Dose-related effects of dexamethasone on liver damage due to bile duct ligation in rats

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Abstract

AIM: To evaluate the effects of dexamethasone on liver damage in rats with bile duct ligation.

METHODS: A total of 40 male Sprague-Dawley rats, weighing 165-205 g, were used in this study. Group 1 (sham-control, *n* = 10) rats underwent laparotomy alone and the bile duct was just dissected from the surrounding tissue. Group 2 rats (untreated, *n* = 10) were subjected to bile duct ligation (BDL) and no drug was applied. Group 3 rats (low-dose dexa, *n* = 10) received a daily dose of dexamethasone by orogastric tube for 14 d after BDL. Group 4 rats (high-dose dexa, *n* = 10) received a daily dose of dexamethasone by orogastric tube for 14 d after BDL. At the end of the two-week period, biochemical and histological evaluations were processed.

RESULTS: The mean serum bilirubin and liver enzyme levels significantly decreased, and superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) values were significantly increased in low-dose dexa and high-dose dexa groups when compared to the untreated group. The histopathological score was significantly less in the low-dose and high-dose dexa groups compared to the untreated rats. In the low-dose dexa group, moderate liver damage was seen, while mild liver damage was observed in the high-dose dexa group.

CONCLUSION: Corticosteroids reduced liver damage produced by bile duct obstruction. However, the histopathological score was not significantly lower in the high-dose corticosteroid group as compared to the low-dose group. Thus, low-dose corticosteroid provides a significant reduction of liver damage without increased side effects, while high dose is associated not with lower fibrosis but with increased side effects.

INTRODUCTION

Obstruction of bile flow through the extrahepatic biliary system results in development of oxidant injury, hepatic fibrosis, biliary cirrhosis, and portal hypertension[1,2]. Patients with obstructive jaundice are at significant risk for severe complications, particularly sepsis[3,4]. Although preoperative percutaneous biliary drainage has been attempted to reduce perioperative complications, it failed to demonstrate a significant benefit despite successful reduction in serum bilirubin levels[5]. Preventing or minimizing the deleterious effects of bile acids might represent a potential therapeutic target for patients with obstructive jaundice. However, effective pharmacological therapy in chronic liver diseases is not yet available.

Since the inflammatory process is important in the development of liver injury following biliary obstruction, the purpose of this study was, therefore, to evaluate the effects of dose-related dexamethasone (dexa) on liver damage caused by bile duct ligation in rats.

MATERIALS AND METHODS

Forty male Sprague-Dawley rats (Dicle University Research Center), weighing 165-205 g, were used in the study. All of the experimental protocols were performed according to the guidelines for the Ethical Treatment of Experimentation Animals. The animals were divided into 4 groups, each group containing 10 animals. Each rat was anesthetized with ketamine (50 mg/kg) and xylazine (4 mg/kg). The rats were subjected to either bile duct ligation (BDL) or sham operation using aseptic

techniques, as previously described by Criado *et al*[6]. Group 1 rats (sham-control) underwent laparotomy alone and the bile duct was dissected from surrounding tissue. Group 2 rats (untreated) were subjected to bile duct ligation alone and received 1 mL of saline by orogastric tube. Group 3 rats (low-dose dexa) received a daily dose of dexamethasone (0.125 mg/kg per day) by orogastric tube for 14 d after BDL. Group 4 rats (high-dose dexa) received a daily dose of dexamethasone (0.400 mg/kg per day) by orogastric tube for 14 d after BDL. The rats were housed in standard cages in a room at 12 h:12 h light-dark condition, temperature (20℃) and humidity (60%), and maintained on a standard rat pellet diet. At the end of the two-week period, all animals were anesthetized with 100 mg/kg inactin ip, placed on a thermoregulated table, and a short segment of polyethylene (PE)-240 catheter was inserted into the trachea to assist the spontaneous respiration. After opening the abdomen through a midline incision, the abdominal aorta was punctured and 5 mL of blood was taken into heparinized tubes. Plasma was separated by centrifugation for biochemical studies, and the activities of alanine aminotransferase (ALT) (IU/L), aspartate aminotransferase (AST) (IU/L), alkaline phosphatase (ALP) (IU/L), γ-glutamyltranspeptidase (GGT) (IU/L) and the concentrations of total bilirubin (TB) (mg/dL) in plasma were determined by standard auto-analyzer methods on an Abbot Aeroset (USA). Just before the rats were sacrificed, the livers were extracted for histopathological evaluation. During this period of surgical preparation, the rats in all groups received 1% of their body weight of Ringer's lactate solution.

