The Protective Effects of Magnesium on Liver Tissue Alteration Induced by Bile Duct Ligation in Rat

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***Abstract*—** Magnesium is the fourth most abundant cation in the body and the second most abundant cation in intracellular fluid. Magnesium plays an important role in numerous biological functions. In the present study we investigated the effect of magnesium sulfate ($MgSO_4$) on histological abnormalities induced by bile duct ligation in male Wistar rats. The effects of 28-day oral administration of $MgSO_4$ (at doses of 0.01, 0.05, 0.1, and 0.2 g/kg bw) on the levels of liver parameters, were evaluated in normal and BDL-induced cholestatic rats. Histopathological studies confirmed the protective effects of $MgSO_4$ on cholestasis-induced hepatic injury in rats. the results of this study suggest that $MgSO_4$ treatment may be beneficial in cholestasis-induced hepatotoxicity.

***Index Terms*—** Magnesium Sulfate, Extrahepatic Cholestasis, Bile-duct Ligation, Rat.

I. Introduction

Magnesium (Mg^{2+}) is the second most abundant cation (after potassium) in the cell and plays an important role in various biological functions, including cell cycle, channel regulation, ATPase activity, metabolic regulation, etc. [9],[14],[27]. Numerous enzymes require the presence of Mg^{2+} ions for their catalytic activity, such as all enzymes utilizing or synthesizing ATP or those that use other nucleotides to synthesize DNA and RNA [7]. On these bases, it is not unexpected that many symptoms and diseases are associated with altered Mg^{2+} homeostasis. Mg^{2+} levels influence the synthesis of nitric oxide [12], intracellular calcium release [35], uptake and metabolism of low-density lipoproteins [35], permeability to water and albumin [30], and proliferation of endothelial cells [2]. A large body of evidence has been accumulating showing that Mg^{2+} deficiency can promote inflammation, exacerbate immune stress response, and reduce specific immune response [23],[22]. Magnesium deficiency elevates phospholipid levels in rabbit liver [38], increases permeability of mitochondrial inner membrane, and weakens coupling between oxidation and phosphorylation [16]. Therefore, decreased magnesium by altering mitochondrial function could play a role in lipid peroxidation, induction of cytokines and Fas ligands and well-known pathways for the development of steatohepatitis and fibrosis [1].

Cholestasis can be defined as bile flow impairment, which is caused by the obstruction of the bile duct or functional defect in bile formation [5]. Studies have revealed that failure of bile acids excretion due to cholestasis leads to accumulation of toxic bile salts within hepatocytes [13] and thus oxidative stress [19]. Reactive oxygen species (ROS) further cause cell death through necrosis or apoptosis [28]. In the early stages of cholestasis, toxic bile salts damage hepatocytes due to their surfactant properties, and the damaged hepatocytes generate ROS [6]. ROS amplify inflammation and also stimulate hepatic stellate cells to promote fibrosis through lipid peroxidation. Therefore, decrease of oxidative stress can play an important role in the treatment of obstructive cholestasis. Cholestasis can cause inflammatory reactions, excessive oxidative stress [4], and eventually periductular fibrosis [33]. The number of patients suffering from metabolic disorders with hepatic cholestasis and cholestatic liver fibrosis is growing [25]. Therapeutics for cholestatic diseases and hepatic fibrosis are thus urgently needed, but a potent drug has not been developed yet. Bile duct ligation (BDL) is an appropriate model to evaluate therapeutic efficacy in animal cholestatic liver injury [20]. BDL can also cause accumulation of hydrophobic bile acids, which results in oxidative stress in the liver [29].

The objective of this study was therefore to investigate the effect of magnesium sulfate ($MgSO_4$) on cholestasis-induced hepatic injury in BDL rat model.

II. Material and Methods

A. Animals

Male Wistar rats weighing 250-300 g were housed under standard laboratory conditions and had free access to food and water. This investigation was conducted according to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996), and was approved by our Institutional Animal Ethics Committee.

B. Chemicals

$MgSO_4$ was obtained from Sigma Chemicals (Poole, UK).

C. BDL surgery and BDL induced extra-hepatic cholestasis

BDL surgery was carried out under intraperitoneal anesthesia with a mixture of ketamine hydrochloride

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(50 mg/kg) and xylazine (5 mg/kg). After laparotomy, the common bile duct was double-ligated by silk threads and cut between the ligatures to prevent regeneration. Sham-operation was composed of laparotomy, bile duct identification and manipulation without ligation or resection (to equalize groups for the possible stress that is induced by surgery, per se). In the BDL group, the main bile duct was first ligated with two ligatures approximately 0.5 cm apart and then transected at the middle point between the two ligatures. All surgeries were done under the aseptic condition. Immediately after the operation, each animal was placed in a cage by itself to prevent wound dehiscence and then returned to its original home cage 4 h later [3].

