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World J Gastroenterol 2006 June 14; 12(22): 3593-3596
World Journal of Gastroenterology ISSN 1007-9327

CLINICAL RESEARCH

Effect of abdominal trauma on hemorrhagic shock-induced acute lung injury in rats

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Received: 2006-02-08 Accepted: 2006-02-28

Abstract

AIM: To evaluate the effects of abdominal trauma on hemorrhagic shock-induced acute lung injury in rats.

METHODS: Five groups were allocated (n = 8) in the study. Group I was taken as the control group, group II as the hemorrhagic shock group, group III as hemorrhagic shock + laparotomy, group IV as hemorrhagic shock + splenectomy and group V as splenectomy + omentectomy + hemorrhagic shock group. Hemorrhagic shock was induced by drawing blood and reducing mean arterial pressure (MAP) to 40 mmHg within 10 min. After a hypotensive period of 1 h, animals were resuscitated. Bronchoalveolar lavage (BAL) was performed to recover cells from the alveolar space with 40 mL of BAL fluid after resuscitation malondialdehyde (MDA) and L-γ-glutamyl-L-cysteinyl-glycine (GSH) levels were measured in serum, erythrocytes and lung tissue.

RESULTS: Serum, erythrocyte, lung tissue MDA and GSH levels were significantly increased in hemorrhagic shock groups II-V (P < 0.05). Lymphocyte, neutrophil and alveolar macrophage counts in BAL fluid indicated a significant difference between control and shock groups (P < 0.05).

CONCLUSION: The degree of trauma increases hemorrhagic shock-induced acute lung injury.

Key words: Hemorrhagic shock; Acute lung injury; Abdominal trauma

Kilicoglu B, Eroglu E, Kilicoglu SS, Kismet K, Eroglu F. Effect of abdominal trauma on hemorrhagic shock-induced acute lung injury in rats. *World J Gastroenterol* 2006; 12(22): 3593-3596

http://www.wjgnet.com/1007-9327/12/3593.asp

INTRODUCTION

Acute respiratory distress syndrome (ARDS) and acute lung injury (ALI) frequently occur after major trauma with a high mortality[1,2]. Clinically, acute lung injury is characterized by altered gas exchange, dyspnea, decreased static compliance, and nonhydrostatic pulmonary edema[3]. Firmly implicated mediators are endotoxin, cytokines, and products of arachidonic acid metabolism, leukocyte-derived proteolytic enzymes, and toxic oxygen species. The basic mechanisms of lung injury are focused on oxidant-mediated damage[4]. Recent evidence supports that leukocyte activation and the subsequent release of large quantities of highly reactive oxygen radicals and hydrogen peroxide are partially responsible for diffuse microvascular and tissue injury in patients with hemorrhagic shock. It has been shown that neutrophils in trauma patients release greater amounts of superoxide anion, proteases, and proinflamatory cytokine[5]. In addition to neutrophils, alveolar macrophages and circulating macrophages as well as endothelial cells aggravate lung injury and alveolar neutrophil sequestration[6]. In response to various inflammatory stimuli, lung endothelial cells, alveolar cells, airway epithelial cells, and alveolar macrophages, produce both nitric oxide and superoxide[7].

To address these issues we designed a trauma model of controlled hemorrhagic shock. Free oxygen radical metabolism of serum, erythrocyte, lung tissue and cellular change in alveolar epithelium was investigated in this model, but the main objective was to understand the role of abdominal injury in hemorrhagic shock.

MATERIALS AND METHODS

Animals

Adult male Wistar Albino rats weighing 243-325 g were used in experiments. Animals were housed and kept at 25℃ in a 12 h light/dark cycle and used in studies after an acclimatization period of 7 d. Rats were allowed to have

Table 1 Serum, erythrocyte, tissue MDA and GSH levels, and lymphocyte, neutrophil, alveolar macrophage counts in BAL fluid of different groups (mean ± SD)