Histopathological examination

The extracted liver was divided into two pieces in each rat. One of the pieces was immediately placed into 100 g/L formaldehyde solution overnight, embedded in paraffin, and cut into 5-mm thick sections; stained either with hematoxylin-eosin (HE) or Masson's trichrome for light microscopic analysis. Histopathological scoring of groups for degree of fibrosis (ductular proliferation, focal ductular cholestasis, portal tract expansion, mixed inflammation, necrosis, and fibrosis) was scored as: 0 = absent; 1 = slight; 2 = moderate; and 3 = severe[7]. Histopathological evaluation was performed twice in four sections per slide from all animals in each group. In addition, the number of infiltrating neutrophils per portal tract was assessed by counting neutrophils manually at a 400 × magnification (Olympus Eyepiece Micrometer®) in 10 portal tracts per slide ($n = 10$ in each group).

The other piece was washed in ice-cold saline and homogenized in 0.1 mol/L Tris-HCI buffer (pH 7.4). The homogenate was then centrifuged and the supernatant obtained was used for the assay of various enzymes. Superoxide dismutase (SOD) was assayed as previously described by Misra and FridoVich[8]. Based on the inhibition of epinephrine auto-oxidation by the enzyme, catalase (CAT) activity was measured by following decomposition of H_2O_2 according to the method described by Beers and Sizer[9]. Glutathione peroxidase (GSH-Px) was assayed using H_2O_2 as the substrate as previously described[10].

Table 1 Comparative biochemical measurements at the two week of the study (mean ± SD)

Groups	Biochemical parameters				
	ALP (IU/L)	GGT (IU/L)	TB (mg/dL)	AST (IU /L)	ALT (IU/L)
Sham-control	177 ± 20	1.9 ± 0.3	0.3 ± 0.2	117 ± 3.3	55 ± 10
Untreated	386 ± 44[a]	49 ± 10[a]	11 ± 1.3[a]	1378 ± 256[a]	258 ± 62[a]
Low-dose dexa	297 ± 78[c]	20 ± 2.3[c]	8 ± 0.8[c]	1037 ± 215[c]	198 ± 39[c]
High-dose dexa	300 ± 60[e]	19 ± 2[e]	8 ± 1.1[e]	963 ± 139[e]	150 ± 43[e]

ALP: Alkaline phosphatase; GGT: γ-glutamyltranspeptidase; TB: Total bilirubin; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase. [a]$P < 0.05$ *vs* sham-control; [c]$P < 0.05$ *vs* untreated; [e]$P < 0.05$ *vs* untreated.

Table 2 Comparative SOD, CAT and GSH-Px measurements at the two week of the study (mean ± SD)

Groups	SOD (IU/L)	CAT (IU/L)	GSH-Px (mg/dL)
Sham-control	16 ± 2.5	5.1 ± 0.9	430 ± 31
Untreated	6.9 ± 0.9[a]	0.8 ± 0.1[a]	142 ± 13[a]
Low-dose dexa	10.6 ± 1.2[c]	2.7 ± 0.6[c]	254 ± 15[c]
High-dose dexa	10.7 ± 0.9[e]	2.6 ± 0.6[e]	255 ± 19[e]

SOD: Superoxide dismutase; CAT: Catalase; GSH-Px: Glutathione peroxidase. [a]$P < 0.05$ *vs* sham-control; [c]$P < 0.05$ *vs* untreated; [e]$P < 0.05$ *vs* untreated.

