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RESEARCH ARTICLE

Protective role of taurine against hepatotoxicity induced by pyrazinamide in rats

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ABSTRACT

Background: Pyrazinamide is a widely used antituberculosis drug. However, associated with its clinical use, hepatotoxicity is a life-threating side effect reported in some patients, but the exact mechanism by which pyrazinamide induces hepatotoxicity is not clear yet. **Aims and Objectives:** The present investigation was conducted to study the exact mechanism of subchronic toxicity induced by pyrazinamide and protective role of taurine in rats. **Materials and Methods:** Markers such as alanine transaminase (ALT) and aspartate aminotransferase (AST) levels, lipid peroxidation, reactive oxygen species (ROS) formation, hepatocytes glutathione (GSH) content, and apoptosis were examined. Furthermore, pathological changes were evaluated. **Results:** The results showed that pyrazinamide administration caused hepatotoxicity as revealed by elevation in ALT and AST levels. Pyrazinamide increased ROS generation and malondialdehyde derivative levels, and also, it reduced intracellular GSH contents. Pyrazinamide induced apoptosis in rats liver tissue. **Discussion:** Administration of taurine effectively decreased the intensity of hepatotoxicity induced by pyrazinamide in rats.

KEY WORDS: Taurine; Pyrazinamide; Oxidative Stress; Hepatotoxicity; Apoptosis

INTRODUCTION

Hepatotoxicity is a major cause for drug withdrawal in clinical use. For the reason that strategic situation of liver in the body. It is constantly exposed to drugs and other toxins.[1] Over thousands of drugs have been implicated in causing liver injury at least on the rare incident.[2,3] Pyrazinamide is a potent antimycobacterium tuberculosis. It is used from1956. However, hepatotoxicity is a serious adverse reaction reported in some patients. The beginning of liver damage due to this drug is usually after 4–8 weeks.[4]

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The previous study showed that pyrazinamide was associated with an acute liver injury that can be severe and even fatal.[5] The result of another study showed coadministration of rifampin and pyrazinamide for 2 months inducing severe liver injury that was fatal.[6]

Taurine, a natural aminosulfhonic acid that is present in many mammalian tissues such as skeletal muscle, brain, retina, heart. and platelet, has several physiological roles including membrane stabilization, osmoregulation and cytoprotective effects, antioxidant and anti-inflammatory actions as well as modulation of intracellular calcium concentration and ion channel function.[7] Variety of experiments has been showed that taurine has protective effects against different drugs and

toxins induced hepatotoxicity. In many studies, it was reported that taurine could act as a potent antioxidant in biological systems.[2,7-14] Consequently, the hepatoprotective effects of taurine against different toxins - induced hepatotoxicity could be due to the antioxidant ability of this amino acid. Furthermore, taurine has the ability to scavenge the reactive oxygen species (ROS), diminish lipid peroxidation, and therefore, stabilize biological membranes.[2,14] This study was designed to evaluate the cytotoxic mechanism(s) of pyrazinamide in rat's liver, and also, the protective effects of taurine on this toxicity were investigated. Aminotransferase levels, ROS generation, malondialdehyde (MDA) derivatives levels, and reduced glutathione (GSH) levels were monitored as toxicity markers also pathological changes and apoptosis were evaluated.

MATERIALS AND METHODS

Chemicals and Materials

Pyrazinamide and taurine were purchased from Sigma-Aldrich Chemical Co. 5-5 dithionitrobenzoic acid and thiobarbituric acid and Trichloroacetic acid were purchased from Merck Chemical Co. The kit for alanine transaminase (ALT) and aspartate aminotransferase (AST) analysis was obtained from Pars Azmon Company (Tehran-Iran). The kit for apoptosis was purchased from Roche, and ROS generation was purchased from Co-Mybiosource. All salts were purchased from Merck Chemical Co.

Animals

Male Sprague–Dawley rats with 250–300 g weight were kept in ventilated plastic cages with environmental temperature 21–23°C and 50–60% relative humidity and with 12 h darkness photoperiod. Animals were fed a normal chow diet and water *ad libitum*. The animals were handled and used according the animal handling protocol that approved by a local Ethics Ccommittee in Gorgan University of Medical Sciences, Gorgan, Iran.

