Original Paper

Beraprost Sodium, a Stable Analogue of PGI₂, Inhibits the Renin-Angiotensin System in the Renal Tissues of Rats with Chronic Renal Failure

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Key Words

Pgi2 analogue • Chronic renal failure • Renin-angiotensin system • Beraprost sodium

Abstract

Background/Aims: Chronic renal failure (CRF) is a prolonged kidney condition characterized by decreased kidney function, and can develop into total kidney failure. The renin-angiotensin system (RAS) helps regulate the balance between human bodily fluids and electrolytes. The aim of this present study was to investigate the effects of a prostacyclin analogue (beraprost sodium [BPS]) on the expression of key factors associated with local RAS activities in the renal tissues of rats with CRF. **Methods:** After a CRF rat model was successfully established, the levels of BUN, SCr, phosphorus, and calcium were detected by an automatic biochemical analyzer. Furthermore, the activities of malondialdehyde (MDA) and superoxide dismutase (SOD) in rat renal tissues were measured using a colorimetric method, while the activity of angiotensin-converting enzyme (ACE) was determined by ultraviolet (UV) spectrophotometry. In situ hybridization was employed to determine the expression of angiotensin type 1 receptor (AT). Finally, the positive expression rates of cells expressing pro-apoptotic proteins (Bax and Bcl-2) were determined, and the protein and mRNA levels of phosphatidylinositol 3-kinase (AKT) and key factors involved in the RAS (AT1, AT2, angiotensinogen [AGT], ACE and angiotensinogen [AGT]) were evaluated by RT-qPCR and western blot analysis. **Results:** Initial observations revealed that treatment with BPS decreased the levels of BUN, SCr and phosphorus but increased calcium levels in the renal tissues of CRF rats. Additionally, BPS reduced the levels of MDA while increasing the levels of SOD, ACE activity, and AT1 expression in the renal tissues of CRF rats. BPS inhibited glomerular hypertension and hyperfiltration; increased the mRNA and protein levels of AKT and AT2; and decreased the mRNA and protein levels of AT1, AGT, and ACE in the renal tissues of CRF rats. **Conclusion:** The results of this study demonstrate that BPS, a PGI₂ analogue, inhibits the expression of

key factors involved in the local RAS, resulting in a delay in the occurrence and development of CRF. The key findings of the present study ultimately highlight the potential of this PGI2 analogue as a promising therapeutic strategy for treating CRF.

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Introduction

Chronic renal failure (CRF) is widely regarded as a condition characterized by irreversible degeneration of renal function associated with oxidative stress (OS) and inflammatory responses [1]. The mortality rate of CRF is approximately 0.64 cases per 100,000 per year, with higher rates observed among females and the elderly [2]. Evidence has suggested a correlation between CRF and low serum levels of 1, 25-dihydroxyvitamin D₃ (calcitriol or 1, 25[OH]₂D₃) as well as the reactive metabolite of vitamin D₃ due to a reduction in renal 1 α -hydroxylase (CYP27B1) [3]. CRF shares a strong association with older age, along with an age-induced decline in renal functions resulting from conditions including vascular disease, hypertension, hepatitis C (HCV) infection, and diabetes mellitus [4–6]. Investigation into mesenchymal stem cells as a potential therapy for kidney injuries reveals that when end-stage kidney disease is reached, renal transplantation is not the optimal therapeutic approach, but there are several limitations, including a lack of organ donors and immune reactions observed within the five-year posttransplantation period, and these limitations vary across different age groups [7, 8]. Treatments for CRF, including many current existing therapies that remain limited for a variety of reasons, could slow the progression to end-stage renal disease [9].

