxidative stress leading to acute lung injury, ranging from mild pulmonary dysfunction to severe lung injury. It should be borne in mind that rapid onset of the acute respiratory distress syndrome (ARDS) may also be due to increased oxidative stress following alcohol abuse, especially when ischemic disturbances, e.g. coronary heart disease, acute ischemia of the extremities and traumatic accidents, are concomitantly present. Therefore, precautions against ARDS may prevent morbidity and mortality in alcohol-induced lung damage in at-risk patients.

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Introduction

Alcohol is very well recognized as a systemic toxin, and it is one of the most common drugs of abuse worldwide [1–3]. It is capable of generating oxygen radicals, inhibiting glutathione synthesis, inducing reduced glutathione levels in the tissue, increasing malondialdehyde (MDA) levels and impairing antioxidant defense mechanisms in humans and experimental animals [4–6]. In addition, acetaldehyde, the metabolite of ethanol, is a very reactive molecule with a high affinity for sulfhydryl groups, enabling binding to a variety of intracellular proteins. It can impair enzyme functions by forming Schiff bases with sulfhydryl groups at the active sites of several enzymes. Acetaldehyde also enhances lipid peroxidation and inter-

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feres with protective responses in the cell, including interference with glutathione synthesis and reduction in the activity of free radical scavenging enzymes [7].

The lungs are one of the target organs most vulnerable to oxidative stress due to their unique structure and function [8]. We speculate that alterations induced by alcohol intake render the host susceptible to acute lung injury due to impaired protective responses, and we tested this hypothesis in rats treated intraperitoneally with ethanol and compared the results to those of controls.

Materials and Methods

Fourteen adult male Wistar rats (weighing 250–300 g) were used in this study. All animal procedures used were in strict accordance with the Helsinki Declaration. Approval of the Ethics Committee of the Mersin University School of Medicine was obtained before the study.

Study Design

The animals were anesthetized with intramuscular ketamine (100 mg kg^{-1}). They were divided into two groups (control and ethanol-treated rats). Rats in the ethanol group received 2 g/kg ethanol (3 ml) by an intraperitoneal injection (as described by Rodrigo et al. [9]). The control rats were given the same amount of 0.9% NaCl. After 3 h, under semisterile conditions, midsternotomy was performed in all animals. Blood (5 ml) was drawn from the ascending aorta for biochemical assays, and the lungs were harvested and fixed in 10% formalin for histopathological examination.

Analysis

Blood Ethanol Determination. Ethanol levels were determined enzymatically using alcohol dehydrogenase (Cobas integra 800 biochemical analyzer; Roche Diagnostics, Mannheim, Germany).

Erythrocyte Reduced Glutathione (GSH) Determination. Virtually all of the non-protein sulfhydryl compounds of red cells are in the form of GSH. 5,5′ Dithiobis (2-nitro benzoic acid) is a disulfide compound that is readily reduced by sulfhydryl compounds, forming a highly colored yellow anion. The optical density of this yellow substance is measured at 412 nm [10].

Measurement of MDA. As an index of lipid peroxidation, MDA levels in serum and homogenized tissues were determined using a thiobarbituric acid reaction according to Yagi [11]. The principle of the method depends on the measurement of the pink color produced by interaction of the barbituric acid with MDA elaborated as a result of lipid peroxidation. The colored reaction 1,1,3,3-tetraethoxypropane was used as a primary standard [10, 12].

Measurement of Myeloperoxidase (MPO) Activity. MPO is a haem-containing enzyme within the azurophil granules of neutrophils, and MPO activity was measured as a simple quantitative method detecting leukosequestration. The determination of serum MPO activity in the lung depends on the fact that it reduces *o*-dianazodine. Reduced *o*-dianozidine was measured at 410 nm by spectrophotometry [13].

Measurement of Na^+-K^+ ATPase Activity. Measurement of specific activity of Na^+-K^+ ATPase is based on the principle of the inorganic phosphate released (nmol P_i) in 1 h per milligram of protein in the presence of 3 m*M* disodium adenosine 5′-triphosphate (ATP) added to the incubation medium. The inorganic phosphate released from ATP to the incubation medium was measured according to the method suggested by Reading and Isbir [14]. The protein amount in the sample was determined according to the method developed by Lowry et al. [15]. Results of the ATPase enzyme system were given in nmol $P_i \cdot mg^{-1}$ protein $\cdot h^{-1}$.