Statistical analysis

Data were entered and analyzed on an IBM compatible personal computer using SPSS version 9.0. All values were expressed as mean ± SD. The significance of the data obtained was evaluated by using analysis of variance (ANOVA). Differences between means were analyzed by using the post-ANOVA (Tukey's b) test. $P < 0.05$ was considered statistically significant.

RESULTS

The mean weights of the animals from the four groups before and after BDL were, respectively, as follows: 192 ± 15 g *vs* 196 ± 9 g, 204 ± 12 g *vs* 166 ± 3 g, 203 ± 12 g *vs* 167 ± 9 g, and 190 ± 9 g *vs* 119 ± 11 g. These data showed that the mean weights of the three study groups decreased significantly compared to the control group after BDL ($P < 0.0001$ for all). In addition, the mean weight of group 4 rats decreased significantly compared to groups 2 and 3 ($P < 0.0001$ for all).

The values of biochemical measurements for the different groups are shown in Table 1. Serum levels of ALP, GGT, TB, AST and ALT were significantly increased in the untreated, low-dose dexa and high-dose dexa groups in comparison with the sham-control group (all $P < 0.05$). ALP, GGT, total bilirubin, AST and ALT values were decreased in the low-dose dexa group and high-dose dexa group when compared to the untreated group (all $P < 0.05$). However, no significant difference was found between the low-dose dexa and high-dose dexa groups.

The values of SOD, CAT and GSH-Px measurements for the different groups are shown in Table 2. Mean values were significantly decreased in the untreated, low-dose
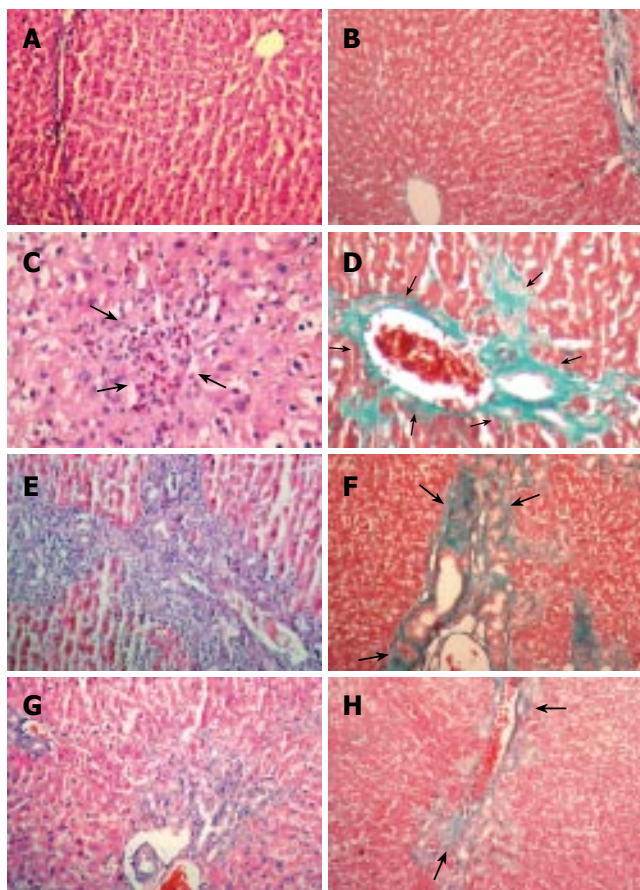
Figure 1 Morphological changes in the livers of different group rats (HE × 200; Masson's trichrome × 200). No morphological damage was observed in any of the rats in the sham-control group (**A** and **B**). In the untreated group, proliferation of portal and periportal biliary ductules with disorganization of the hepatocytes plates, dilated portal spaces and areas of polymorphonuclear leukocyte infiltrate, hepatocytes necrosis and fibrosis were observed (**C** and **D**). Moderate hepatocytes necrosis and fibrosis were present in the low-dose dexa group (**E** and **F**). The high-dose dexa group showed a remarkably less necrosis and fibrosis (**G** and **H**). **A**, **C**, **E** and **G** are stained with HE; **B**, **D**, **F** and **H** are stained with Masson's trichrome.

dexa and high-dose dexa groups in comparison with the sham-control group (all $P < 0.05$). However, these values were significantly increased in low-dose and high-dose dexa groups when compared to the untreated group (all $P < 0.05$).