Prostacyclin (PGI₂) is widely considered a relaxing factor derived from endothelium-derived relaxing factor (EDRF) [10]. Beraprost sodium (BPS), a stable analogue of PGI₂, is capable of preventing apoptosis caused by hyperoxia and smoke-induced pulmonary endothelium-induced alterations in rats [11]. Furthermore, BPS suppresses the proliferation of high glucose-induced cells as well as the development of reactive oxygen species (ROS) to improve the antioxidant capacities of peritubular interstitial cells in rats [12]. The renin-angiotensin system (RAS), a constantly evolving part of the endocrine system, is often connected with the important balance between the generation and catabolism of angiotensin peptides, largely because of the extensive effects of angiotensin as well as the activation of the local RAS in certain cells, including pancreatic islet cells, macrophages and adipocytes, which can lead to dysregulated functions with promotion of OS, cell apoptosis, and inflammation [13]. RAS hyperactivity is closely related to the development of renal damage [14]. Thus, in the current study, we explored whether an analogue of PGI₂, exerts significant effects on the local RAS in the renal tissues of rats with CRF.

Materials and Methods

Experimental Statement

All procedures in this study were conducted in strict compliance with the Guide for the Care and Use of Laboratory Animals.

Establishment of CRF models

Fifty Sprague Dawley (SD) male rats weighing approximately 200 $\pm$ 30 g were provided by the Experimental Animal Center of the Chinese Academy of Sciences (Shanghai, China), raised in a Specific Pathogen-Free (SPF) environment and permitted free access to food and water. Following a week-long adaptive feeding process, the rats were separated into sham (n = 10) and CRF (n = 40) groups. A CRF rat model was established by performing a 5/6 renal resection and applying Platt's method. First, the rats were anesthetized via intraperitoneal injection of 100 mg/kg phenobarbital (HengYuan Biological Technology Co., Ltd., Shanghai, China). Next, ether (Liguan Chemical Co., Ltd., Shenzhen, China) was applied to induce

anesthesia, while the lower limbs of the rats were fixed on the operation table, with the rats placed in a supine position. The left kidney and left renal pedicles of the rats were exposed, and a 1.5 cm incision was made along the left rib and outer chamber from the peritoneum to expose the left kidney. After the perirenal fat and membrane were removed, 5/6 of the renal tissue was removed using a curve-shaped incision. A gelatin sponge was employed to help stop bleeding. Proper fibrinogen solution and thrombin solution (Baomeng Biotechnology Co., Ltd, Shanghai, China) were administered, and the remaining kidney was placed in its original position and sutured. The rats were observed to confirm the absence of active bleeding from the resection. After one week, 80% of the right kidney was resected. The sham operation comprised a ventral laparotomy and manipulation of the kidneys and renal pedicle without destruction of renal tissues. During this experiment, blood was collected from the rat tail vein every two weeks. After 30 d, the blood pressure of the rats was measured, and blood was collected from the celiac artery. A routine method was adopted to assess rat kidney function by measuring the levels of blood urea nitrogen (BUN) and serum creatinine (SCr). The results of these tests demonstrated that the kidney function indices in the CRF group were significantly higher than those in the sham group ($p < 0.05$), ultimately indicating the successful establishment of the CRF model. The experiment was repeated three times.

Treatment regimens

CRF rats were further randomly assigned to receive a high-, moderate- or low-dose of BPS (Tide Pharmaceutical Co. Ltd., Beijing, China; $n = 10$ per group). Intragastric administration was performed in rats in the CRF group and comprised 10 ml/mg normal saline every day and 50, 40 or 20 μ g BPS twice per day in the high-, moderate-, and low-dose groups, respectively. All rats were administered BPS continuously for 8 weeks. During the experiment, the sample size of each group was 10, and the data were measured three times.

Evaluation of renal hemodynamics

At eight weeks postoperation, a micropuncture study was conducted to evaluate renal hemodynamics. After ether and anesthesia methods were applied, the rats were placed on a thermoregulation table maintained at 37°C. A polyethylene pipe was used to cannulate the trachea, jugular veins, femoral arteries, and left ureter. The left kidney was exposed by a 1.5 cm incision in the waist. The left kidney was placed in a Lucite bracket, sealed, and covered with 0.9% saline water. A femoral catheter was used for blood sampling, and an additional catheter was used for a pressure sensor (Model p23 Db, Gould, Puerto Rico, USA) that was used to monitor mean arterial pressure (MAP), which was recorded on a polygraph (Grass Instruments, Quincy, MA, USA). During the procedure, the rats were injected with 6% albumin (1% body weight) through the jugular catheter using 5% glucose at a rate of 2.2 ml/h. Moreover, blood extracted from normal rats was used to replace the rat's own blood. After the experiment was completed, rat kidneys were removed and weighed. A micropuncture oil-based sealing was subsequently used to determine the flow speed and polyfructose content, which allowed us to calculate the single-nephron glomerular filtration rate (SNGFR). Samples from proximal tubule were then obtained from seven different nephrons, and polyfructose was detected in plasma and urine samples. The results were used to calculate the glomerular filtration rate.