Histopathological Examination

Lung tissues were fixed in 10% formalin, routinely processed for light-microscopic evaluation and embedded in paraffin. Sections (4 μm thick) were cut using a microtome and stained with hematoxylin and eosin to assess the oxidative changes in relation to the number of leukocytes. Slides were examined via an Olympus BX50 light microscope and photographed using an Olympus PM10SP photograph system. Leukocyte infiltration was evaluated to determine the severity of oxidative damage that resulted from ethanol intoxication. Each section was divided into 10 subsections, and leukocytic infiltration was assessed using the following scale for comparison at a magnification of × 400: 0 = no extravascular leukocytes; 1 = <10 leukocytes; 2 = 10–45 leukocytes, and 3 = >45 leukocytes.

Statistical Analysis

The differences in the biochemical results among the groups were analyzed by Student's t test using the Statistical Package for Social Sciences, version 9.0 (SPSS, Chicago, Ill., USA), and p values <0.05 were regarded as significant. The results are expressed as means ± SD. χ^2 and z-approximation tests were used to compare two independent results in histopathological studies.

Results

Biochemical Evaluations

In the ethanol group, blood ethanol levels amounted to 49.2 ± 3.7 m*M*. MDA levels in serum and lung tissue and serum MPO levels were significantly increased in the ethanol group compared to the controls. Na^+-K^+ ATPase activity in the lung tissue and erythrocyte GSH levels were significantly reduced in the ethanol group (table 1).

Histopathological Evaluation

Biochemical tests in both study groups demonstrated that only in the ethanol group median scores of peribronchial and perivascular leukocytic infiltration were increased. In the controls, median scores of leukocyte infiltration were mainly under 0 and 1, being 3 and 4 in the ethanol group. The difference between both groups was statistically significant (p = 0.001). The data are presented in table 2. The bronchiolar and normal alveolar structure was preserved in the control specimens (fig. 1), whereas degenerative alveolar structures, leukocytic infiltration and erythrocyte extravasation were readily observed in the lung tissue of the ethanol group (fig. 2).

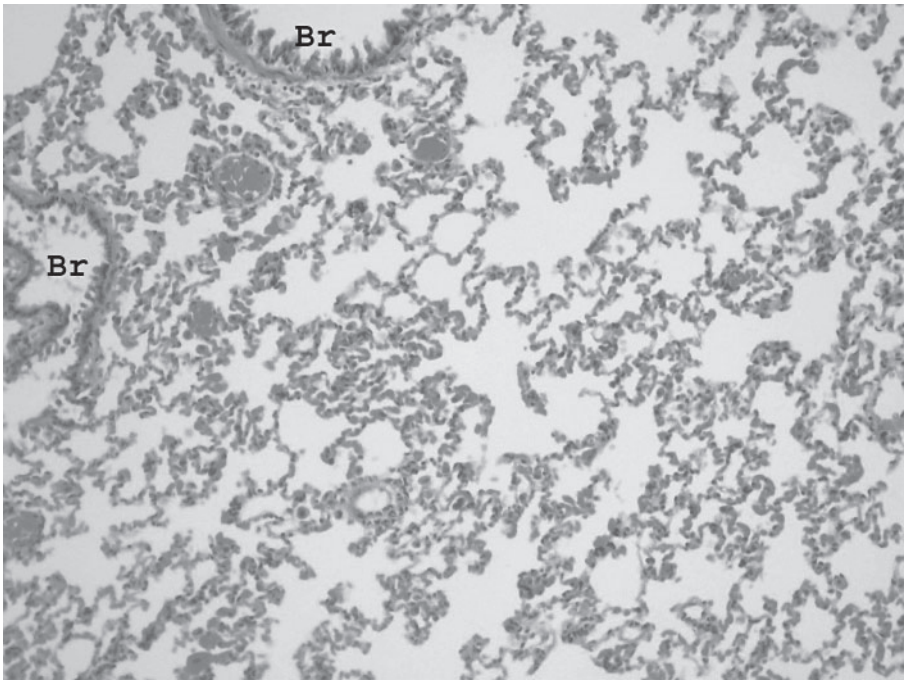

Fig. 1. Histopathologic findings in the lungs of the controls (HE. ×200). The bronchiolar and normal alveolar structure was preserved in the controls. Br = Bronchiole.

