Nusret AKYÜREK[1]
Mustafa KEREK[2]
Erdoğan M. SÖZÜER[2]
Murat ALPER[1]
Sabahattin MUHTAROĞLU[4]
Mustafa KEÇELİ[5]

Effects of PAF Antagonist BN52021 on Systemic and Renal Oxidative Stress in Experimentally Induced Obstructive Jaundice

[1]Department of General Surgery, Faculty of Medicine Gazi University, Ankara,
Departments of [2]General Surgery, [4]Biochemistry, Faculty of Medicine , Erciyes University, Kayseri,
[3]Department of Pathology, Faculty of Medicine, Abant İzzet Baysal University, Düzce,
[5]Department of 4th Surgery Ankara Training and Research Hospital, Ankara – TURKEY

Abstract: The aim of this study is to investigate the effects of PAF antagonist BN52021 on systemic and renal oxidative stress in experimentally induced obstructive jaundice.

A total of 30 Wistar-Albino type rats used in the study were divided into sham, control and study groups, each consisting of ten subjects. A laparotomy was performed on the study and control groups, and the choledochus was ligated and dissected. In the sham group, the choledochus was dissected by laparotomy and not ligated. The subjects in the control group were administered physiological saline intraperiotoneally between the 2nd and 7th days, and subjects in the study group were administered BN52021 at a dose of 1 mg/kg/day. Blood and kidney samples were taken postoperatively on the 7th day. In the blood sample, bilirubine, SOD, GSH-PX, BUN, creatinine determination, and in renal tissue, MDA and histopathologic examination, were performed.

Serum bilirubine, blood urea nitrogen, creatinine, renal and, within erythrocyte, MDA levels were all measured on the 7th day in the study and the working groups' levels were significantly higher than those in the sham group. In BN52021 administered subjects, except the billirubine parameter measured on the 7th day, other parameters were found to be significantly lower than in the control group. The administration of BN52021 brought about a significant rise in the levels of endogenous antioxidant, but it did not cause any significant change in renal hystopathologic scoring.

The administration of BN52021 in experimental obstructive jaundice, improves renal functions by increasing endogenous antioxidant enzyme levels and by decreasing lipid peroxidation.

Key Words: Obstructive jaundice, PAF antagonist, renal failure, systemic stress

Introduction

Acute Renal Failure (ARF) is one of the most frequently encountered complications of obstructive jaundice (1). In about 60-75% of subjects with obstructive jaundice, a decrease in the Glomerule Filtration Rate (GFR) is observed in the postoperative period. Some 8-18% of subjects with obstructive jaundice present acute renal failure (ARF) (2). Mortality rate in the patients who develop renal failure is between 25 and 100% (1, 2).

The mechanism in the development of ARF in patients with extrahepatic cholestasis is of a multi-factor type; decrease in cardiac output, reduced peripheral vasoconstriction, hypovolemia and elevated renal vasoconstriction cause decreases in GFR (3). In additon, increased prostaglandin E_2, prostaglandin I_2 and thromboxane A_2 are also held responsible for the decrease in GFR (3). Biliary acids and conjugated bilirubine result in tubular necrosis by their ischemic effects and via direct toxic effects (4).

An increase in the levels of free oxygen radicals (FOR) and in the levels of endogenous antioxidant enzyme play an important role in renal malfunctions observed in obstructive jaundice (5).

Platelet activating factor (PAF) is a phospholipid origin mediator, which plays an important role in the tissue damage observed in obstructive jaundice. It is formed as a result of the activation of phospholipase A_2, and has the chemical structure of acetyl glycerol ether phosphocholine (6). It causes vasoconstriction and permeability increase by altering the shape of PAF endothelial cells (7).

In this study, the effects of BN52021, a PAF antagonist, on endogenous anti-oxidant enzyme levels and on renal functions were investigated in rats whose extrahepatic biliary duct was ligated.

Materials and Methods

This study was carried out in Erciyes University, Experimental and Clinical Research Centre, upon the approval of the Ethics Committee of the Faculty of Medicine, Erciyes University.