Preparation of renal tissues from normal rats and rats with CRF

Eight weeks after intragastric administration, blood was obtained from the heart after the rats were anesthetized by 1.0 g/kg ethyl carbamate (EC) (Tong Xing Technology Co. Ltd., Wuhan, China). After the rats were sacrificed, the entire aorta abdominalis and kidney were dissected. Next, the collected aorta abdominalis was dried in an oven at 80°C and preserved at room temperature, while the left kidney samples were stored in a freezer at -80°C. The separated blood serum was then preserved in a freezer at -20°C.

Hematoxylin-eosin staining

Certain parts of the renal tissues were promptly extracted, fixed in 4% formaldehyde (Zone Young Chemical Sales Co. Ltd., Shandong, China) for 6 h and embedded in paraffin. The embedded tissues were then sliced into 5 μ m-thick sections. After the sections were dried at 60°C overnight, they were dewaxed in xylene I and xylene II for 20 min each; dehydrated in 100%, 100%, 95%, 80% and 70% ethanol in

succession for 5 min each, and finally placed in distilled water. The sections were stained with hematoxylin for 10 min, washed with running water to allow the color to return to blue, stained with eosin for 30 s and washed with double-distilled water to remove residual red staining. Next, the sections were dehydrated using ethanol, cleared with xylene, and mounted in neutral balsam. The pathological tissues were observed and photographed under a light microscope ($\times 200$) (cx40, Olympus Optical Co., Ltd., Tokyo, Japan).

Detection of BUN, SCr, calcium and phosphorus before and after BPS treatment

Serum (3 ml, nonanticoagulation) was obtained from the abdominal artery and permitted to stand at 4°C. After blood stratification, the serum was centrifuged at 1600 r/min at 4°C for 15 min and preserved at -80°C in tubes (110 μ l). The levels of BUN, SCr, phosphorus, and calcium were subsequently determined using an automatic biochemistry analyzer (Center for Drug Safety Evaluation, Shanghai University of Traditional Chinese Medicine, Shanghai, China).

Chemistry colorimetric method

A total of 3 ml blood serum was collected from the abdominal artery for hepatic free amino acid regulation (Scientan Co., Ltd, Beijing, China) and promptly centrifuged at 3500 r/min at 4°C for 15 min. The blood plasma was then collected and preserved at -70°C. The OS indices were detected based on the following steps: malondialdehyde (MDA) was detected by evaluating thiobarbituric acid reactive substances (TBARS), and superoxide dismutase (SOD) was determined by chromatometry. A microplate reader (Model550, Bio-Rad, Massachusetts, USA) was used for detection according to the instructions included with the kit (MDA: OxiSelect™, TBARS Assay kit, Jiamay Biotechnology, Beijing, China), with specific procedures completed by laboratory technicians at the Shanghai University of Traditional Chinese Medicine (Shanghai, China).

Ultraviolet (UV) spectrophotometry assay

Approximately 100 mg renal tissue was collected from each rat and added to 5 times the volume of 0.1 mol/l Tris-HCl buffer solution (pH = 7.8), completely ground in a tissue bath, and centrifuged. The supernatant was then discarded. Benzoyl glycolyl-glycylglycine was hydrolyzed in accordance with the procedure for benzoyl glycolyl and glycolyl-glycine to measure the effects of angiotensin-converting enzyme (ACE). Benzoyl glycolyl was extracted using a kit (Jiamay Biotechnology, Beijing, China) to a benzoyl glycolyl content of 228 mm, and a UV spectrophotometer (Model UV-2401PC, Shimadzu Corporation, Japan) was used to detect ACE activity.

