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Effect of beraprost sodium (BPS) in a new rat partial unilateral ureteral obstruction model

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ABSTRACT

Unilateral ureteral obstruction (UUO) is a representative model for investigating the common mechanism of decreasing renal function in chronic renal failure. In this study, we present a new partial UUO model in adult rats and evaluated the effect of beraprost sodium (BPS: stable prostaglandin I_2 (PGI_2) analog). We could make reproductive and uniform partial UUO by ligating the left ureter together with a 0.5 mm diameter stainless steel wire with nylon thread, and withdrawing the stainless wire. One week later, the ureteral obstruction was released. After 3 weeks from the release of UUO, all animals of control group, without BPS administration, developed basophilic degeneration of tubular epithelium, tubular dilatation and interstitial fibrosis. The areas of tubular degeneration and fibrosis were significantly reduced in the BPS group, orally administered BPS 300 μg/kg twice a day from the next day of the release of obstruction, than in control group.

In conclusion, we can established the adult rat partial UUO-release model and revealed that BPS can inhibit renal tubular damage and tubulointerstitial fibrosis.

1. Introduction

Congenital ureteral obstruction is the most important cause of end-stage renal failure especially in children [3]. In adults, acquired ureteral obstruction due to prostatic hyperplasia can be the cause of decreased renal function [8]. On the other hand, it is believed that there is a common mechanism that does not depend on causal disease for the progression of renal failure. It has been reported that histological evaluations revealed that the degree of tubulointerstitial damage is more closely associated with decreasing renal function than glomerular damage [16] in humans and other animals. Then studies are being actively encouraged to elucidate the mechanism of the progression of tubulointerstitial damage in various animal models.

The unilateral ureteral obstruction (UUO) is widely studied in animals, and this model is a suitable investigative tool for the common mechanism of decreasing renal function, since it can reliably induce tubular damage and interstitial fibrosis [9]. In the rat UUO model, the rapid expansion of the renal pelvis area and significant thinning of the renal parenchyma develop in the obstructed side of the kidney, and in the renal parenchyma, tubular damage and interstitial fibrosis are induced [27]. However, since most of the clinical ureteral obstruction is incomplete, partial UUO models have been developed to better simulate clinical conditions to elucidate the progression mechanism of kidney damage and study the effects of pharmaceuticals [23,26]. In this study, we tried to produce a new partial unilateral ureteral obstruction model in adult rats. Reliable and uniform partial ureteral obstructions were made by ligating the left ureter together with a 0.5 mm diameter stainless steel wire with nylon thread, and withdrawing the stainless wire. One week later, the rats underwent a laparotomy and the ureteral obstruction was released. It was demonstrated that, after 3 weeks of the release of ureteral obstruction, the rats developed renal tissue damage, i.e., tubular desquamation and interstitial fibrosis similar to human ureteral obstruction.

Prostaglandin I_2 (PGI_2) is one of the most important arachidonic acid metabolites generated in the kidney [14]. It has recently been noted again that endogenous PGI_2 has an important role in the renal development and functional retention, since prostacyclin synthase knockout mice develop significant renal damage [33]. Beraprost sodium (BPS) is a stable PGI_2 derivative that can be orally administered. It acts through PGI_2 receptors and expresses pharmacological effects similar to those of PGI_2 [17,22], including the following: antiplatelet effect [17]; vasodilating effect in a variety of organs [1]; inhibitory effect on growth of

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smooth muscle cells [6]; protective effect for vascular endothelial cells [7]; and repression of proinflammatory cytokines [13]. While BPS is clinically used in patients with chronic arterial occlusive disease and pulmonary arterial hypertension, it is also used in patients with chronic renal disease to suppress the decline in renal blood flow and function in patients with chronic renal insufficiency [4] and to inhibit an increase in urinary protein in patients with diabetic nephropathy [21]. BPS has demonstrated efficacy in rat models of GBM nephritis [11,28], diabetic nephropathy [30], contrast-induced nephropathy [31], and drug-induced nephrotoxicity [12]. However, no study has been reported so far using a UUO model.

In this study, we investigated the effect of BPS on the ureteral obstruction in a newly developed adult rat partial UUO-release model, focusing on the changes of tissue lesions.

2. Methods

2.1. Animals

We used Wistar male rats aged 8 weeks (Charles River Japan, Tokyo, Japan) after conditioning for 1 week.

2.2. Methods

2.2.1. Preparation of partial unilateral ureteral obstruction-release rats

The studies were performed in accordance with the University of Tokyo Agriculture and Technology "Guide for Care and Use of Laboratory Animals." After premedication with propionylpromazine 0.5 mg/head and Ketalar 2.5 mg/head, rats were anesthetized with fluothane and maintained. Partial unilateral ureteral obstruction was created by performing an abdominal midline incision, separating the left ureter, ligating ureter with 4-0 nylon thread together with a 0.5 mm diameter stainless steel wire, and withdrawing the stainless wire after the ligation. One week later, the rats underwent a laparotomy under anesthesia and the ureteral obstruction was released. The rats were divided into two groups: those that received BPS (Totay Industries, Inc., Tokyo, Japan) 300 μg/kg in distilled water at 12-h intervals for 3 weeks from the next day of the release of partial ureteral obstruction (BPS group) and those without the BPS treatment (Control group). After 3 weeks from the release of ureteral obstruction, blood, urine, and kidneys were collected from both the BPS and control rats anesthetized with pentobarbital sodium for analysis. We also established a group where we performed a laparotomy without a ureteral obstruction, maintained for 4 weeks, and then performed an autopsy (sham group).

