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Research Paper

Effects of pentoxifylline on oxidative stress in rats with abdominal compartment syndrome model

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ABSTRACT

Background: Abdominal compartment syndrome (ACS) causes severe pathology in the cardiovascular, renal and pulmonary systems. Recent studies showed that pentoxifylline (PTX) has effects on increasing tissue oxygenation, healing capillary refill and reducing superoxides and hydroxyl radicals by inhibiting xanthine oxidase. In this study, our aim was to study the effects of PTX on free oxygen radicals and oxidative damage in rats with ACS model.

Materials and methods: ACS model was created in 32 male Wistar-Albino-rats, which were randomized into one of the four study groups: Group A (n:8), having ACS; Group B (n:8), having ACS and receiving PTX (50 mg/kg/day) intraperitoneal for 10 days; Group C (n:8), receiving PTX (50 mg/kg/day) intraperitoneal for 10 days without having ACS; Group D (n:8), having no ACS and not receiving PTX. On the 11th day blood samples were collected to measure alanine-amino-acid-transferase (ALT) and aspartate-amino-acid-transferase (AST) in the heart, malondialdehyde (MDA), myeloperoxidase (MPO) and glutathione (GSH) in the liver, lung and small bowel. Histopathologic injury scoring was done.

Results: Groups were compared in pairs. Group A compared to Group B: ALT increase, liver MDA, lung GSH and MPO decrease were statistically meaningful in Group B. Group A compared to Group C: ALT and liver MPO decrease and liver MDA increase were statistically meaningful in Group A. Group B compared to Group C: ALT increase, MDA and GSH decrease in the lung were statistically meaningful in Group B. Group B compared to Group D: ALT and MPO increase in the small bowel and MPO decrease in the lung were statistically meaningful in Group B. Group A had the highest histopathologic injury scoring.

Conclusion: Histopathologically confirmed pentoxifylline was effective in the treatment of ACS in these rat models.

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1. Introduction

Abdominal compartment syndrome (ACS) is an independent marker for mortality and is associated with an increased rate of multiple organ dysfunction. ACS is defined as increased intra-abdominal pressure equal to or more than 20 mmHg [1]. The pathophysiology of ACS is directly related to the increased intraabdominal pressure (IAP). Increased IAP compresses the thoracic cavity through the diaphragm and reduces ventilation. Increased IAP compresses the vena cava and decreases the cardiac output by decreasing venous return. Decrease in cardiac output and venous return cause decreased kidney, splenic and liver perfusion. This multi-organ hypoperfusion causes pathologies as systemic inflammatory response and adult respiratory distress syndrome [2–6]. IAP may increase with various reasons like trauma, abdominal surgery, laparoscopic surgery, over fluid replacement, severe retroperitoneal hemorrhage, acute renal failure, acute pancreatitis, retroperitoneal malignancies, and massive ascites [6,7].

Ischemia is defined as insufficient arrive or interruption of blood flow to certain tissues or organs [8]. Hypoxia-related tissue damage occurs. Prolonged ischemia impairs the structural integrity of cells and cell death may occur [8,9]. Reperfusion is tissue restoration of blood flow [8,10]. Previous studies reported that severity of the tissue

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injury varies according to the duration and grade of the ischemia [11,12]. Tissue injury occurs in the ischemic period and also in the reperfusion period. When ischemic tissue blood flow is resupplied, free oxygen radicals are released from polymorphonuclear leukocytes in the tissue, and tissue damage is exacerbated [8,10,13]. Ischemia/ reperfusion damage is not only seen in the affected tissue, but also in other organs due to systemic inflammatory response [14,15]. To limit the potential damage of free oxygen radicals, enzymatic and non-enzymatic antioxidants are released from the cells.

Pentoxifylline is a methlxanthine derivative drug, similar to theophylline. Pentoxifylline shows its effect by inhibiting phosphodiesterase enzymes and this increases the cyclic AMP on polymorph nuclear leukocytes and decreases the free oxygen radical production [16]. It is commonly used to improve microvascular circulation in patients with vascular insufficiency, because it reduces platelet aggregation [17]. Recent studies showed that pentoxifylline inhibits xanthine oxidase, thus reduces superoxide (SO) and hydroxyl (OH) radicals, heals capillary circulation and tissue oxygenation. Besides it also inhibits free oxygen radicals and Phospholipase A2, which increases prostacyclin release [17,18].

Harmful physiological changes that occur as a result of ACS clinically affect most cardiovascular, renal and pulmonary systems. Reduction in cardiac output, increase in peripheral resistance, oliguria, anuria and hypoxia may occur. Fatal organ failure may occur in untreated cases. ACS working model experimentally carried out is not possible except for animal.

We designed an animal model of ACS to study the effects of a vasodilating agent, pentoxifylline, on the treatment of oxidative damage caused by ACS.

2. Methods

This study was approved by the Animal Experiments Local Ethics Committee of İstanbul University (Process number 2014/101). The experiments were performed in adherence with the international guidelines for the care and use of laboratory animals at the Laboratory of Surgical Physiopathology.

