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Hepatoprotective and antifibrotic effects of sodium molybdate in a rat model of bile duct ligation

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ABSTRACT

Project: Cholestasis liver fibrosis has been increasingly recognized as a cause of high morbidity and mortality in humans. The accumulation of toxic bile salts in a bile duct ligation (BDL) animal model plays a pivotal role in the induction of liver fibrosis. Cholestatic liver fibrosis is characterized by excessive collagen production and deposition, which is mediated by reactive oxygen species (ROS). Molybdenum is an essential micronutrient trace element which acts as a cofactor in many detoxification system enzymes. The aim of the present study was to evaluate the antifibrotic effect of sodium molybdate on liver cholestasis induced by bile duct ligation in rats.

Procedure: After BDL, rats were given sodium molybdate (0.05 or 0.1 or 0.2 g/kg) or urosodeoxycholic acid (UDCA, 25 mg/kg) via intragastric gavage for 45 consecutive days (once per day).

Results: BDL drastically increased the serum levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin and direct bilirubin, whereas it reduced the levels of antioxidant enzymes, superoxide dismutase and catalase in the liver. Treatment of BDL rats with sodium molybdate significantly attenuated these changes. As determined by Masson's trichrome staining, BDL markedly induced the liver fibrosis. These alterations were also significantly attenuated by sodium molybdate administration.

Conclusions: The results of this study indicate the hepatoprotective and antifibrotic effect of sodium molybdate in the cholestatic liver. Sodium molybdate, by inhibiting the activation of Ito cells, decreases the collagen production in the liver. The antifibrotic effect of sodium molybdate is likely due to the antioxidative and free radical scavenging effects of this trace element.

Introduction

Recently, liver fibrosis has been increasingly recognized as a cause of high morbidity and mortality in humans [1]. Bile duct ligation (BDL) is a suitable experimental model for research to evaluate the pathogenesis, pathophysiology and treatment of cholestatic liver fibrosis [2,3]. Although the mechanisms of fibrosis in cholestatic liver are complicated, it has been well established that accumulation of toxic and hydrophobic bile salts in the liver, shifts the oxidant/pro oxidant balance in favor of increased activities of reactive oxygen species (ROS) [4–6] and these free radicals then promote the inflammatory response, which induces the collagen production in the liver [7]. The oxidative stress in cholestatic liver disease serves as a link between hepatic injury and liver fibrosis in both humans and animals [8,9]. Therefore, any agent that has the capacity of normalizing the oxidative damage, would be able to decrease fibrosis in a cholestatic liver. Molybdenum is an essential micronutrient trace element for plants, animals and microorganisms [10]. In mammals, molybdenum is a constituent of molybdenum-containing enzymes (molybdoenzymes) including sulfite oxidase, aldehyde oxidase, xanthine oxidoreductase and the mitochondrial amidoxime reducing component (mARC) [11]. Although molybdenum can form complexes with

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numerous physiologically important compounds, this trace element is preferably absorbed, transported and excreted in a simple molybdate form [12]. It has been reported that molybdate prevents lipid oxidation and improves antioxidant systems during diabetes mellitus [13]. The findings of previous researches have revealed that sodium molybdate has an insulin-mimicry activity and improves immune dysfunction associated with diabetes in rats [14]. Sodium molybdate has been demonstrated to protect the cell membrane against oxidative stress by elevation of glutathione (GSH) levels [15]. Sodium molybdate may also prevent certain forms of cancer induced by N-nitroso compounds such as forestomach, oesophageal and mammary gland cancer in experimental animals [16,17]. The beneficial effects of molybdate treatment on post ischemic cardiac function of diabetic rats was also observed [12]. Considering the possible role of sodium molybdate in detoxification of xenobiotic compounds, animals and humans stressed by an exposure to certain xenobiotics or endotoxins may have an enhanced need for molybdenum [18].

With an increasing number of patients exhibiting hepatic cholestasis and liver fibrosis, a proper therapeutic is, thus, urgently required [19,20]. The results of our previous study suggested that sodium molybdate could serve as a hepatoprotective agent against toxicity caused by carbon tetrachloride (CCl_4) [21]. To date, no studies have examined whether sodium molybdate is effective in treating the liver fibrosis. The aim of the present study was to evaluate the hepatoprotective and antifibrotic effect of sodium molybdate in a rat model of bile duct ligation.

Materials and methods

Animals

Eighty-one adult male Wistar rats weighing approximately 230–250 g were used in this study. The animals were housed under standard laboratory conditions with a 12 h light–dark cycle and were fed commercial pellets and tap water ad libitum for 1 week before being exposed to the experiments. Animal welfare and experimental procedures were carried out strictly in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council of USA, 1996), and the approval has been received from the Islamic Azad University, Science and Research Branch Animal Ethics Committee.