	Group I	Group II	Group III	Group IV	Group V
Serum MDA (μmol/L)	1.374 ± 0.24[a]	2.095 ± 0.246	2.54 ± 0.18	3.04 ± 0.12	3.57 ± 0.23[c]
Serum GSH (μmol/L)	29.005 ± 4.14	15.374 ± 4.369	11.149 ± 1.71	8.240 ± 2.65	5.055 ± 0.86
Erythrocyte MDA (μmol/L)	411.048 ± 31.61[a]	665.676 ± 173.609	723.287 ± 89.0	792.28 ± 235.6	900.28 ± 254.9[c]
Erythrocyte GSH (μmol/L)	74.145 ± 7.35	52.702 ± 10.725	52.412 ± 9.684	46.831 ± 6.408	27.852 ± 10.438
Tissue MDA (μmol/L)	11.721 ± 1.79[a]	16.651 ± 3.702	17.315 ± 1.485	18.178 ± 2.364	20.327 ± 5.786[c]
Tissue GSH (μmol/L)	0.318 ± 0.053	0.170 ± 0.056	0.101 ± 0.020	0.082 ± 0.001	0.019 ± 0.001
Lymphocyte/100 cells	0.0 ± 0.0	0.5 ± 1.07	1.0 ± 0.0	2.75 ± 0.46	3.5 ± 1.07
Neutrophil/100 cells	2.88 ± 0.35	1.88 ± 1.36	2.63 ± 0.74	3.38 ± 0.74	4.75 ± 0.89
Alveolar macrophage/100 cells	1.38 ± 0.52	4.0 ± 2.27	4.57 ± 1.58	3.25 ± 0.71	3.63 ± 0.52

[a]$P < 0.05$ *vs* hemorrhagic shock groups; [c]$P < 0.05$ *vs* other groups.

free access to rodent chow and water before experiment. All animals were maintained in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals. The Süleyman Demirel University Medical School Animal Use and Care Committee approved all experiments. In the experiments, forty rats were used and allocated into five groups ($n = 8$). Group I was taken as the control group, group II as the hemorrhagic shock group, group III as hemorrhagic shock + laparotomy group, group IV as splenectomy + hemorrhagic group, and group V as splenectomy + omentectomy + hemorrhagic shock group.

Methods

Animals were intraperitoneally anesthetized with 80 mg/kg ketamine and 8 mg/kg xylazine. The right carotid artery was cannulated with a 24-gauge angiocathether for monitoring the mean arterial pressure (MAP), blood sampling, and resuscitation. Hemorrhagic shock was induced by drawing blood and reducing MAP to 40 mmHg for 10 min. Blood was collected into 0.1 mL citrate to prevent clotting. After a hypotensive period of 1 h, animals were resuscitated by transfusion of blood and Ringer lactate in a volume equal to that of blood. After a period of two hours blood samples were taken *via* carotid artery, and the catheter was removed with the artery ligated. The cervical incision was extended towards midline to isolate trachea. Trachea was catheterized with a feeding tube (No: 8) and stabilized with 4/0 silk suture to perform bronchoalveolar lavage (BAL). BAL was performed to recover cells from the alveolar space with 40 mL of BAL fluid. Lung tissue was resected to measure tissue malondialdehyde (MDA) and L-γ-glutamyl-L-cysteinyl-glycine (GSH) levels after bronchoalveolar lavage. Control animals underwent the same procedures, but hemorrhage was not induced.

In groups III-V, additional surgical procedures were added. In group III (hemorrhagic shock + laparotomy) after hemorrhagic shock was maintained, a midline incision in 2 cm length was performed on the abdominal wall. The incision was sutured with 4/0 silk suture. In group IV (hemorrhagic shock + laparotomy + splenectomy) after laparotomy splenectomy was performed with its vessels ligated, laparotomy incision was sutured as in group III. In group V (hemorrhagic shock + laparotomy + splenectomy + omentectomy) omentectomy was added. All vessel boundaries were ligated when the omentum was removed.

BAL fluid and its analysis

The lungs were lavaged using the feeding tube with BAL fluid containing cold phosphate buffered saline (PBS), 8 mmol/L sodium phosphate, 2 mmol/L potassium phosphate, 0.14 mol/L sodium chloride, and 0.01 mol/L potassium chloride (pH7.4) and 0.1 mmol/L ethylenedia minetetraacetic acid (EDTA). Forty mL of BAL solution was used for lavage in each animal, and about 90% was re-collected. The fluid was centrifuged at 700 r/min for 15 min. Supernatant was collected and re-suspended in 1 mL of the same solution. Two slides were prepared from each sample, and stained with Wright-Giemsa stain. A total number of 100 cells were counted in each slide with immersion objective (100 ×) and the number of neutrophils, lymphocytes and alveolar macrophages was counted respectively.

MDA and GSH levels in serum, erythrocyte and lung tissue

Hemolysate and serum of the blood specimens were prepared and stored at -70℃ until assay. The lung specimens were homogenized with a mechanic homogenizator (Ultra-Turrax T25) and sonicated (Bandelin D.12207) in phosphate buffered saline (pH7.4). The homogenate was centrifuged at 10000 r/min for 15 min at 4℃ and supernatant was used for determination of the MDA and GSH concentrations. MDA, an end product of lipid peroxidation, was assayed by the method of Drapper and Hadley[8]. GSH levels were determinated by the method of Beutler *et al*[9].