2.2.2. Measurement of blood and urine parameters

Blood and urine samples of the animals of all groups were measured immediately after collection at autopsy. Red blood cell (RBC) count, white blood cell count, platelet count, hemoglobin (Hb), packed cell volume (PCV), sodium (Na), potassium (K), and chloride (Cl) as blood parameters using an automatic cell counter (Nihon Kohden, Tokyo, Japan). Also, blood chemistry parameters such as total protein (TP), total cholesterol (Tchol), blood urea nitrogen (BUN), creatinine (Cre), and urinary pH were measured (Fukuyama Rinsho, Hiroshima, Japan). Urine protein were estimated by test paper and graded 0 (none) to 3 (severe).

2.2.3. Histopathology

Tissue segments were cut in round slices at a thickness of 3 mm from the renal hilus of the left kidneys collected at autopsy, in parallel with the flow direction of renal arteries and veins, and immersed and fixed in methanol-Carnoy's solution. The fixed tissue segments were then paraffin embedded according to the standard procedure and serial sections of 5 μm thickness were prepared. The renal tissues were stained with periodic acid—Schiff, hematoxylin–eosin (HE), and Sirius red for the evaluation of renal damage. Basophilic degeneration of tubular epithelium and tubular dilatation of the sham, control, and BPS groups were semi-quantitatively evaluated. Briefly, both alteration were separately observed in 20 randomly chosen high-power fields (40 ×) and graded according to the area of alteration from 0 to 5+ as follows: 0 no lesion, 1+ less than 10%, 2+ 10–25%, 3+ 25–50%, 4+ 50–75%, 5+ more than 75% in each left kidney. For the quantification of fibrosis areas, images of Sirius red-stained kidney sections of the sham, control and BPS groups imported into a computer were used. Briefly, five high-power fields in each left kidney were randomly selected to extract stained areas and calculate fibrosis area rate (except glomeruli) by an image analysis system (MacSCOPE, Mitani Corporation, Tokyo, Japan).

2.2.4. Statistical analysis

Basophilic degeneration of tubular epithelium, tubular dilatation, and fibrosis area of each animal was calculated by averaging value of each field. The average and standard deviation values of the sham, the control, and the BPS group were calculated and compared between the groups by the Wilcoxon test (semi-quantitative analysis) and Welch's test (quantitative analysis) (EXSUS, Arm Systex Co., Ltd., Osaka, Japan). Also, blood and urine parameters were compared between the groups and Student's *t* test was used for the comparison. Differences were considered statistically significant with the *P* value less than 0.05.

3. Results

Table 1 lists the blood and urine parameters of the sham, control, and the BPS group. Only the blood total protein was significantly low in control group compared with the sham group. This decrease was reversed by the BPS treatment. As for renal parameters, BUN, Cre, and urinary protein were slightly high in the control group compared with the sham group, but there was no significant difference.

In the sham group, no significant tissue change was seen in kidney (Fig. 1). In the control group, all animals revealed interstitial fibrosis in the renal parenchyma area lateral to the renal pelvis. Quantitative evaluation revealed fibrosis area rate was high compared with the sham group (Fig. 2). There were

Table 1

	Beraprost group Mean ± SE	Control group Mean ± SE	Sham group Mean ± SE
BW (g)	401.7 ± 5.2	385.0 ± 18.1	370 ± 4
RBC (10^6/μl)	752.0 ± 27.4	730.2 ± 19.2	705 ± 25
PCV (%)	45.7 ± 2.1	44.1 ± 0.9	43.6 ± 1.2
Hb (g/dl)	14.2 ± 0.4	13.6 ± 0.3	14.2 ± 0.4
Na (meq/l)	143.2 ± 0.6	140.7 ± 1.5	141.0 ± 0.8
K (meq/l)	4.7 ± 0.8	4.5 ± 0.7	3.9 ± 0.16
Cl (meq/l)	93.3 ± 0.5	94.3 ± 1.3	95.8 ± 0.66
TP (g/dl)	6.1 ± 0.1**	5.5 ± 0.1##	6.1 ± 0.2
Tchol (mg/dl)	65.0 ± 3.1	59.7 ± 5.4	64.0 ± 2.7
BUN (mg/dl)	27.3 ± 1.2	41.7 ± 12.0	23.7 ± 0.9
Cre (mg/dl)	0.28 ± 0.01	0.77 ± 0.35	0.23 ± 0.01
Urine pH	7.8 ± 0.1	7.6 ± 0.2	8.1 ± 0.3
Urine protein (score)	1.8 ± 0.3	1.3 ± 0.5	0.3 ± 0.4

** $P < 0.01$ vs. control group.
$P < 0.01$ vs. sham group