In situ hybridization

Paraffin-embedded sections were dewaxed in a routine manner and treated with 3% H₂O₂ at room temperature for 1 h to inactivate endogenous peroxidase. The sections were later treated with a compound digestive solution with the addition of 10 μ l prehybridization solution for 30 min. The mixture was then incubated at 37°C for 1 h before 15 μ l probe was added. A special cover glass made for in situ hybridization was placed on the sections, which were then placed in a wet box at 42°C and incubated overnight. The sections were then covered with biotin-labeled mouse antidigoxin and incubated in a wet box at 37°C for 1 h. An avidin-biotin-peroxidase complex (ABC) was then added to the sections, and the sections were incubated in a wet box at 37°C for 40 min. The color was developed by adding diaminobenzidine (DAB). The tissues were counterstained with hematoxylin, dehydrated, cleared, and mounted. The experiments were conducted in accordance with the instructions of the in situ hybridization kit (Boster Biological Technical Co., Ltd., Wuhan, Hubei, China), and the results were analyzed using a Motic Med 6.0 pathology image analysis system (Beihang University, Beijing, China). Ten fields of renal tubular interstitial tissue were selected in each section to detect the proportion of the whole visible area that was positively labeled [(positive area/whole area) $\times$ 100%] in an attempt to obtain the mean value postdetection.

Immunohistochemistry

Preserved renal tissues were collected, fixed in 4% formaldehyde, embedded in paraffin using a routine procedure, and sliced into 5 μ m-thick serial sections. Paraffin-embedded sections were dewaxed and incubated with 3% H₂O₂ for 20 min to eliminate endogenous peroxidase activity. The sections then underwent antigen retrieval with citrate buffer (pH = 6.0) and were sealed with 5% bovine serum albumin

(BSA) sealing solution (Shanghai BioSun Sci & Tech Co., Ltd, Shanghai, China) before an overnight incubation at 4°C with primary antibodies (Bcl-2-associated X (Bax), ab32503, 1: 1000; B cell lymphoma 2 (Bcl-2), ab32124, 1: 1000; Abcam, Inc., Cambridge, MA, USA). Next, a biotin-labeled goat antirabbit immunoglobulin G (IgG) (H + L) (1: 1000, ComWin Biotech Co., Ltd., Beijing, China) secondary antibody was added, and the tissues were incubated at 37°C for 30 min. After the addition of streptavidin-biotin complex (SABC), the tissues were incubated at 37°C for 30 min and then developed using DAB (Jiuzhou Technology Co., Ltd., Beijing, China), counterstained with hematoxylin, dehydrated, cleared, and sealed using routine methods. Phosphate-buffered saline (PBS) was applied to wash the sections, and this treatment was used instead of the primary antibody as a negative control. The immunoreactive substance was represented by a brown-yellow color, with strong positive labeling indicated by a dark brown-yellow color, medium positive labeling indicated by a medium brown-yellow color, and weak positive labeling indicated by a light brown-yellow color.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

Based on the instructions included in a RT-qPCR kit (Solarbio Biotech Co., Ltd., Shanghai, China), total RNA was extracted from the renal tissues of rats in each group. The extracted RNA was used to determine OD260/280 values on a NanoDrop2000 ultramicrospectrophotometer, and RNA concentrations were calculated with the remaining content, which was preserved at -80°C. The Primer database and Primer Premier 5.0 were employed to design RT-qPCR sequences, which were then synthesized by the Beijing Genomics Institute (BGI) (Table 1). Reverse transcription was performed using total RNA based on the experimental procedure described for a Reverse Transcription System A3500 (Promega, Wisconsin, USA). After reverse transcription, extracted cDNA was mixed with a certain amount of diethyl phosphoryl cyanide (DEPC) solution and fully mixed. The RT-qPCR reaction mixture consisted of 5 µl Premix Ex Taq or SYBR Green Mix (2 ×), 0.5 µl forward primer (10 µM), 0.5 µl reverse primer (10 µM), and 4 µl cDNA template. A two-step method was also performed to facilitate the following required conditions: predenaturation at 95°C for 30 s, followed by 30 cycles of denaturation at 95°C for 30 s, annealing at 72°C for 1 min, and extension at 72°C for 5 min. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the internal reference, and the reliability of the PCR was evaluated using a melting curve. After the RT-qPCR reaction, the specificity of the primers was detected using a melting curve. The 2^{-ΔΔCt} assay was used to detect the mRNA expression of key RAS factors in renal tissues as follows: changes in the rate of multiplication = relative expression of the targeted genes calculated by the formula among which the ΔCt = Ct value / (target gene Ct value - reference gene Ct value). RT-qPCR was also applied to evaluate the expression of other apoptosis-related genes.