Statistical analysis

All data were analyzed by one-way ANOVA with *post hoc* test Bonferroni. Data were expressed as mean ± SD. $P \leqslant 0.05$ was considered statistically significant.

RESULTS

There was no difference in MAP values before and after hemorrhagic shock between the groups ($P > 0.05$). Serum, erythrocyte and lung tissue MDA levels were significantly higher in hemorrhagic shock groups (groups II-V) than in control group ($P < 0.05$, Table 1). MDA levels were also significantly different in groups II-V. Serum MDA

levels had a trend to increase with hemorrhagic shock and additional surgical injury ($P < 0.05$). The same trend could be seen in erythrocyte and lung tissue MDA levels ($P < 0.05$, Table 1).

Serum, erythrocyte and lung tissue GSH values were significantly lower in groups II-V than in control group ($P < 0.05$, Table 1). All GSH levels were decreased especially in group V ($P < 0.05$), but the difference in groups II-IV was not significant ($P > 0.05$). The decreased serum GSH levels were parallel to the severity of trauma, but the difference in groups II-V was not statistically significant ($P > 0.05$, Table 1). Similar GSH levels in lung tissue were obtained. Tissue GSH level in group V was significantly lower than that in other groups ($P < 0.05$).

Lymphocyte, neutrophil and alveolar macrophage counts in BAL fluid indicated a significant difference between groups ($P < 0.05$, Table 1). The highest lymphocyte and neutrophil counts were found in group V. Although there was no significant difference in group II-V, lymphocyte, neutrophil and alveolar macrophage counts were significantly higher in groups II-V than in control group.

DISCUSSION

Many mediators of acute lung injury in animal models and ARDS in human beings have been implicated as causative factors. Selection of agents that can abrogate these mediators is based on the efficacy of these agents in whole animal models, and their relative potency and potential toxicity. Through examination of metabolic processes, GSH has been shown to be important in host defenses against oxidative stress[3]. Another important agent showing oxidative stress is MDA, a marker of free radical activity[3]. After ischemia-reperfusion, lipid peroxides induce a substantial drop in intracellular GSH levels[10,11]. In this study, hemorrhagic shock and resuscitation significantly elevated serum, erythrocyte and lung tissue MDA levels ($P < 0.05$). GSH levels were significantly lower in hemorrhagic shock groups than in control group ($P < 0.05$). It was reported that oxidative stress significantly elevated MDA levels and reduced GSH levels[12,13]. These significant alterations were also seen in our study. MDA levels were significantly higher in group V (laparotomy + splenectomy + omentectomy) than in other groups ($P < 0.05$). Serum GSH levels of erythrocyte and lung tissue were also altered in hemorrhagic shock groups ($P < 0.05$). All these findings support the hypothesis that ischemia-reperfusion affects free oxygen radical metabolism and additional surgical trauma increases this effect. Lipid peroxidation seems the main mechanism of free radical toxicity and results in alterations of structural integrity and function of cellular membranes[14]. Intracellular overproduction is not the only mechanism of free radical-induced lung injury[15]. Hypoxia can damage alveolar macrophages, which may release chemotactic and activating factors for polymorphonuclear neutrophils[16]. The release of toxic oxygen may result in endothelial injury, which is an early event in the course of oxygen-induced lung damage.

Neutrophils sequestered in the lung play a central role in the pathogenesis of ARDS. Lung polymorphonuclear neutrophile accumulation in ARDS occurs as a result of a cascade of cellular events initiated by either infectious or noninfectious inflammatory stimuli[17]. Neutrophil and lymphocyte counts were significantly increased in BAL fluid of hemorrhagic shock groups in this study ($P < 0.05$). There was also a significant difference between these groups. The count of these cells increased significantly with the degree of surgical trauma ($P < 0.05$). Alveolar macrophage count was similarly increased in BAL fluid in shock groups ($P < 0.05$), the difference between these groups was not significant. There was no relation between the degree of trauma and alveolar macrophage count increase ($P > 0.05$). These observations can be explained with the findings in the studies of Jorens *et al*[18] and Bernard *et al*[19]. Acute lung injury in ARDS is largely due to activated neutrophils, which not only aggravate oxidative stress in the lungs but also release mediators, including cytokines and lipids.

In conclusion, oxidative stress and surgery increase free oxygen radicals in serum, erythrocytes and lung tissue and lead to epithelial injury in the lungs. The observed injury seems related with the degree of trauma.

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S- Editor Pan BR **L- Editor** Wang XL **E- Editor** Bi L