Effects of lexipafant (BB-882), a platelet activating factor receptor antagonist, on liver damage due to bile duct ligation in rats

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Abstract

Background and study aims : Extrahepatic cholestasis is one of the main factors causing liver fibrosis. In this study, we aimed to evaluate the effects of lexipafant (BB-882), a platelet activating factor receptor antagonist, on liver damage in rats with bile duct ligation.

Methods : A total of 30 male Sprague-Dawley rats weighing 160-190 g were used in this study. Group 1 (Sham-control, n = 10) rats were undergone laparotomy alone and bile duct was just dissected from the surrounding tissue. Group 2 rats (BDL/Untreated, n = 10) were subjected to bile duct ligation and no drug was applied. Group 3 rats (BDL/BB-882, n = 10) received a daily dose of BB-882 intraperitoneally for 14 days after BDL. At the end of the two-week period, biochemical and histological evaluation was processed.

Results : The mean serum bilirubin and liver enzymes level significantly decreased, and Superoxide dismutase, catalase and glutathione peroxidase values were significantly increased in BDL/BB-882 group when compared to BDL/Untreated group. The histopathological score was significantly less in the BDL/BB-882 group compared to the BDL/Untreated rats. In the BDL/BB-882 group was observed less fibrosis and neutrophil infiltration

Conclusions : These results suggest that BB-882 (lexipafant) may reduce the severity of the inflammatory response to liver injury produced by bile duct ligation in rats. (Acta gastroenterol. belg., **2006**, 69, **197-202**).

Key words : bile duct ligation, BB-882, lexipafant, rat.

Introduction

Bile duct ligation (BDL) in rats induces portal fibrosis, which begins with an early proliferation of biliary duct epithelial cells and portal periductular fibroblasts (1,2). Experimental and clinical data indicate that oxidative stress and endotoxaemia represent important mechanisms in the development of liver injury in conditions of biliary obstruction (3,4). 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.

Platelet activating factor (PAF) is an inflammatory chemical mediator, and has various biologic actions such as stimulation of platelets and neutrophils, and increases in response to various inflammatory stimuli (5). This substance is implicated as a mediator of various types of liver diseases, such as endotoxin liver injury (6,7), ischemia-reperfusion liver injury (8-10), and hepatic resection (9,10). In addition, PAF is an inducer of prostaglandins and oxygen radicals' synthesis. PAF can also increase the toxicity of other proinflammatory cytokines, including tumor necrosis factor (TNF) and interleukin (IL)-2, and may contribute to the induction of nitric oxide (11,12). It is suggested that BB-882 (lexipafant) block PAF receptors on the surface of endothelial cells, and so down-regulating the activation of leucocytes/macrophages (13). On these data base we aimed to evaluate the effects of the PAF antagonist (BB-882, lexipafant) on liver damage caused by bile duct ligation in rats.

Materials and methods

Thirty male Sprague-Dawley rats weighing 160-190 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 3 groups. Each rat was anesthetized with 50 mg/kg ketamine (Ketalar, Eczacıbası, Turkey) and 4 mg/kg xylazine (intramuscular) (Rompun, Bayer, Leverkusen, Germany) administered intraperitoneally.

The rats were subjected to either bile duct ligation (BDL) or sham operation using aseptic techniques, as previously described by Criado *et al.* (14). Group 1 rats (Sham-control, n = 10) underwent laparotomy alone and bile duct was dissected from surrounding tissue. Group 2 rats (BDL/Untreated, n = 10) subjected to bile duct ligation alone and no drug were given. Group 3 rats (BDL/BB-882, n = 10) received an intraperitoneal injection every day of PAF receptor antagonist BB-882 (5 mg/kg) (Lexipafant, British Biotech Pharmaceuticals Ltd, Oxford, UK), for 14 days after BDL. The rats were housed in standard cages in a room controlled for daylight (12 h), temperature (20 °C) and humidity (60%), and maintained on a standard rat pellet diet.