Table 1. Biochemical data of the control and ethanol groups

	MDA (lung) $nmol \cdot g^{-1}$	MDA (serum) $nmol \cdot ml^{-1}$	MPO (lung) $U \cdot mg^{-1}$ protein	ATPase (lung) nmol $P_i \cdot mg^{-1}$ protein $\cdot h^{-1}$	GSH (erythrocyte) μmol GSH/g Hb
Controls (n = 7)	7.2 ± 1.23	1.4 ± 0.51	1.37 ± 0.44	226.6 ± 22.64	6.24 ± 0.68
Ethanol (n = 7)	12.62 ± 2.01	4.68 ± 1.43	4.58 ± 0.71	158.6 ± 24.84	4 ± 0.5
p value	0.001	0.007	0.000	0.001	0.000

Differences were assessed between control and ethanol groups.

Discussion

Reactive oxygen species (ROS) are constantly produced in the cells, but under normal physiological conditions the enzymatic and non-enzymatic antioxidant mechanisms of the cell overcome the destructive potential of ROS. There is a delicate balance between the production of ROS and endogenous protection mechanisms. Overproduction of ROS or a decrease in antioxidants results in oxidative stress, e.g. in inflammation, ischemia-reperfusion injury and in the presence of catalytic iron ions. ROS formed may cause cellular damage by peroxidation of membrane lipids, sulfhydryl enzyme inactivation, protein cross-linking and DNA breakdown. This damage may be involved in the etiology of diverse human diseases, e.g. coronary heart disease, acute ischemia of the extremities, infection, neurodegenerative disease, cancer and ethanol intoxication [8, 16–20]. Sometimes a combination of these factors may coexist, i.e. ethanol intoxication and coronary artery disease and/or critical leg ischemia. Consequently, organ damage may further increase ROS production.

Table 2. Leukocyte infiltration scores of the control and ethanol groups

Group	Leukocyte infiltration score				
	0	1	2	3	total
Control rats	6 (86%)	1 (14%)	–	–	7 (100%)
Ethanol rats	–	1 (14%)	4 (57%)	2 (29%)	7 (100%)

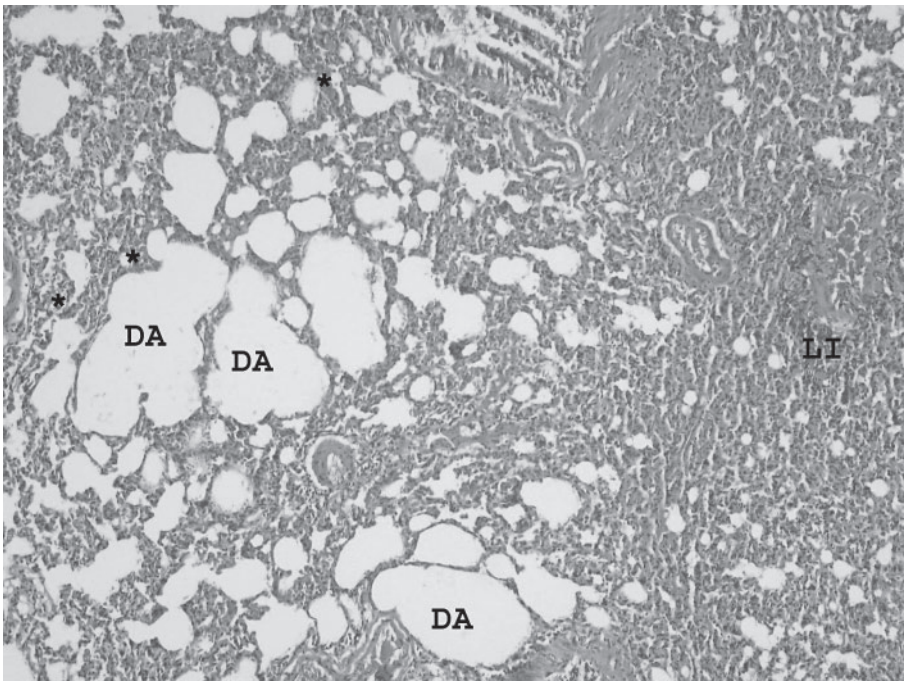

Fig. 2. Histopathologic findings in the lungs of the ethanol-treated rats (HE. ×200). Degenerative alveolar structures, leukocytic infiltration and erythrocyte extravasation were observed. DA = Degenerative alveolar structure; LI = leukocyte infiltration. Asterisks show erythrocyte extravasation.