Hepatotoxicity Study

Animals were randomly divided equally into four groups of seven animals for 4 weeks. In the normal control group, normal rats were orally administered 1 ml/kg (of body weight) of saline daily. In the PZA group, normal rats were orally administered 1 ml/kg of 500 mg/kg PZA in saline daily. In the PZA and taurine group, normal rats were orally administered 1 ml/kg of 500 mg/kg taurine and 1 ml/kg of 500 mg/kg PZA in saline. In the taurine group, normal rats were orally administered 1 ml/kg of 500 mg/kg PZA in saline daily. Rats were treated with 1 ml/kg of 500 mg/kg PZA in saline daily, which was reported as a hepatotoxic dose of pyrazinamide in previous investigation.[6]

Serum Biochemical Analysis

Blood was collected from the abdominal vena cava under ketamine and xylazine anesthesia, and the liver was removed. The blood was allowed to clot at 25°C and serum was prepared by centrifugation (1000 g for 20 min). Serum ALT and AST levels were measured with a commercial kit.[15]

Histopathological Evaluation

For histopathological evaluation, samples of liver were fixed in formalin (10%). Paraffin-embedded section of liver was prepared and stained with hematoxyline and eosin (H and E) before light microscope viewing. A pathologist, who was blind to the treatment groups and the corresponding liver biochemistries, assessed liver histology.

Liver GSH Content

The excised livers were immediately frozen at −70°C and analyzed for GSH within 24 h. Briefly, samples of liver (200 mg) were homogenized in 8ml of 20 mM EDTA. The GSH contents were assessed by determination of non-protein sulfydryl contents with the Ellman reagents.[16]

Lipid Peroxidation

MDA derivative levels were detected by measuring thiobarbituric acid reactive substances (TBARS) that were formed during the peroxidation of polyunsaturated fatty acid. Briefly, the liver was removed and homogenized with cold KC1 (1.15%) to make a 10% homogenate. Then, 3 ml of phosphoric acid 1% and 1 ml of TBA 0.6% aqueous solution were added to 0.5 ml of 10% homogenate tissue. The mixture was heated for 45 min in a boiling water bath. After cooling, 4 ml of n-butanol was added and mixed vigorously. The n-butanol phase was separated by centrifugation and absorbance was measured at 535 nm using an Ultrospec 2000 UV spectrophotometer.[17]

ROS Formation

ROS was determined by Elisa kit (Mybiosource Co., product no MBS 039665) according to the kit protocol.

Apoptotic Cells in Liver

Animals anesthetized with ketamine and xylazine and their livers were withdrawn, and then, the sections were prepared. The sections were examined for apoptotic cells by the terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) method, using an *in situ* cell death detection kit (Roche, product no11684817910), according to the manufacturers protocol. A photograph of each section was produced using a microscope (BX53, Olympus, Tokyo) and a DP73 digital camera under a magnification of 400. To measure the area density of the apoptotic cells, the images were transferred on to a computer. Using OLYSIA cell sense

software (Olympus), then the cells were counted manually statistical analysis.

Results are shown as a mean ± standard error of the mean of at least three independent experiments. Comparisons between multiple groups were determined using one-way analysis of variance followed by Tukey's *post hoc* test. The minimal level of significant differences was considered statistically significant when $P < 0.05$.[18]

RESULTS

Histological Study

Rats were treated with PZA at a dose of 1 ml/kg of 500 mg/kg PZA in saline over 4 weeks which was reported as a hepatotoxic dose in previous study histologically,[6] and when animals were treated with pyrazinamide, the extensive necrosis of liver paranchymal cells and inflammatory cells infiltration was occurred [Figure 1].

Biochemical Results

When toxicants stimulate parenchymal damage, hepatocytes die because of apoptosis or necrosis. Subsequent to initial changed membrane permeability or final cell disruption that accompanies necrotic death, cytosolic enzymes such as ALT and AST are released into the blood serum ALT and AST levels were measured after PZA administration.[19] Plasma ALT and AST levels were elevated in rats treated with PZA as compared with the control group. This indicates the hepatotoxic effects of PZA. Then, the hepatotoxic effect of PZA was investigated in taurine-treated animals. Taurine (1 ml/kg of 500 mg/kg/day) administration diminished PZA-induced ALT and AST elevation in animals effectively ($P < 0.05$).

Lipid Peroxidation Test

The probability of lipid peroxidation in liver tissue was evaluated. Our data showed that PZA-induced lipid peroxidation in rat hepatocytes and the amount of TBARS increased greatly. Treatment of rats with taurine prevented TBARS production drastically ($P < 0.05$) [Figure 2].

GSH Levels

Hepatic GSH content was assessed to study the toxic effects of PZA on hepatic GSH reservoirs. According to Figure 3, PZA caused a significant reduction in cellular GSH content as compared with control group. When rats were treated with taurine, GSH content was notably increased ($P < 0.05$).