At the end of two-week period, all animals were anesthetized with 100 mg/kg Inactin i.p., placed on a thermoregulated table, and a short segment of polyethylene (PE)-240 catheter was inserted into the trachea to

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Table 1. — **Comparative biochemical measurements at two week of the study**

	Biochemical parameters				
Groups	AP (units/l)	GGT (units/l)	TB (mg/dl)	AST (units/l)	ALT (units/l)
Sham-control	179 ± 23	1.8 ± 0.4	0.3 ± 0.2	119 ± 8	57 ± 9
BDL/Untreated	396 ± 64*	59 ± 12*	13 ± 1.3*	1306 ± 246*	358 ± 42*
BDL/BB-882	230 ± 48*°	21 ± 2.5*°	7 ± 0.8*°	940 ± 115*°	180 ± 40*°

Values are means ± S.D. Abbreviations: AP, alkaline phosphatase ; GGT, γ-glutamyltranspeptidase ; TB ; Total bilirubin, AST, aspartate aminotransferase ; ALT, alanine aminotransferase.
* $p < 0.05$ compared with Sham-control.
° $p < 0.05$ compared with BDL/Untreated.

assist the spontaneous respiration. After opening the abdomen by 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) (units/l), aspartate aminotransferase (AST) (units/l), alkaline phosphatase (AP) (units/l), γ-glutamyltranspeptidase (GGT) (units/l) and the concentrations of total bilirubin (TB) (mg/dl) in plasma were determined by standard auto-analyzer methods on a 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 10% formaldehyde solution overnight, embedded in paraffin, and cut into 5-mm thick sections ; stained either with hematoxylin-eosin (H&E) or Masson's trichrome for light microscopic analysis. Histopathological scoring of groups, for degree of hepatic 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 (15). 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 (Olympus Eyepiece Micrometer) magnification in 10 portal tracts per slide (n = 10 in each group).

The other piece was washed in ice-cold saline and homogenized in Tris-HCI buffer (0.1 M) pH 7.4. The homogenate was then centrifuged and supernatant obtained was used for the assay of various enzymes. Superoxide dismutase (SOD) was assayed according to the method of Misra and FridoVich (16) based on the inhibition of epinephrine auto-oxidation by the enzyme, Catalase (CAT) activity was measured by following decomposition of H2O2 according to the method of Beers and Sizer (17). Glutathione peroxidase (GSH-Px) was assayed by the method of Rotruck *et al.* (18) using H2O2 as substrate, those of control rats.

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* values of less than 0.05 were considered significant.

Results

The values of biochemical measurements for the different groups are shown in Table 1. AP, GGT, TB, AST and ALT levels were significantly increased in the BDL/Untreated and BDL/BB-882 groups in comparison with the sham-control group ($p < 0.05$, $p < 0.05$ *respectively*). However, AP, GGT, total bilirubin, AST and ALT values were decreased in BDL/BB-882 group when compared to BDL/Untreated ($p < 0.05$).

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 BDL/Untreated and BDL/BB-882 groups in comparison with the Sham-control group ($p < 0.05$, $p < 0.05$ *respectively*). However, these values were significantly increased in BDL/BB-882 group ($p < 0.05$) when compared to BDL/Untreated.

The mean count of polymorphonuclear leukocyte (PNL) in control group was less than one in portal spaces of hepatic parenchyma. The mean count of PNL was found as 35 ± 6 and 6 ± 2 in the BDL/Untreated and BDL/BB-882 groups, respectively. The number of PNL was significantly decreased in BDL/BB-882 group when compared to BDL/Untreated group ($p < 0.0001$).

No morphological damage was observed in any of the rats in the Sham-control group (Fig. 1A, B). In BDL/Untreated group, severe damage (dilated central veins, proliferation of portal and periportal biliary ductules with disorganization of the hepatocytes plates, dilated portal spaces and areas of PNL infiltrate and hepatocytes necrosis) was observed (Fig. 2A, B). In

