The Protective Effects of Sodium Molybdate on Cholestatic Liver - Cellular Approach

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***Abstract*—** Cholestatic liver has been increasingly recognized as a cause of high morbidity and mortality in humans. 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 protective effect of sodium molybdate on liver cholestasis induced by bile duct ligation (BDL) in rats. After BDL, rats were given sodium molybdate (0.05 or 0.1 or 0.2 g/kg) for 45 days. BDL markedly induced necrosis, inflammation, collagen deposition and bile duct hyperplasia in the liver. These alterations were also significantly attenuated by sodium molybdate administration. The results of this study indicate the hepatoprotective effect of sodium molybdate in the cholestatic liver. Sodium molybdate, by inhibiting the activation of Ito cells, decreases the collagen production in the liver and its protective effect is likely due to the antioxidative and free radical scavengering effects of this trace element.

***Keywords*—** Bile duct ligation (BDL), Cholestatic Liver, Sodium molybdate

I. Introduction

Bile duct ligation (BDL) is a suitable experimental model for research to evaluate the pathophysiology and treatment of cholestatic liver [1]-[3]. The accumulation of toxic bile salts in the liver, shifts the oxidant/ pro oxidant balance in favor of increased activities of reactive oxigen species (ROS) [4], [5] and these free radicals then promote the inflammatory response and injury in the liver [6]. Molybdenum is an essential micronutrient trace element for plants, animals and microorganisms [7]. In mammals, molybdenum is a constituent of molybdenum-containing enzymes (molybdoenzymes) [8]. 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 [9]. The aim of the present study was to evaluate the hepatoprotective effect of sodium molybdate in a rat model of bile duct ligation.

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II. Materials and Methods

A. Animals

Eighty-one adult male rats weighing 230–250 g were used in this study. The rats were divided into nine groups 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; (7,8) 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 gavage for 45 days. BDL surgery was performed using a standard technique [10]. Briefly, after the rats were anesthetized, a midline abdominal incision was made and the common bile duct was doubly ligated. Then the bile duct was cut between these two points, followed by suturing of the peritoneum and muscle layers as well as the skin wound [11]. In sham-treated rats, an abdominal incision was made without ligation of the common bile duct.

B. Histopathological Examination

At the end of the 45-day treatment, the histopathological alterations such as necrosis, Inflammation, ductal hyperplasia and fibrosis were defined and graded with Masson's trichrome stained sections [12].

C. Statistical Analysis

Results are expressed as mean ± S.E.M. Statistical analysis was performed using one-way ANOVA test followed by a Tukey post hoc test. $P< 0.05$ was considered statistic]ally significant.

III. RESULTS

As shown in Table 1, there is a significant difference between the pathologic scores of the sham operated group and the BDL group, whereas sodium molybdate treatment (0.1 and 0.2 g/kg b.w.) markedly attenuated the pathologic scores in the BDL groups. Histopathological evaluation with Masson's trichrome staining indicated the liver injury indexes including hepatocellular necrosis, infiltration of inflammatory cells,

collagen proliferation and bile-duct hyperplasia in the BDL group. There were no histological abnormalities in the sham+.

TABLE I
HISTOPATHOLOGICAL INJURY SCORES IN BILE DUCT LIGATION (BDL)-INDUCED LIVER FIBROSIS IN RATS

Groups	Injury of Score [a]			
	Necrosis	*Inflammation*	*Collagen Deposition*	*Bile ducts hyperplasia*
Sham	0	0	0	0
Sham +sodium molybdate				
0.05 g/kg	0	0	0	0
0.1 g/kg	0	0	0	0
0.2 g/kg	0	0	0	0
BDL	3^{***}	3^{***}	3^{***}	3^{***}
BDL + sodium molybdate				
0.05 g/kg	2.6±0.3	1^{+++}	2.6±0.3	2.3±1.2
0.1 g/kg	$1.8±0.7^{++}$	1^{+++}	$1.8±0.2^{++}$	$1.8±0.7^{+}$
0.2 g/kg	$1.4±0.3^{+++}$	$1.2±0.2^{+++}$	$1.4±0.3^{+++}$	$1.8±0.21^{+}$
BDL + UDCA (25 mg/kg)	3	1^{+++}	2.6±0.3	3