ROS Generation

As Figure 4 illustrates, pyrazinamide caused an increase in ROS formation in liver tissue and orally administration of taurine, decreased ROS generation ($P < 0.05$).

Apoptosis

Furthermore, our data showed that the number of apoptotic cells in treated groups with pyrazinamide was more than the control group drastically, but there are no statistically significant differences between control groups and the other experimental groups that received taurine [Figure 5].

DISCUSSION

The aim of this study was to investigate the exact mechanisms of PZA-induced liver injury and the protective role of taurine. Our results showed that PZA-induced hepatotoxicity represents a state of increased oxidative stress, which is interfered with the evidence of motivating ROS generation in rat's liver.

In previous finding, it was documented that oxidative stress is linked with the pathogenesis of various types of drug-induced

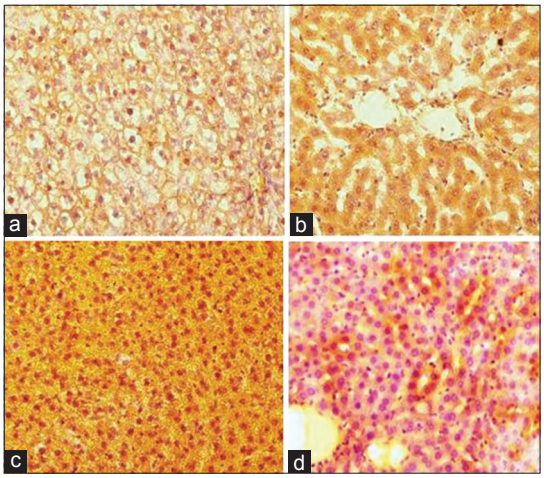

Figure 1: Histopathological evaluation of rat's liver treated with pyrazinamide. Hematoxyline and eosin staining (H and E). (a) Control, (b) pyrazinamide-treated group, (c) pyrazinamide + taurine group, (d) taurine

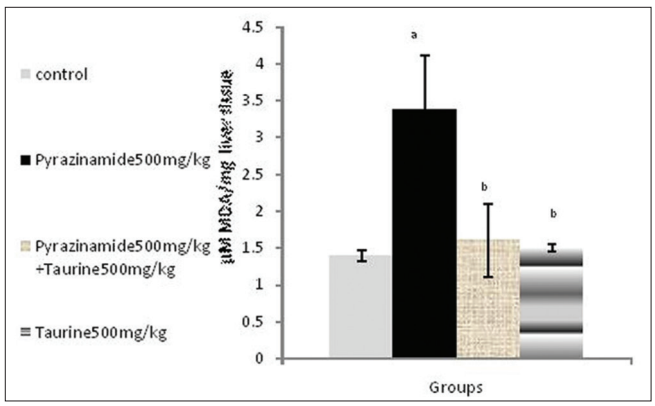

Figure 2: Effect of pyrazinamide alone and with taurine in the MDA level of rats. Values are expressed as the mean ± SED. [a]$P < 0.05$, significant difference from control; [b]$P < 0.05$, significant difference from pyrazinamide-treated rats

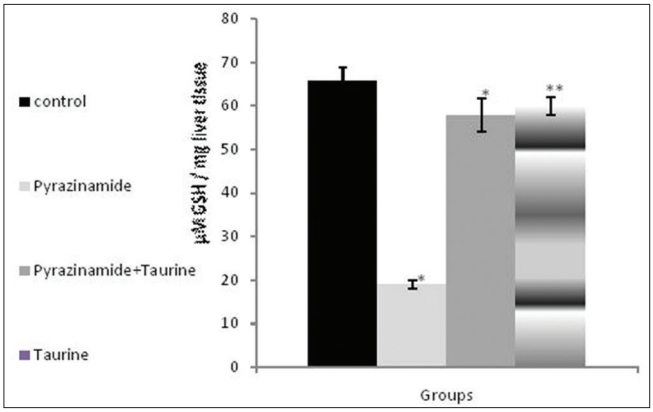

Figure 3: Effect of pyrazinamide alone and with taurine in the glutathione levels of rats. Values are expressed as the mean ± SED. [a]$P < 0.05$, significant difference from control; [b]$P < 0.05$, significant difference from pyrazinamide-treated rats