The test subjects were randomly enrolled in one of the 4 study groups. The rats were fasted before the surgery night. Rats were subsequently anesthetized with ketamine hydrochloride 50 mg/ml and Xylazine hydrochloride 20 mg/ml given intraperitoneally in a dosage of 0.1 ml/100 g. Following this, the rats were fixed in the supine position on a regularly disinfected and surgically draped operating table. Model of ACS was stimulated by insertion of a 16 Gauge cannula under sterile conditions into the peritoneal cavity and insufflation of 20 mmHg CO2 for two hours. Ten days after this procedure the rats were sacrificed. Blood samples were taken from the heart apex for ALT and AST, and tissue samples were taken from the lung, liver and small bowel.

Group A (n:8), having ACS. The rats were fasted before the surgery night. Rats were subsequently anesthetized with ketamine hydrochloride 50 mg/ml and Xylazine hydrochloride 20 mg/ml given intraperitoneally in a dosage of 0.1 ml/100 g. Following this, the rats were fixed in the supine position on a regularly disinfected and surgically draped operating table. Model of ACS was stimulated by insertion of a 16 Gauge cannula under sterile conditions into the peritoneal cavity and insufflation of 20 mmHg CO2 for two hours.

Group B (n:8), having ACS and receiving pentoxifylline (50 mg/kg/day) intraperitoneal for 10 days. The rats were fasted overnight prior to surgery, and were subsequently anesthetized with ketamine hydrochloride 50 mg/ml and xylazine hydrochloride 20 mg/ml given intraperitoneally in a dosage of 0.1 ml/100 g. Following this, the rats were fixed in the supine position on a regularly disinfected and surgically draped operating table. Model of ACS was stimulated by insertion of a 16 Gauge cannula under sterile conditions into the peritoneal cavity and insufflation of 20 mmHg CO2 for two hours. After desufflation of the peritoneal cavity, pentoxifylline was given (50 mg/kg/day) in one cc of 9% NaCl intraperitoneal for 10 days.

Group C (n:8), receiving pentoxifylline (50 mg/kg/day) intraperitoneal for 10 days without having ACS. The rats were fasted overnight prior to surgery, and were subsequently anesthetized with ketamine hydrochloride 50 mg/ml and xylazine hydrochloride 20 mg/ml given intraperitoneally in a dosage of 0.1 ml/100 g. Following this, pentoxifylline was given (50 mg/kg/day) in one cc of 9% NaCl intraperitoneal for 10 days.

Group D (n:8) having no ACS and not receiving pentoxifylline was the sham group. The rats were fasted overnight prior to surgery, and were subsequently anesthetized with ketamine hydrochloride 50 mg/ml and xylazine hydrochloride 20 mg/ml given intraperitoneally in a dosage of 0.1 ml/100 g.

32 Male Wistar rats (250–300 g) were purchased from the İstanbul University, Institute of Experimental Medicine. Male rats were preferred, because menstrual cycle may have affected the blood test results.

All rats were stored in metal cages and maintained in 12-hour dark/light cycle at a controlled temperature of 22 °C (±1). All rats were fed with 21% protein food and fresh tap water, only rats to get IAP will be unfed one day before. All cages were cleaned daily, 4 rats were put in a cage and the rats that were in the same group were stored together.

All rats were sacrificed on the 11th day with high dose ketamine and blood samples were collected to measure alanine-amino-acid-transferase (ALT) and aspartate-amino-acid-transferase (AST) in the heart, malondialdehyde (MDA) and glutathione (GSH) in the liver, lung and small bowel. Histopathologic injury scoring was done in the liver, lung and small bowel. All tissues were put in a cryotube into the deep freezer at –80 °C after being washed at +4 °C phosphate buffer saline (PBS) and stored until the biochemical analysis. Blood samples were taken from the heart apex while the heart rate is presented and centrifuged at 3000–35,000 rpm for 15 minutes in the dry yellow cap biochemistry tube. All samples of serum obtained from each animal were put into three separate eppendorf tubes and kept into the deep freezer at –80 °C again until the biochemical analysis. MDA and GSH levels were used as oxidative stress and MPO activity was used as inflammatory parameters.

2.1. ALT and AST analyses

ALT and AST analyses were measured with cobas 8000 e602 modular automatic analyzer (Roche Diagnostics, Mannheim, Germany).

2.2. Glutathione (GSH) analysis

Dithiobisnitrobenzoic acid and GSH sulfhydryl (-SH) group makes a reaction. The product of this reaction is yellow. For one mole -SH group, one mole dithiobisnitrobenzoic acid forms and this product has an absorbance at 412 nm at UV spectrophotometer. After ten minutes of waiting at room temperature GSH activity was calculated according to the formula described in the manufacturer's protocol.

2.3. Myeloperoxidase (MPO) analysis

Tissue homogenites were made with 1 ml of cold hexdecyltrimethly ammonium bromide buffer (50 mM KPO+ and 0.5% hexdecyltrimethly ammonium bromide [pH 6.0]). Homogenites were sonicated on ice for 10 seconds, and centrifuged at 10,000 rpm for 15 min at 4 °C. Twenty ml of supernatant solution was transferred into well plate, and 200 µl of O-dianisidine hydrochloride solution at 37 °C (16.7 mg O-dianisidine, 100 ml: 90% water and 10% 50 mM KPO4 buffer + 0.0005% H^2O^2) was added. Immediately after

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