D. Experimental design

A total of 99 rats were divided into 11 groups of 9, as follows:

Group I: Normal control rats were administered 1 ml of distilled water intragastrically.

Group II: Sham-operated control rats were administered 1 ml of distilled water intragastrically.

Groups III-VI: Normal experimental rats were administered $MgSO_4$ dissolved in distilled water (intragastrically, daily) at doses of 0.01, 0.05, 0.1, and 0.2 g/kg bw, respectively.

Group VII: BDL control rats were administrated 1 ml of distilled water intragastrically.

Groups VIII-XI: BDL experimental rats were administrated $MgSO_4$ dissolved in distilled water (intragastrically, daily) at doses of 0.01, 0.05, 0.1, and 0.2 g/kg bw, respectively.

The volume of administration was 1 ml, and the treatments lasted for 28 consecutive days [17]. The animals were carefully monitored every day and weighed every week. The dosages used in this study were based on dosing experiments of our previous study, which demonstrated magnesium protection against carbon tetrachloride (CCl_4)-induced liver injury in rats [8].

E. Liver Samples Prepararion

Following 28 days of treatment, the rats were anesthetized by inhalation of diethyl ether, and were sacrificed after blood and tissue samples were obtained. Liver slices were taken from a part of the left lobe of liver in each group and then fixed in a 10% buffered formalin phosphate solution, and embedded in paraffin. The block was cut into 5 µm sections and stained with Masson's trichrome. The typical histopathological alterations, such as inflammation, necrosis, bile duct hyperplasia, and fibrosis were evaluated using trichrome stained liver section [11].

F. Statistical Analysis

Statistical analysis was carried out using SPSS 10 program for windows (SPSS, Chicago, Ill). Data were expressed as mean±SEM. Statistical analysis was performed by one-way analysis of variance followed by Tukey post hoc test. The criterion for statistical significance was $p<0.05$.

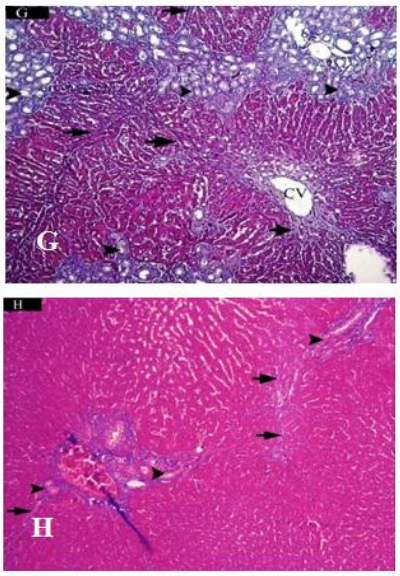
III. Results

The liver tissue architecture in the sham-operated rats remained intact with no morphological alterations, whereas hepatocyte necrosis, infiltration of mononuclear inflammatory cells, fibrosis, and bile duct hyperplasia were observed in the BDL rats (Fig. 1). A Massive bridging fibrosis around the portal and central vein was also seen in the BDL rats. The administration of $MgSO_4$ at doses of 0.1 and 0.2 g/kg prevented this histological alteration and significantly reduced bile duct hyperplasia, fibrosis, hepatocyte necrosis, and inflammation (Fig. I, Table I).

TABLE I.
EFFECT OF $MGSO_4$ ON HISTOLOGICAL INJURY SCORE OF LIVERS IN NORMAL AND BDL RATS

Groups	Injury of score [a]			
	Necrosis	Inflammation	Collagen deposition (fibrosis)	Bile ducts hyperplasia
Normal Control	0	0	0	0
Sham operated	0	0	0	0
$MgSO_4$ (g/kg)				
0.01	0	0	0	0
0.05	0	0	0	0
0.1	0	0	0	0
0.2	0	0	0	0
BDL control	2.6 ± 0.2 ***	2.8 ± 0.1 ***	3.0 ± 0.0 ***	3.1 ± 0.1 ***
BDL + $MgSO_4$ (g/kg)				
0.01	2.2 ± 0.1 ***	2.5 ± 0.2 ***	2.4 ± 0.2 ***	2.6 ± 0.2 ***
0.05	2.1 ± 0.1 ***	2.0 ± 0.3 ***+	2.1 ± 0.1 ***+	2.1 ± 0.1***+
0.1	1.6 ± 0.2 ***++	1.1 ± 0.1***+++	1.1 ± 0.1 ***+++	1.6 ± 0.1***+++
0.2	1.3 ± 0.2***+++	1.0 ± 0.0***+++	1.0 ± 0.0***+++	1.0 ± 0.0***+++

*** $p<0.001$ significantly different from the normal control group.
+ $p<0.05$, ++ $p<0.01$, +++ $p<0.001$ significantly different from the BDL control 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.



