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The protective effects of ozone therapy in a rat model of acetaminophen-induced liver injury

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ABSTRACT

Objectives: Acetaminophen (APAP) overdose may cause acute liver injury. Ozone therapy (OT) is shown to reduce inflammation and necrosis in several entities. Thus, we have designed this study to evaluate the efficacy of OT in a rat model of APAP-induced liver injury.

Methods: Twenty-seven Sprague-Dawley rats were divided into three groups: sham, APAP and APAP+OT groups. In the APAP and the APAP+OT groups, liver injury was induced by oral administration of 1 g/kg APAP. The APAP+OT group received a single dose ozone/oxygen mixture (0.7 mg/kg) intraperitoneally 1 h after APAP administration. All animals were killed at 24 hour after APAP administration. Blood samples and liver tissues were harvested to determine liver injury and oxidative stress parameters. Liver tissues and blood samples were obtained for biochemical and histopathological analyses.

Results: APAP administration caused necrosis in the liver after 24 h. The degrees of liver necrosis of the APAP group were higher than the other groups (in both $p < 0.05$, respectively). In the APAP+OT group, liver antioxidant enzymes activities were significantly higher than the APAP group ($p < 0.05$), but were lower than the sham group ($p < 0.05$). In the sham group, serum neopterin, a marker of cell-mediated immunity, concentrations (4.8 ± 1.2 nmol/L) were lower than the APAP (14.7 ± 1.4 nmol/L) and APAP+OT groups (7.5 ± 2.4 nmol/L) (in both $p < 0.05$, respectively).

Conclusion: Our results showed that OT prevented liver necrosis in rats and reduced neopterin levels. These findings suggest that the use of OT as an adjuvant therapy which might improve the outcome in APAP induced liver injury.

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1. Introduction

Acetaminophen (N-acetyl-p-aminophenol, paracetamol, APAP) is a widely used agent for its analgesic and antipyretic actions. Therapeutic doses of APAP of approximately 4 g daily are considered to be safe while overdose produces severe centrilobular liver injury that can lead to fatal fulminant hepatic failure (Yaman et al., 2011). APAP is eliminated almost entirely as nontoxic glucuronic acid and sulfate conjugates when used at therapeutic doses (Pacifici et al., 1988). However, a small proportion of APAP is converted to N-acetyl-p-benzoquinoneimine (NAPQI) by cytochrome P-450 mediated oxidation. This forms NAPQI, which is normally detoxified by conjugation with reduced glutathione (GSH) (Nelson, 1995). At exposure to high dose APAP, much more NAPQI is formed; subsequently the hepatic GSH stores are consumed. Eventually, the remaining NAPQI binds to cellular macromolecules covalently and leads to cell death (Jollow et al., 1973).

Elevated serum alanine aminotrasferase (ALT) and aspartate aminotrasferase (AST) activities indicate liver damage (Bruss et al., 2004). Necrosis of the liver usually results in leakage of AST and ALT from the hepatocellular membrane into the bloodstream. Although the elevated activities of these enzymes are indicators of hepatocellular damage, they are poor prognostic indicators for the severity of the liver injury or acute liver failure (Huang et al., 2008). Recently, it was shown that serum neopterin, indicator of the liver injury, levels were elevated in a rat model of APAP induced liver injury and that the increased neopterin levels were correlated with the dose of APAP (Demirbas et al., 2011). Thus, serum neopterin levels are regarded as a better prognostic marker for liver injury than the activities of aminotransferases in APAP toxicity.

Administration of a gas mixture comprising ozone/oxygen (O_3/O_2) is known as ozone-therapy (OT) (Bocci et al., 2011). O_3/O_2 mixture exhibits various effects on the immune system, such as the modulation of phagocytic activity of peritoneal and alveolar macrophages (Bocci, 2004, 2006a). So far, clinical studies have shown that OT appears useful in conditions ranging from peritonitis, to infected wounds, chronic skin ulcers, initial gangrene, burns and advanced ischemic diseases (Re et al., 2008). Administration of ozone induced a sort of cross-tolerance to free radicals released after hepatic and renal ischemia-reperfusion injury in experimental studies (Ajamieh et al., 2002; Chen et al., 2008). It was also demonstrated that ozone increased antioxidant enzyme activities, such as glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT), preparing the host to face with physiopathological conditions mediated by reactive oxygen species (ROS) (Bocci, 1996, 2006a).

Our group recently reported that OT had an ameliorative effect in reducing oxidative stress in caustic esophageal burn, necrotizing enterocolitis, methotrexate induced intestinal injury and acute necrotizing pancreatitis in experimental rat models (Guven et al., 2008, 2009; Kesik et al., 2009; Uysal et al., 2010). In our previous study, it was also shown that OT had an ameliorative effect on APAP induced renal injury (Demirbag et al., 2010), although APAP hepatic overdose is known to cause especially acute liver injury rather than renal injury. Therefore, we designed this experimental study to evaluate the efficacy of OT in the liver injury caused by APAP overdose in rats.

2. Methods

2.1. Animals

Adult male Sprague-Dawley rats (Health Sciences Institute, Gulhane Military Medical Academy, Ankara, Turkey), weighing 200–250 g each, were used in our study. They were randomly assigned into three groups containing nine rats each: sham, APAP and APAP+OT groups. Before the experiment, all animals were fed standard rat chow and water ad libitum and were kept in an air-conditioned room at 21 °C, with a 12 h:12 h light:dark cycle and were handled humanely, in accordance with the European Union Directive 609/86 for care and use of laboratory animals. Animals were fasted for 12 h before APAP treatment. This project was approved by the Experimental Ethics Committee of Gulhane Military Medical Academy, Ankara, Turkey.

2.2. Surgery and experimental protocol

The acetaminophen (APAP) and APAP+OT groups of animals were given as single dose 1 g/kg body weight of APAP (Ordu Ilac Fabrikasi, Ankara, Turkey) suspended in hot distilled water was administered by gastric tube. Rats in the sham group received distilled water by gastric tube. All animals were sacrificed, under light diethyl ether anesthesia, at time points of 24 h after APAP treatment. Whole blood was drawn from the heart. The abdomen was opened, and livers were removed and cleaned. Liver tissue samples were stored in 10% formalin solution for histological analysis. The remaining liver tissues were immediately frozen in liquid nitrogen and stored in a deep freezer at −80 °C until all assays.

2.3. Ozone treatment

OT was performed immediately after the induction of liver injury. After 1 h the induction of liver injury, the rats in the APAP+OT group were administrated O_3/O_2 mixture at a dose of 0.7 mg/kg via intraperitoneal route. O_3 was generated by the ozone generator (OZONOSAN Photonik 1014, Hansler GmbH, Nordring 8, Iffezheim, Germany), allowing control of the gas flow rate and O_3 concentration in real time by a built-in UV spectrometer. The O_3 flow rate was kept constant at 3 L/min representing concentration of 60 mg/mL and gas mixture of 97% O_2 + 3% O_3. Tygon polymer tubes and single-use silicon-treated polypropylene syringes (ozone resistant) were used throughout the reaction to ensure containment of O_3 and consistency of concentrations.

2.4. Serum and tissue preparations

Whole blood samples were collected into tubes without anticoagulant. The serum fraction was obtained by centrifugation (2000 × *g*, 10 min, 4 °C) after storing the whole blood at room temperature for ∼1 h. All sera were stored at −80 °C until