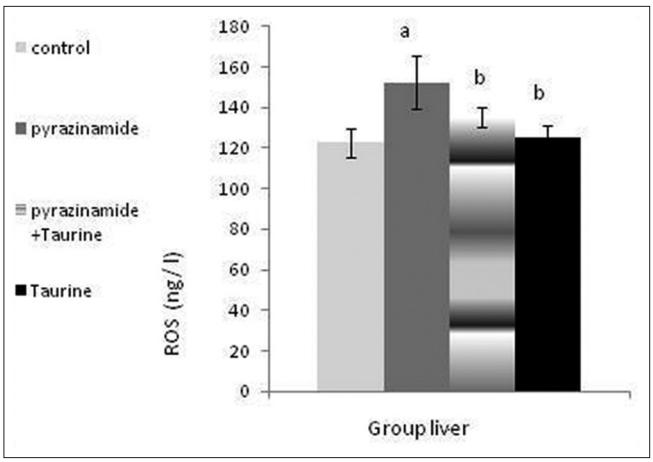

Figure 4: Effect of pyrazinamide alone and with taurine in the ROS formation of rats. Values are expressed as the mean ± SED. [a]$P < 0.05$, significant difference from control; [b]$P < 0.05$, significant difference from pyrazinamide-treated rats

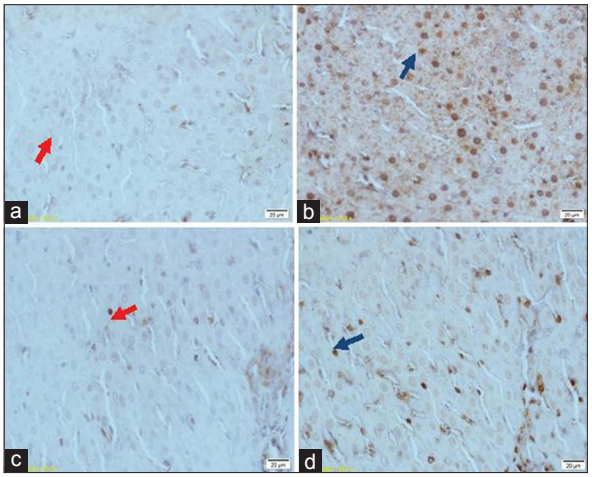

Figure 5: The apoptotic cells (black arrow) in all groups (mag. 9100), Red arrow showed the normal hepatocytes. (a) Control group; (b) PZA group: (c) Taurine group; (d) PZA + Taurine group

liver injuries. Our results are in line with the previous finding established that overproduction of free radicals turns on several downstream signaling cascades leads to subcellular organelles and eventually induction of cellular necrosis and apoptosis.[8,19-21] In previous finding, it has been established that taurine could scavenge ROS.[8-11,22] We found that taurine efficiently diminished ROS generation induced by PZA that is in accordance with the other studies showing that taurine is a ROS scavenger.[14,23] The present results show that PZA induced lipid peroxidation significantly [Figure 2]. Lipid peroxidation is usually one of the consequences of ROS formation and oxidative stress in biological systems Taurine is a successful anti-LPO agent, and it is interfered with the propagation of LPO. The effect of taurine in decreasing the level of MDA derivative produced by PZA might be due to its ability in scavenging the ROS and modulating the oxidative stress caused by PZA that supports the previous studies.[8,22] Furthermore, according to our finding, PZA diminished GSH reservoirs significantly. GSH is a prominent antioxidant defense molecule systems and neutralizes free radicals. GSH conjugates free radicals through GSH peroxidase. GSH depletion was occurred subsequent to ROS formation.[2,10] Recently, investigation demonstrated that PZA-induced ROS formation leads to attenuated GSH contents [Figure 3]. In additon, taurine prevented GSH consumption through scavenging free radicals. It can be concluded preventing of the depletion of GSH reservoirs might be another possible mechanism by which taurine exerts protective effects in PZA-induced hepatotoxicity which is in accordance with other findings.[2,8]

Our finding showed that PZA increased the number of apoptotic cells in the liver and oral treatment of taurine can have a protective role in liver and prevented PZA-induced apoptosis that is a new result. All in all, the toxicity induced by PZA was accompanied by increased ROS formation, lipid peroxidation, GSH depletion, and induction of apoptosis. Based on diagnostic indicators of liver damage (AST and ALT), histopathological finding confirms that rat's co-administration of taurine reduced the hepatotoxicity of PZA efficiently. According to these findings, taurine might be a good antioxidant for prevention or treatment of hepatotoxicity induced by PZA, but feature clinical studies are necessary.

CONCLUSION

Furthermore, hepatoprotective roles of taurine against PZA-induced hepatotoxicity might encourage their continued use in the treatment of the hepatotoxicity induced by other drugs or any chemical agents, especially where oxidative stress is involved. In addition to taurine, the use of other antioxidants against PZA-induced hepatotoxicity might be the subject of future studies, especially in patients who use PZA and other antituberculosis drugs for a long time.

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