Table-1 *** P< 0.001 compared with the sham group. $^{+}$P< 0.05, $^{++}$ P< 0.01, $^{+++}$ P< 0.001 compared with the BDL group.[a] Necrosis: 0 = none, 1 = focal necrosis on less than 25% of the tissue, 2 = focal necrosis on 25–50% of the tissue, 3 = extensive, but focal necrosis; Inflammation: 0 = none, 1= focal inflammation on less than 25% of the tissue, 2= focal inflammation on 25–50% of the tissue, 3= extensive, but focal inflammation; Fibrosis: 0= none, 1= fibrous portal expansion, 2= septal formation, 3= marked bridging fibrosis; Bile duct hyperplasia: 0= none, 1= hyperplasia on less than 25% of each liver lobule, 2= hyperplasia on 25–50% of each liver lobule, 3= extensive, but focal hyperplasia.

sodium molybdate groups, compared with sham group and also the morphology of the liver was regular with intact hepatocytes, sinusoids, and portal tract and no fibrosis was observed (Fig.1). Sodium molybdate treatment in the BDL groups significantly reduced all liver injury indexes in a dose-dependent pattern, compared with the BDL-untreated rats.

I. DISCUSSION

In this study, study bile duct ligation was induced in rats, to evaluate the hepatoprotective effect of sodium molybdate on cholestatic liver injury. The usual molybdenum intake of a young man is approximately 120 µg/day. It is already known that sodium molybdate can be useful for certain nutritional purposes in the food supplement industry. The molybdenum compounds toxicity is low, the probable reasons for which include its quick excretion in the urine (36-90%), especially at higher intake levels [13]. Considering the possible role of sodium molybdate in detoxification of cholestatic liver from bile salts toxicity, the BDL rats may have an enhanced need for sodium molybdate. Based on our histopathological evaluation, BDL markedly induced the liver fibrosis as determined by accumulation of collagen in trichrome staining. In contrast, this effect was significantly attenuated by sodium molybdate treatment (Fig.1). Sodium molybdate also markedly attenuated the other pathologic scores of cholestatic liver, as well as hepatocellular necrosis, infiltration of inflammatory cells and bile-duct hyperplasia (Table 1). Hepatic fibrosis is usually initiated by hepatocyte damage, leading to attraction and recruitment of inflammatory cells, activation of kuppfer cells and subsequently transformation of quiescent Ito cells to an activated phenotype, which activate the collagen production and deposition in the liver [14]. The oxidative stress is thus truly known as an important factor in cholestatic liver fibrosis [15]. sodium molybdate prevents oxidative stress through its anti-free radical activities [15]. Sodium molybdate may exert its antifibrotic effect by decreasing the oxidative stress and inhibition of Ito cells activation. It has been suggested that the administration of molybdate to diabetic rats protects cells from further peroxidative damage through its antioxidative action [13]. It has been reported that the administration of molybdate resulted in elevated GSH levels, which protects the cell membrane against oxidative damage. Sodium molybdate helps reducing the risk of cancer and also slowing the ageing process [16].

V. CONCLUSION

The results of this study indicate the hepatoprotective and antifibrotic effects of sodium molybdate in the cholestatic liver. Sodium molybdate, by inhibiting the activation of Ito cells, decreases the collagen production and deposition in the liver. The antifibrotic effect of sodium molybdate is likely due to the antioxidative and free radical scavengering effects of this trace element. Sodium molybdate could likely be used as an hepatoprotective supplement for patients with cholestatic liver disease in the future.