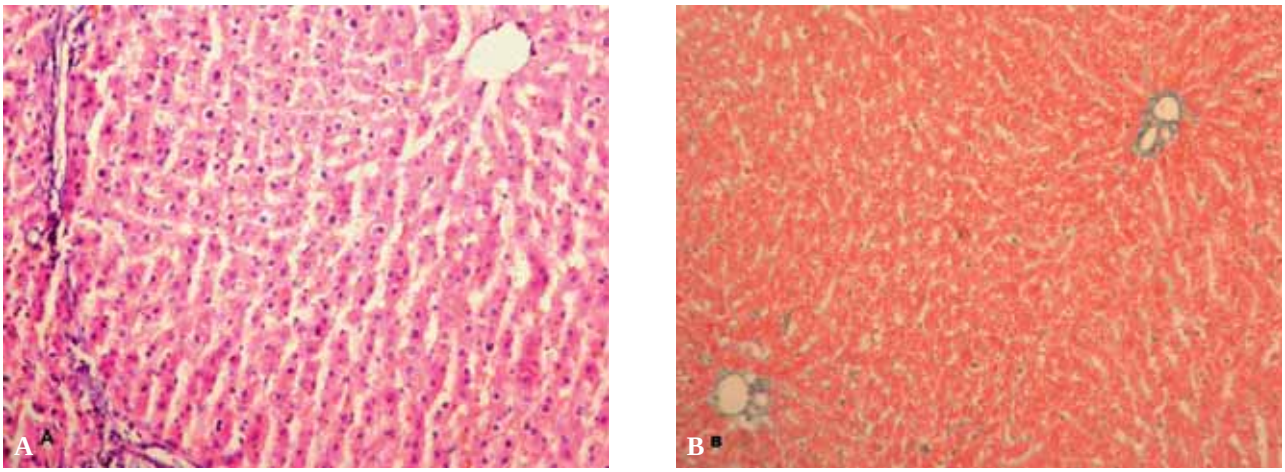

Fig. 1. — No morphological damage was observed in any of the rats in the sham-control group (A ; X200 H&E, B ; X200 Masson's trichrome).

Table 2. — **Comparative SOD, CAT ve GSH-Px measurements at two week of the study**

Groups	SOD (mg/dl)	CAT (mg/dl)	GSH-Px (mg/dl)
Sham-control	18 ± 2.7	6.1 ± 0.9	410 ± 57
BDL/Untreated	5.7 ± 1.9*	0.9 ± 0.1*	136 ± 17*
BDL/BB-882	9.6 ± 1.3*°	3.7 ± 0.8*°	264 ± 35*°

SOD ; Superoxide dismutase, CAT ; Catalase, GSH-Px ; Glutathione peroxidase.
* $p < 0.05$ compared with Sham-control.
° $p < 0.05$ compared with BDL/Untreated.

BDL/BB-882 group, mild damage (dilated central veins and minimal disorganization of the hepatocytes plates, rare PNL and hepatocytes necrosis) was seen (Fig. 3A, B).

The histopathological score was found as 0.1 ± 0.2 ; 3.2 ± 0.5 ; and 1.9 ± 0.6 in the Sham-control, BDL/Untreated and BDL/BB-882 groups, respectively. The histopathological score tended to be more in the BDL/Untreated and BDL/BB-882 groups compared with that in the Sham-control group rats *($p < 0.0001$ and $p < 0.0001$, respectively)*. However, the histopathological score was significantly less in the BDL/BB-882 group compared to the BDL/Untreated rats *($p < 0.05$)*.

Discussion

Here we explore the possibility that the administration of the PAF antagonist BB-882 could attenuate the effects of liver injury in BDL rats. Animals receiving BB-882 showed a marked reduction in disorganization of the hepatocytes plates, PNL infiltration and hepatocytes necrosis. We also found that treatment with BB-882 prevented the reduction in SOD, CAT and GSH-Px, probably by its free radical scavenging activity. The beneficial effects of BB-882 suggest that PAF contributes to the progression of hepatocytes necrosis in this model.

During cholestatic liver injury, the accumulation of bile acids in liver is thought to play a role causing hepatocyte necrosis (19). This is a dynamic process implying different rates of progression or regression. Therefore the histological examination of liver is essential for a diagnosis but biochemical tests are found to be necessary to assess the activity of process and monitoring its evolution. In this experimental study, the rats subjected to BDL for two weeks showed changes in plasma levels of bilirubin, AST, GGT and AP that indicate presence of cholestasis and diffuse hepatic injury. These observations are in agreement with those of several authors (20,21). The values of biochemical measurements were decreased in BDL/BB-882 group when compared to BDL/Untreated.