This study demonstrated that high-dose ethanol increased oxidative stress and also resulted in impaired function of endothelial cells reflected by increased vascular permeability, neutrophil infiltration and hemorrhage in the lung. These histopathological findings were corroborated by increased tissue MDA and MPO activity, increased serum MDA, decreased erythrocyte GSH levels and decreased Na^+-K^+ ATPase activity in the lung.

The data presented indicate that acute ethanol intoxication induces different effects on rat sera and indicators of lung injury related to oxidative stress as well as tissue morphology. Ethanol-induced oxidative stress in the lung and sera can be assessed on the basis of both enzymatic and non-enzymatic pathways for ROS clearance. Ethanol-induced erythrocyte GSH depletion may render lipids more susceptible to ROS attacks. In addition, the ethanol-induced fatty acid ethyl esters (the end products of a non-oxidative pathway for ethanol metabolism) play a role in the development of alcohol-related injury to the lung [21]. This view is supported by the significant increase in lung and serum lipid peroxidation indicators found in the ethanol group. Ethanol-induced tissue damage occurs in a variety of organs, including the liver, where ethanol is actively oxidized, and in extrahepatic tissues of the rats, such as the central nervous system, heart and testes, at least in vitro. These findings lead to the assumption that ethanol may likewise induce oxidative stress in a variety of organs in vivo, too [21–25].

Impaired structure and function of the plasma membrane have long been considered as a major cause of tissue injury, which is reflected by the Na^+-K^+ ATPase activity [26]. Increased lipid peroxidation combined with decreased tissue Na^+-K^+ ATPase activity may be associated with an impairment in the interaction of Na^+-K^+ ATPase with membrane phospholipids [27, 28]. In addition, the contribution of oxidative modifications of thiol groups of the enzyme itself may be of importance, as well as the direct inhibitory effect of ethanol reported in vitro [29]. Therefore, ethanol may alter the regulation of the activity of Na^+-K^+ ATPase, an enzyme that participates in lung fluid clearance by exerting the active transport of sodium. If this process persists, the decreased enzyme activity will give rise to the disintegration of the cells and consequently to the thickening of the air-blood barrier, alveolar degeneration, erythrocyte extravasation and leukocyte infiltration, as observed histopathologically in the lungs of the ethanol rats (fig. 1).

ROS, which prevails due to the decreased antioxidant defense mechanisms following ethanol intake, stimulate the release and the formation of various inflammatory mediators with powerful chemotactic potential. These mediators lead to leukocyte activation, expression of endothelial adhesion molecules and vascular endothelial

damage [30]. MPO reflects the activity of neutrophils, and hence we studied the MPO levels in the lung tissue and found that they were higher in the ethanol group ($p = 0.000$).

The association between alcohol abuse and the acute respiratory distress syndrome (ARDS) was first reported by Moss et al. [31], who demonstrated that chronic alcohol abuse in humans independently increases the incidence of ARDS in at-risk patients, and is associated with increased mortality due to multiorgan failure. As was seen in humans, chronic ethanol ingestion increased endotoxin-mediated acute edematous injury in rat lungs isolated and perfused ex vivo [32]. In addition, ethanol increased sepsis-mediated lung dysfunction in vivo [6]. These pathophysiological events were associated with the decrease in GSH, which is a very important antioxidant in the lung. Furthermore, Rodrigo et al. [9] have shown that acute ethanol intoxication gives rise to oxidative damage with GSH depletion and lipid peroxidation, in agreement with our study.

In agreement with previous studies, our results suggest that high-dose acute alcohol administration appears to significantly aggravate systemic and local oxidative stress and lead to acute lung injury. Therefore, in the presence of high-dose alcohol intake, onset of ARDS might be accelerated and, in case of accompanying ischemic conditions such as coronary heart disease, acute ischemia of the extremities, traffic or occupational traumatic accidents, precautions regarding the prevention of ARDS might be helpful in decreasing morbidity and mortality.

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