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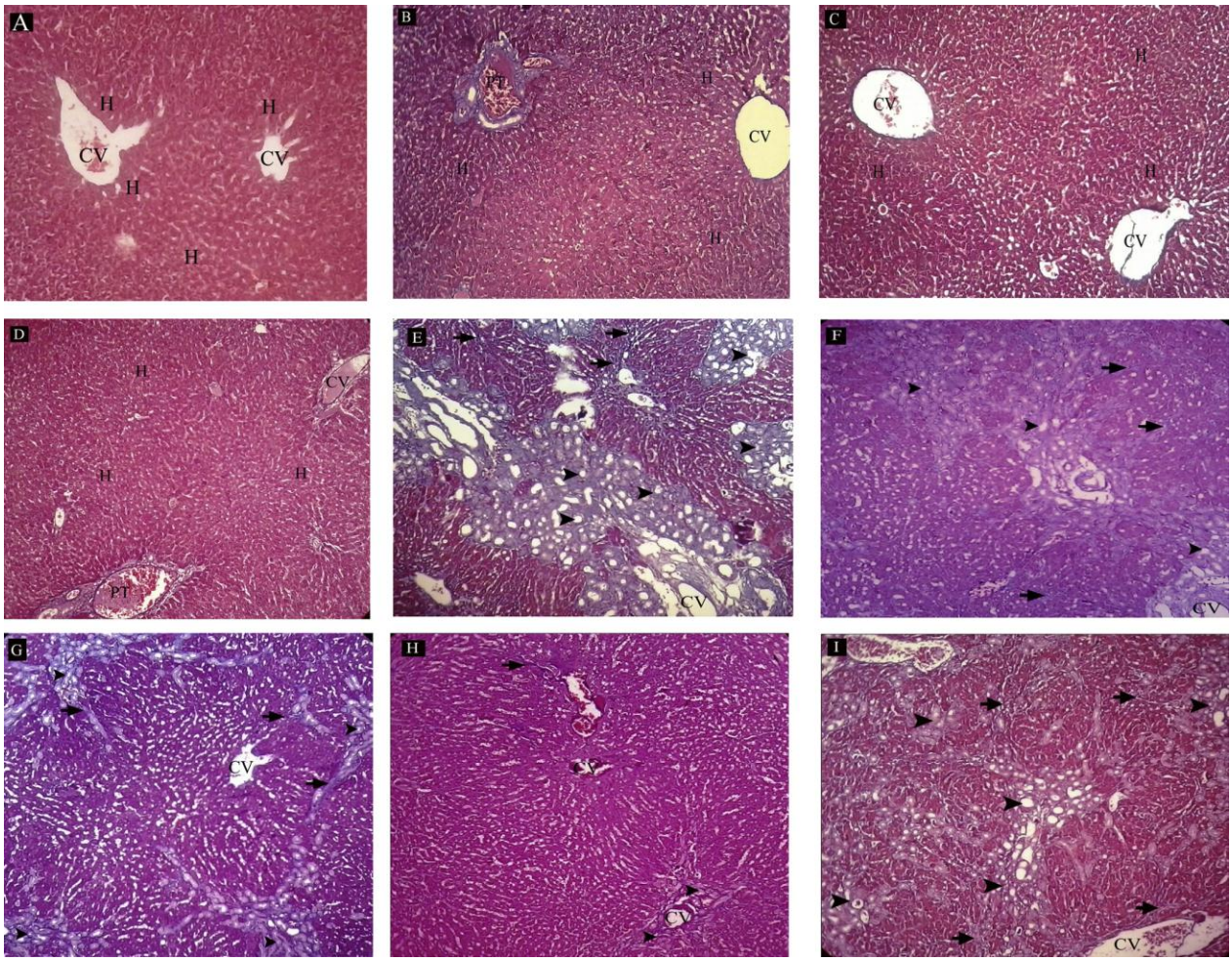

Fig. 1. Histopathological findings of BDL-induced liver fibrosis in rats. 45 days after experiment, hepatic tissue samples were evaluated by staining of Masson's trichrome. (A) sham, (B-D) sham+ Sodium molybdate (0.05, 0.1 and 0.2 g/kg respectively), (E) BDL, (F-H) BDL+ Sodium molybdate (0.05, 0.1 and 0.2 g/kg respectively), (I) BDL+ UDCA (25 mg/kg). No histopathological changes were observed in liver tissues with sham (A) and sham+ sodium molybdate (B-D) and the central vein (CV), periportal space (PT) and hepatocytes (H) are all seen in normal pattern. In BDL group (E) extensive bridging fibrosis (arrow) and bile duct hyperplasia (arrow heads), are shown. All these lesions were markedly attenuated in sodium molybdate (F-H) or UDCA treatment (I) (Trichrome*160).

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