Some authors questioned the role of lipoperoxidation in hepatic damage induced by BDL (20,22). It was reported that BDL increases production of free radicals (23) and decrease free radical scavengers (GSH-Px, SOD and CAT), as we showed similar results in our study. Lipid peroxidation is one of the results of toxicity mediated by oxygen free-radicals. Lipid peroxidation represents oxidative tissue damage by hydrogen peroxide (H_2O_2), superoxide (O_2^-) and hydroxyl radicals (OH^-), resulting in structural alteration to membranes with release of cell and organelle contents, loss of essential fatty acids with formation of cytosolic aldehyde and peroxide products (24,25). The cell is endowed with several antioxidant systems to limit the extent of lipid peroxidation ; these include the enzyme CAT, SOD and GSH-Px (26). The development of tissue injury probably depends on the balance of the generation of reactive oxygen species and the tissue antioxidant defense mechanism (27). PAF is an oxidized phospholipid. Oxidative mechanisms associated with vascular damage have been reported in studies of PAF-mediated cellular injury both in vivo and in vitro (28-31). This PAF-induced effect can be inhibited by antioxidants such as SOD and catalase (28). The protective effect of SOD has also been

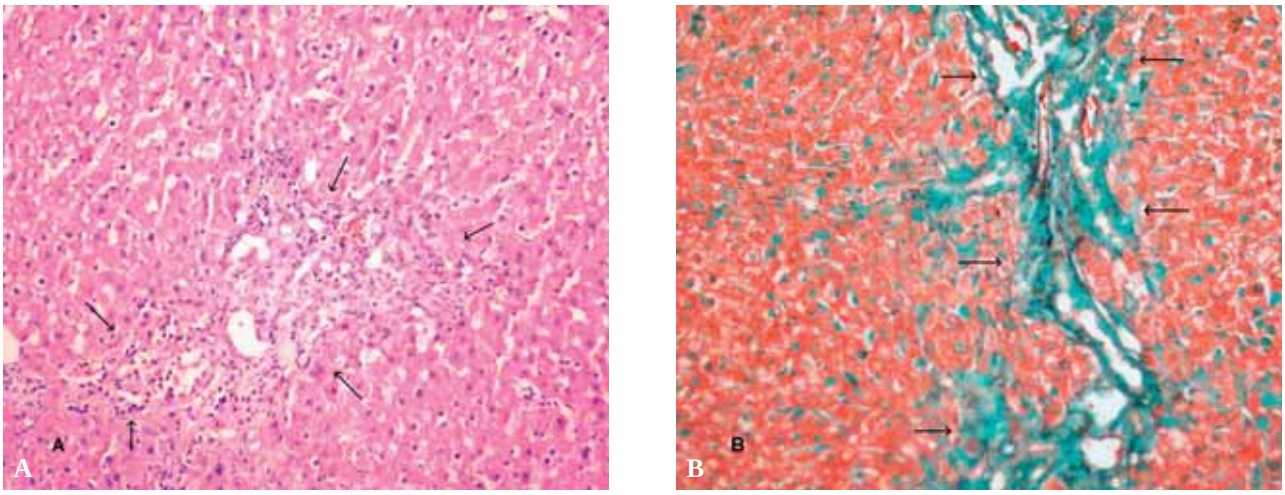

Fig. 2. — In the BDL/Untreated group, proliferation of portal and periportal biliary ductules with disorganization of the hepatocytes plates, dilated portal spaces and areas of polymorphonuclear leukocyte infiltrate, hepatocytes necrosis and fibrosis were observed (A ; X200 H&E, B ; X200 Masson's trichrome).