The mean count of polymorphonuclear leukocytes (PNL) in the control group was less than one in portal spaces of hepatic parenchyma, whereas it was 15 ± 3, 4.6 ± 0.9 and 3.4 ± 0.8 in the untreated, low-dose dexa and high-dose dexa groups, respectively. The number of PNL was significantly decreased in low-dose and high-dose dexa groups when compared to the untreated group ($P < 0.0001$). However, no significant difference was found between the high-dose dexa and sham-control group.

No morphological damage was observed in any of the rats in the sham-control group (Figure 1A, 1B). In the untreated group, severe damage (dilated central veins, proliferation of portal and periportal biliary ductules with disorganization of the hepatocyte plates, dilated portal spaces and hepatocyte necrosis) was observed (Figure 1C, 1D). In the low-dose and high-dose dexa groups, moderate damage (dilated central veins and minimal disorganization of the hepatocyte plates, and hepatocyte necrosis) was

observed (Figure 1F). Mild damage (less necrosis and less fibrosis) was observed in the high-dose dexa group (Figure 1G, 1H).

The histopathological scores found in the sham-control, untreated, low-dose dexa and high-dose dexa groups were 0.1 ± 0.3, 2.6 ± 0.5, 1.9 ± 0.7 and 1.8 ± 0.6, respectively. The histopathological score tended to be more in the untreated, low-dose dexa and high-dose groups as compared with the sham-control groups (all $P < 0.0001$). However, the histopathological score was significantly less in the low-dose and high-dose dexa groups compared to the untreated rats ($P < 0.05$, $P < 0.02$, respectively). Moreover, no significant difference was observed in the histopathological score between the low-dose dexa and high-dose groups.

DISCUSSION

Bile duct ligation (BDL) in rats induces portal fibrosis, which begins with an early proliferation of biliary duct epithelial cells and portal periductular fibroblasts[11]. In the early phases, there is neither substantial hepatocyte necrosis, nor fibrotic reaction in the centrilobular region[12]. Later, fibrosis distributes more widely and alters intrahepatic vascular resistances and blood flow, leading to the establishment of portal hypertension[13]. In this experimental study, the rats subjected to BDL for two weeks showed changes in plasma levels of bilirubin, AST, GGT and ALP, thereby indicating the presence of cholestasis and diffuse hepatic injury. These observations are in agreement with those of several authors[14,15]. After BDL, we observed a significant decrease in the mean weights of rats, including group 4. It has been demonstrated in previous studies that the administration of dexamethasone was ineffective in increasing the liver weight in advanced liver fibrosis in rats or in improving body weight or body weight gain[16]. In fact, the body weight (or body weight gain) was further decreased by dexamethasone treatment in rats with liver fibrosis[17], which is in agreement with our study.

BDL affects the balance between antioxidant and prooxidant activities and increases the prooxidant activity. It increases production of free radicals[18] and decreases free radical scavengers (GSH-Px, SOD and CAT). High level of free radicals induces lypoperoxidation[19] and alteration of fluidity and functionality of cell membranes[20]. It has been suggested that the increase of intrahepatocyte concentration of biliary acids induces mitochondrial toxicity in chronic cholestasis[24]. Furthermore, increased lipid peroxidation in the BDL model has been described[21]. A significant correlation between TB levels and lipid peroxidation has also been described in hepatic toxicity or biliary tract diseases[22]. In addition, nitric oxide (NO) has been shown to be related to the development of hepatic fibrosis. The hepatocytes are mainly responsible for the production of NO radicals[23]. Dexamethasone can either inhibit the release of inflammatory mediators and consequently the formation of NO radicals or block the up-regulation of inducible NO synthetase[24]. Also, previous studies have indicated a dual role for dexamethasone; besides blocking