Experimental Method

Male Wistar-Albino type rats, having weights between 235 and 280 g, were used in the study. The animals were fed with standard laboratory nutrition. They were divided into three groups, each with 10 subjects. Intraperitoneally applied ketamine-HCl (10 mg/kg) was used as the anaesthetic agent.

Sham Group: After administering the anaesthetic agent, a standard midline incision laparotomy was performed under sterile conditions, and the choledochus was turned, but not ligated. No other substance was given. The abdomen was closed with a two-fold suture using 4/0 silk. On the post-operative 7th day, the rats were given ketamine-HCl at dose of 10 mg/kg, and the abdomen was opened from the previous incision line under sterile conditions. A 4 ml blood sample was taken from the heart, and both kidneys were extracted and the animals were then sacrificed.

Control Group: After administering the anaesthetic agent, a standard midline incision laparotomy was performed under sterile conditions, and the choledochus was ligated-divided, and was ligated with 6/0 silk, near the liver hilus, and the choledochus was dissected (8). The abdomen was closed two-fold using 4/0 suture. Between the post-operative 2nd and 7th days physiological saline was administered intraperitoneally. On the post-operative 7th day, the rats were given ketamine-HCl at dose of 10 mg/kg, and the abdomen was opened from the previous incision line under sterile conditions. A 4 ml blood sample was taken from the heart, and the two kidneys were extracted and the animals were then sacrificed.

Study Group: They were treated in the same way as the control group, and the choledochus was ligated to induce obstructive jaundice. This group was administered BN52021 (Gingolide B, BIOMOL Research Lab) at 0.2 mg/kg/day intraperitoneally on the post-operative 2nd and 7th days. On the post-operative 7th day, tissue and blood samples were taken (9). The subject was then sacrificed.

From the blood sample taken, levels of serum billirubin, BUN and creatinine (Cre) were measured. In erythrocyte, levels of superoxide dismutase (SOD), glutathione peroxidase (GSH-PX) and malondialdehyde (MDA) were measured. In addition, a level of MDA in renal tissue was measured and histopathologic examinations were performed.

Biochemical Analysis

The blood samples taken were separated into two separate tubes, with heparin and without heparin. GSH-PX, SOD and MDA levels were measured from the blood sample in the tube with heparin. From the blood sample in the tube without heparine, levels of serum billirubine, BUN and Cre were measured. The blood samples were centrifuged at 3,000 rpm for 10 min and their sera were separated. Remaining erythrocytes were washed three times with 0.9% NaCl solution. The serum and washed erythrocyte samples were stored at –70oC until the experiment date. Total bilirubine, BUN, CRE in sera were analysed by a Technicon RAXT automatic analyser.

GSH-PX and SOD determination within Erythrocyte Sample

The erythrocyte samples previously stored at –70ºC were allowed to dissolved in the room temperature, and determination of glutathione peroxsidase (RANSEL, RS-504, Randox) and SOD (RANSOD, SD 125, Randox) as performed (U/g Hb).

Determination Of Lipid Peroxidation In Kidney And Within Erythrocyte

One of the extracted kidneys was homogenised by adding 9 ml of 1.15% KCl solution on 1 g wet tissue, using a Bilser BH-105H model homogeniser with a Teflon tip. Kidney and erythrocyte samples were preserved at –70ºC until the experiment date. When making determinations, they were dissolved at room temperature and, using the Ohkawa Method, on 0.1 ml tissue or a blood sample was respectively added to 0.2 ml sodium dodecyl sulphate (SDS), 1.5 ml tiobarbituric acid (TBA) and 1.5 ml acetic acid, and the volume of the mixture was made up to 4 ml with distilled water. The mixture was kept at 95ºC for an hour, and after cooling, 5 ml butanol / pyridine and 1 ml distilled water were added. It was then centrifuged at 4,000 rpm, and the absorbance of the supernatant part was measured at 532 nm, using a Shimadzu–UV 160 A model spectrophotometer (10) (Renal tissue MDA: nmol MDA/g wet tissue and within erythrocyte MDA: nmol/ g Hb).