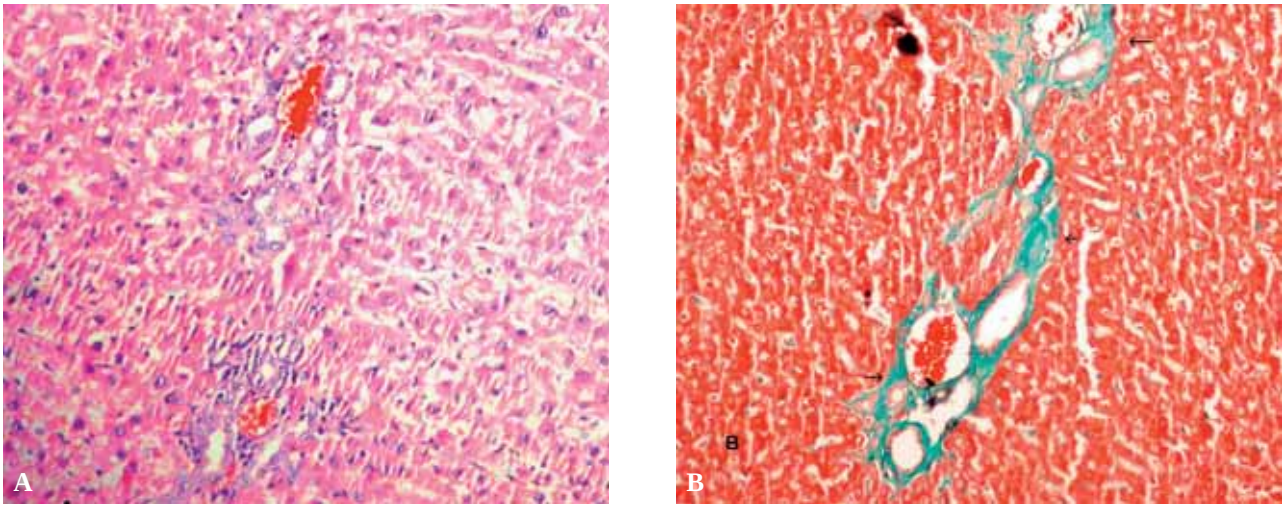

Fig. 3. — Hepatocytes necrosis and fibrosis were mild present in the BDL/BB-882 group (A ; X200 H&E, B ; X200 Masson's trichrome).

demonstrated in experimentally induced small intestinal vascular damage in rats, in which edema and neutrophil invasion induced by ischemia and reperfusion were reduced significantly by SOD and PAF antagonist (29). In our study, BB-882 protected the levels of α antioxidant enzymes such as SOD, CAT and GSH-Px. These results suggest that a balance between superoxide generation and its metabolism may determine endothelial responses to PAF and the consequences induced by PAF damage (30,31).

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 ; experimental studies showed that neutrophils infiltrate the liver within 3 h of BDL (32), and remain there for days to weeks as fibrosis progresses (32-34). A connection between neutrophils and liver fibrosis was first suggested by Parola *et al.* (33). They quantified 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 our study, we evaluated the protective effect of PAF antagonist BB-882 on liver fibrosis caused by bile duct ligation in rats. PAF is a phospholipid chemical mediator that was shown to have a variety of biologic activities and to be produced by inflammatory cells, endothelial cells edema, tissue injury and increase in vascular permeability (34). It may act as an intercellular signal responsible for cell communication and an inflammatory mediator involved in the pathogenesis of inflammation. Major contributions of PAF during the inflammatory reaction

include acting as an inflammatory stimulator to the leukocyte system, causing leukocyte rolling on the endothelium, adhesion and passage through inter-endothelial cells to the interstitium (35). Leukocyte-endothelial cell interaction leads to excessive movement of leucocytes to the tissues, as well as endothelial barrier compromise, responsible for initiation of the inflammatory reaction, serious tissue injury and organ dysfunction. Leukocyte recruitment involves transient tethering and rolling of leucocytes along endothelial cells, triggering of signals that activate up-regulation of leukocyte function, tight adhesion of leucocytes to the vascular endothelium and transendothelial migration (36). BB-882 is one of the most powerful PAF antagonists so far developed. It is suggested that BB-882 blocks PAF receptors on the surface of endothelial cells, thus reducing circulating PAF and so down-regulating the activation of leucocytes/macrophages and the interaction between leucocytes and endothelial cells, and maintaining the integrity of the endothelial barrier (13,37). We, here, observed an important decreased in periductal inflammation, bile duct proliferation and portal fibrosis in the BB-882 treated group in comparison with the BDL/Untreated group. The results show that increases in polymorphonuclear cell infiltration (evaluated by tissue histology) induced in liver by BDL was abolished by the administration of the PAF antagonist. The fact that BB-882 could prevent neutrophil recruitment suggests that PAF is a necessary step for subsequent polymorphonuclear leucocyte accumulation and fibrosis.

In conclusion, these results suggest that BB-882 (lexipafant) may reduce the severity of the inflammatory response to liver injury produced by bile duct ligation in rats and may have a clinical therapeutic role.

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