BDL operation and experimental procedures

After accommodation for one week, the rats were randomly divided into nine groups of nine animals each as follows: (1) the sham operated group: rats underwent laparotomy without BDL and were treated with distilled water; (2–4) the sham operated plus sodium molybdate groups: rats underwent laparotomy without BDL, treated with sodium molybdate at dose levels of 0.05, 0.1 and 0.2 g/kg b.w., respectively; (5) The BDL group: rats with BDL and treated with distilled water; (6–8) The BDL plus sodium molybdate groups: rats with BDL and treated with sodium molybdate at dose levels of 0.05, 0.1 and 0.2 g/kg b.w., respectively; and (9) the BDL + ursodeoxycholic acid (UDCA) group: rats with BDL and administration of UDCA (25 mg/kg b.w.). UDCA was employed as a positive control drug in this study. Sodium molybdate or UDCA was dissolved in distilled water and given by gastric gavage once a day for 45 consecutive days. The sodium molybdate dosages used in this study were based on dosing experiments of our previous study, which demonstrated sodium molybdate protection against carbon tetrachloride (CCl_4)-induced liver injury in rats [21]. Bile duct ligation was performed using a standard technique [22]. Briefly, after the rats were anesthetized intraperitoneally with ketamine (90 mg/kg b.w.) and xylazine (10 mg/kg b.w.), a midline abdominal incision was made. The common bile duct was identified, and doubly ligated with 4-0 nylon suture (AILEE Co., Ltd., Busan, Korea) at two points (just below the junction of the hepatic duct and before the entrance of the pancreatic duct). Then the bile duct was cut between these two points. Two milliliters of sterile saline was instilled into the peritoneum at the end of surgery, followed by careful suturing of the peritoneum and muscle layers as well as the skin wound. Then the animals were allowed to recover on a heat pad [23]. In sham-treated rats, an abdominal incision was made without ligation of the common bile duct.

Chemicals

Sodium molybdate ($Na_2MoO_4^{-2}$) was purchased from Merck Company, Germany. Commercial kits used for determination of total bilirubin, direct bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were all purchased from Pars Azmoon Company, Iran. The superoxide dismutase (SOD) assay kit was purchased from Dojindo Laboratories, Kumamoto, Japan. All other chemicals used were of good quality and analytical grade.

Biochemical evaluation

At the end of the 45-day treatment, the animals were fasted for 18 h and body weights were measured. The animals were anesthetized by inhalation of mild diethyl ether, and after blood and livers were collected, they were sacrificed. The absolute and relative liver weights were determined and livers were processed for further studies, so that a portion of liver tissue was immediately immersion-fixed in 10% formalin buffer solution for histopathology evaluation. Another piece of liver was utilized for the following biological determinations. Liver homogenates (10%, w/v) were obtained in 50 mMol phosphate buffer (pH 7.0) and stored at −80 °C until used. The blood samples were allowed to clot for 30 min at room temperature and centrifuged at 1000 × *g* at 37 °C for 10 min to separate the serum. The serumic levels of total bilirubin, direct bilirubin and the activities of enzymes such as ALT, AST and ALP were determined in the serum using commercially available kits [24].

Measurement of hepatic superoxide dismutase and catalase activities

The liver homogenate was centrifuged at 800 × *g* for 30 min at 4 °C. The supernatant was used to assay the activities of CAT. CAT activity in the liver tissue was assayed as described previously [25]. Briefly, after 0.2 mL of the homogenate was added to 1.2 mL of 50 mM phosphate buffer (pH = 7.0), the reaction started by the addition of 1.0 mL of 30 mM H_2O_2 solution. The decrease in absorbance was measured at 240 nm at 30-s intervals for 3 min. One unit (U) of this enzyme's activity is defined as the amount of the enzyme yielding a value of $K = 1$, where K is the rate constant of the enzyme. Activity is expressed as unit per mg of protein (U/mg protein). SOD activity in the tissue was determined using a SOD assay kit (Dojindo Laboratories, Kumamoto, Japan), according to the manufacturer's protocol.

Histopathological examination

The histopathological alterations such as necrosis, Inflammation, ductal hyperplasia and fibrosis were defined and graded with Masson's trichrome stained sections. The average score taken from 10 random fields per section was used to generate a single score for each specimen [26,27]. The lesions were defined as follows: Necrosis: 0, none; 1, focal necrosis on less than 25% of the tissue; 2,