Histopathological Evaluation

After the tissues were fixed in neutral buffer formaline solution, they were subjected to routine tissue procedures, and paraffin blocks were prepared. Sections of 5-µm thickness were stained with haematoxylin–eosine and examined under a light microscope. Findings concerning acute renal failure were scored from 0 to 10. Under x40 magnification, 100 tubules were counted in 10 different regions. Tubular cytoplasm vacuolisation was scored as 0 or 1 (0 = absent, 1 = present), brush-lie side loss as (1), flattening in tubular epithelium as (1), cell necrosis as (1 or 2), and the means of the scores was taken (11).

Statistical analysis

ANOVA (for Post Anova, Scheffe) test was used in the comparison of parameters among all three groups. Statistical calculations were performed using SPSS Program under Windows. Values of $p < 0.05$ were taken as significant (12).

Findings

Total bilirubine values measured in the control and study groups on the 7th day were found to be significantly higher than in the sham group ($p < 0.001$, Table I). However, there was no difference between the control and study groups ($p > 0.05$, Table I).

BUN and CRE levels measured in the control and study groups on the 7th day were found to be significantly higher than in the sham group ($p < 0.001$, Table I). On the other hand, these values were lower in the study group than in those of the control group ($p < 0.05$, Table I).

Renal MDA levels and MDA levels within erythrocyte in the control and study groups were significantly higher than the sham group, and SOD and GSH-PX levels were significantly lower ($p < 0.001$ or $p < 0.05$, Table I). In addition, renal and erythrocyte MDA levels in the study groups were found to be significantly lower than in the control group, while SOD and GSH-PX levels of erythrocyte were significantly higher ($p < 0.05$, Table 1).

While the histologic scoring performed on the 7th day in the control and study groups was significantly higher when compared to the sham group, these two groups did not have any difference between each other ($p > 0.05$, Table II). Histopathologic changes, especially tubular cytoplasmic vacuolisation, were observed. Necrosis was not seen in any of the subjects.

	Sham (X±SD)	Control (X±SD)	Study (X±SD)	p*	p**	p***
TBIL (mg/dl)	0.62±0.1	8.10±0.4	7.1+0.2	<0.001	<0.001	>0.05
BUN (mg/dl)	21±2	65±12	34±7	<0.001	<0.001	<0.05
CRE (mg/dl)	0.5±0.1	2.1±0.2	1.1±0.2	<0.001	<0.001	<0.05
SOD (U/g Hb)	907±29	714±16	798+23	<0.001	<0.001	<0.001
GSH-PX (U/g Hb)	1321±37	781+24	901±28	<0.001	<0.001	<0.05
MDA (nmol/g Hb)	1.4±0.1	3.2±0.4	2.1±0.5	<0.001	<0.001	<0.05
Renal MDA(nmol MDA/g wet tissue)	66±23	147±38	112±26	<0.001	<0.05	<0.05

Table 1. Comparison of biochemical changes among the groups.

*   Comparison of sham and control groups   *** Comparison of control and study groups
** Comparison of sham and study groups   (ANOVA Post-Hoc Sheffe test)

	Sham (X±SD)	Control (X±SD)	Study (X±SD)	p*	p**	p***
Score	0±0	1.28±0.2	1.21±0.1	<0.05	<0.05	>0.05

Table 2. Comparison of Histologic Scoring.

*   Comparison of sham and control groups   *** Comparison of control and study groups
** Comparison of sham and study groups   (ANOVA Post-Hoc Sheffe test)

Discussion

Although ARF observed in obstructive jaundice was defined several years ago, its pathology has not been clearly defined. However, several factors have been separately shown to have effects. Among these factors are, deposition of bilirubine and biliary salts into tubules, decreased cardiac pulse, hypovolaemia arising from long-term inability of aural feeding, Tumor Necrosis Factor (TNF), cytokines such as PAF, prosto-glandines and endotoxemia (1-3). The secretion of urine and fractional sodium and potassium in the presence of obstructive jaundice increases (13).

Micro-perfusion studies have shown that biliary acids decrease fluid absorption from proximal tubules (14). This effect may arise from the detergent effect of biliary acids, as well as from changes in cyclo-oxygenase (4). On the other hand, in chronic cholestasis, biliary acids containing sulphate, suppress the pump functions of Na-K ATPase (15). It has been shown that in animals with a ligated common biliary duct, water intake decreases by 60%, creatinine clearance and total body fluid decrease by 15%, extracellular fluid by 24%, and plasma volume by 15%, after 12 days (16).