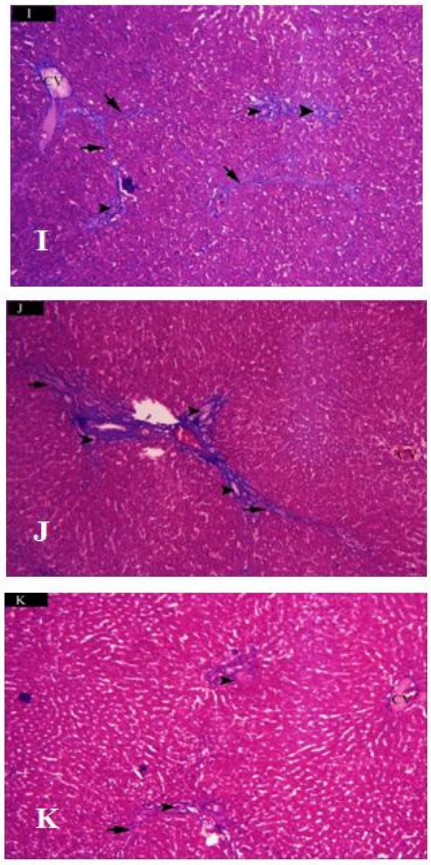


Fig 1 Histopathological findings of BDL-induced liver fibrosis in rats. Hepatic tissue samples were evaluated by staining of Masson'strichrome. (A) Normal control group, (B) sham operated group, (C-F) $MgSO_4$ groups (0.01, 0.05, 0.1 and 0.2 g/kg respectively), (G) BDL, (H-K) BDL+ $MgSO_4$ (0.01, 0.05, 0.1 and 0.2 g/kg respectively). No histopathological changes were observed in liver tissues with control (A) and sham operated (B) and $MgSO_4$ groups (C-F) and the central vein (CV) and hepatocytes are all seen in normal pattern. In BDL group (G) extensive bridging fibrosis (arrow) and bile duct hyperplasia (arrow heads) are shown. All these lesions were markedly attenuated in BDL+$MgSO_4$ treatment groups (H-K) (Trichrome*160).

IV. Discussion

The results of the present study showed that treatment with $MgSO_4$ effectively protected rats against BDL-induced cholestasis, as evidenced by histological observations. Obstruction of the biliary system causes retention of bile. Chronic bile retention leads to bile duct enlargement and proliferation, activates hepatic stellate cells to produce collagen, and finally results in periportal and perineoductular fibrosis [6], which were confirmed by histopathological findings of the present study. The liver tissue from rats that received BDL alone showed extensive ductal proliferation and formation of a fibrous connective tissue bridge between the portal tracts. Treatment with $MgSO_4$ significantly reduced the proliferation of the bile duct and fibrosis. In the BDL model, oxidative stress and impairment of antioxidant defense systems are known to play key roles in hepatocytes damage and the liver fibrosis process [31],[32]. Obstruction of the bile duct leads to the generation of enormous oxidative stress and the depletion of antioxidant enzymes during cholestasis. It has been claimed that prooxidant/antioxidant balance shifts towards lipid peroxidation under BDL conditions [26]. It has been evidenced that Mg deficiency may modulate the levels of antioxidant defense and influence the oxidant-antioxidant balance in the cells [10]. In animal studies, Mg deficiency results in an increased release of substance P and other mediators from nerve endings. These mediators activate immune cells to release histamine and cytokines, inducing a proinflammatory state and increased levels of oxygen free radicals, nitric oxide, and oxidative stress [24],[21]. On the contrary, Mg supplementation was reported to reduce levels of malondialdehyde (MDA), which is a decomposition product of polyunsaturated fatty acid peroxides, and to elevate SOD and GST activities in diabetic rats [15]. It has been reported that Mg prevents the production of oxygen free radicals [37] and inhibit lipid peroxidation both in vitro [18] and in vivo [34].

V. Conclusion

In conclusion, the findings of this study indicate that $MgSO_4$ can attenuate hepatic damage in extrahepatic cholestasis in experimental rats through reducing oxidative stress and inflammatory processes and give an indication of the possible therapeutic effect of $MgSO_4$ on cholestatic liver injur**y.**

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