the release of pro-inflammatory cytokines, it also reduces the release of anti-fibrotic mediators by Kupffer cells and sinusoidal endothelial cells in the liver[25,26]. In our study, dexamethasone protected the levels of such antioxidant enzymes as SOD, CAT and GSH-Px in both doses.

The main liver injury following biliary obstruction is periductal inflammation, bile duct proliferation and portal fibrosis. Prominent among the inflammatory cells that invade obstructed livers are neutrophils; it has been evidenced by several experimental studies that neutrophils infiltrate the liver within 3 h of BDL[27], and remain there for days to weeks as fibrosis progresses[27-29]. In addition, dexamethasone was found to prevent infiltration of inflammatory cells, PMNs and monocytes *in vivo*[16,30]. A connection between neutrophils and liver fibrosis was first suggested by Parola *et al*[27]. They quantitated hepatic neutrophils in an experimental model of bile duct obstruction and found that the number of infiltrating cells correlated directly with the degree of liver fibrosis. In the present study, we found that the number of PNL decreased in the low-dose and high-dose dexamethasone groups, which is in accordance with previous studies.

Glucocorticoids are used as anti-inflammatory and immunomodulatory agents in a wide variety of diseases[31]. Their physiological effects may be accomplished largely by modulating the expression of many cytokine genes, such as IL-1[32], IL-2[33], TNF-α[34], interferon-β[35], interferon-γ[33], and monocyte chemotactic and activating factor[36]. Dexamethasone is also a phospholipase A2 inhibitor and has been shown to inhibit endothelin-1-stimulated arachidonic acid production[37]. Dexamethasone also blocks conversion of arachidonic acid by cyclooxygenase into prostaglandins and suppresses the production of various inflammatory mediators[38]. Although dexamethasone enhances hepatocyte viability and improves the expression of liver-specific genes in liver diseases, there is controversy over the beneficial effects of dexamethasone for liver fibrosis treatment. Dexamethasone has been used as an anti-inflammatory drug in the treatment of chronic active hepatitis for the prevention of liver fibrosis[39]. Glucocorticoids suppress collagen synthesis and gene expression by fibroblasts. Nevertheless, glucocorticoids do not have an inhibitory effect on extracellular matrix synthesis by hepatic stellate cells[16]. In our study, dexamethasone, particularly in high dose, decreased proliferation and infiltration of inflammatory cells in portal and periportal biliary ducts. The results of previous studies provide evidence that dexamethasone fails to therapeutically improve liver function during fibrosis. Thus, the beneficial effect of dexamethasone in ascites formation may be attributed to its anti-inflammatory effect and to a decrease in the production of vasoactive substances from inflammatory cells, but not to improve liver functions and decrease fiber accumulation[16,40]. In our study, however, ALP, GGT, total bilirubin, AST and ALT values were decreased in the low-dose dexa and high-dose dexa groups compared to the untreated group. Although dexamethasone is widely used in the management of inflammatory diseases, it may cause such serious side-effects as immunosuppression and growth retardation, especially when used at high doses. In the present study,

parallel to the literature, dexamethasone caused growth retardation when used at high doses.

In conclusion, using corticosteroids reduced liver damage produced by bile duct obstruction. The finding that no significantly lower histopathological score in the high-dose dexamethasone group compared to the low-dose group suggests that high-dose dexamethasone does not provide any additional protective effect on liver damage. Moreover, high-dose dexamethasone causes obvious growth retardation. Thus, low-dose corticosteroid provides a significant reduction in liver damage without increased side effects, while high dose is associated not with lower fibrosis but with increased side effects.

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