PAF is a mediator playing important roles in the formation of tissue damage and renal failure observed in obstructive jaundice (6). Due to the effects of PAF, liver secretes TNF; interleukin-6 while an increase is also observed in the absorption of endotoxins (17, 18).

Renal intraarterial exogen results in a PAF infusion decrease in GFR and the oliguria. These effects are prevented with PAF antagonist SRI 63-675 (19). In another study, it was observed that this PAF increase effect depends on the dose, and that with BN52021 significant decreases are observed in GFR (20).

Endotoxin plays a significant role in renal malfunction observed in obstructive jaundice. In an ARF model, in which exogen endotoxin data are induced, the PAF Antagonist BN52021 caused significant increases in GFR (19). In post-ischemic ARF, PAF levels were found to be significantly higher than the control group, and decreases were observed in renal functions after the administration of BN 52021 (9). In patients who were followed due to renal failure caused by sepsis, PAF level was found to be significantly high (21).

In obstructive jaundice models induced in rats, liver PAF levels increased 6-fold after 24 hours, and reached a peak on the 7th day. For this reason, we gave PAF antagonist BN52021 to the subjects starting from the 2nd day.

In our study, BUN and creatinine levels in the study group were found to be significantly lower ($p<0.05$, Table 1). However, no significant change was observed in histopathologic scoring ($p>0.05$). This can be explained by the antagonisation of the vasoconstriction effect of BN52021, which has been shown in previous studies. In the experimental obstructive jaundice study of Zhou et al. (6), the level of tromboxane B2, a strong vasoconstrictor, which was found to be high on the 7th day, was significantly reduced with BN52021 and WEB1270 (6).

As a result of the passage of bilirubine and biliary salts into systemic circulation, mitochondrial oxidative phosphorilysis in renal tubules becomes disrupted and causes FOR formation. Biliary salts prevent ATP formation, and cause damage in renal lysosome and cell membranes. This renal damage brings about results similar to those caused by ischaemia. As a result, decreases in glomerule hydrostatic pressure, loss of glomerule permeability and tubular back infiltration are observed. Increased PAF levels in obstructive jaundice and its ischaemic effects may be held responsible for the occurrence of these results.

Several studies have shown that FORs and lipid peroxidase play important roles in tissue and renal damage which occur in obstructive jaundice (5). In rats with the common biliary duct ligated, decreases in plasma A and vitamin E levels have been observed starting from the 4th day of ligation (22). In the same study, decreases in glutathione, GSH-PX and SOD levels have been reported too. In another work, while MDA levels, which is the final lipid peroxidation product in obstructive jaundice, were found to be high, significant decreases were observed in the levels of plasma glutathione, GSH-PX, SOD, catalase, selenium, glutathione and glutathione transferase (23). The occurrence of the suppression in microsomal P-450, an important endogenous antioxidant in obstructive jaundice, increases damage caused by oxygen radicals (24). In a study carried out by Yuceyar et al. (5), a temporary renal ischaemia was induced in rats with the common biliary duct ligated, and renal dysfunction was found to be more profound in this group. It was shown in the same study that decreases in the levels of SOD and GSH-PX, which are among the antioxidant enzymes, played important roles in renal functions (5).

Bautista and Spitzer, in their work, showed that PAF is a very important mediator in the formation of SOR, and that it significantly decreased with SDZ 63-441, a PAF antagonist (25).

In our study, the SOD and GSH-PX levels within erythrocytes determined on the 7th day were significantly lower in the control and study groups than in the sham group. Treatment with BN52021 significantly increased levels of endogenous antioxidant enzymes (Table I). Renal MDA levels and MDA levels within erythrocyte were found to be significantly higher in rats with induced obstructive jaundice than in the sham group (Table I).

In conclusion, the administration of BN52021 in experimental obstructive jaundice improved renal functions by increasing levels of antioxidant enzymes and by decreasing levels of systemic and renal MDA.

Correspondence author:

Nusret AKYÜREK

Kaptanpaşa Sokak No : 27/5

06700 Gaziosmanpaşa/Ankara – TURKEY

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