Western blot analysis

Renal tissues were lysed using a moderate lysis buffer. The bicinchoninic acid (BCA) method (Kongbai Biological Technology Co., Ltd., Shanghai, China) was used to analyze protein concentrations, and protein samples were added to loading buffer for albumin denaturation at 95°C for 8 min. The loaded samples (30 µg/20 µl) were then used for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The gels were blocked in skim milk, and the proteins were transferred to polyvinylidene fluoride (PVDF) membranes, which were incubated with antibodies for key RAS factors (angiotensin II receptor type

Table 1. Primer sequences of related genes analyzed with reverse transcription quantitative polymerase chain reaction (RT-qPCR). Note: AT1, angiotensin II type 1 receptor; AT2, angiotensin II type 2 receptor; ACE, angiotensin-converting enzyme; AGT, angiotensinogen; Bax, Bcl-2-associated X Protein; Bcl-2, B cell lymphoma/leukemia-2; AKT, protein kinase B or PKB; PCR, polymerase chain reaction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase

Gene	Primer sequence
AT1	5'- GCCCTGGCTGATTTATGC-3' 5'- GGAAAGGGAACACGAAGC-3'
AT2	5'- CCCTGGCAAGCATCTTATGTA-3' 5'- TCTAAACACACTACGGAGCT-3'
ACE	5'- GCAAGGAGGCAGGCTATGAG-3' 5'- CGGGTAAAACTGGAGGATGG-3'
AGT	5'- TTGTTGAGAGCTTGGGTCCCTTCA-3' 5'- CAGACACTGAGGTGCTGTTGTCCA-3'
Bax	5'- AACACATGGAGCTGCAGAGG-3' 5'- GAAGTTGCCGTCCTGCAACAT-3'
Bcl-2	5'- TTGAGTTCGGTGGGGTCATG-3' 5'- GATCCAGGTGTGCAGATGCC-3'
AKT	5'- TTTATTGGCTACAAGGAACG-3' 5'- AGTCTGAATGGCGGTGGT-3'
GAPDH	5'- GGGTGATGCTGGTGTGAGTATGT-3' 5'- AAGAAATGGGTGTTGCTGTTGAAGTC-3'

1 (AT1), angiotensin II receptor type 2 (AT2), ACE and angiotensinogen (AGT)) and phosphatidylinositol 3-kinase (AKT) using specific primary antibodies (AT1, ab4471, 1: 1000; AT2, ab2905, 1: 1000; ACE, ab86469, 1: 500; AGT, ab108384, 1: 1000; and AKT, ab81283, 1: 5000, Abcam, Inc., Cambridge, MA, USA). The membranes were then incubated with goat antirabbit IgG (H + L) secondary antibodies (1: 1000, Beijing ComWin Biotech Co., Ltd., Beijing, China) for hybridization at 37°C for 1.5 h. After this reaction, the membranes were washed 3 times for 10 min each. Next, the membranes were incubated with luminescence reagent for 5 min, exposed to an X-ray film, developed and fixed. Finally, Quantity One V4.6.2 software (Bio-Rad, Inc., Hercules, CA, USA) was used to perform a semiquantitative analysis, and the ratio of protein to internal gray value was regarded as the protein expression level of the target gene.

Statistical analysis

All statistical analyses were performed using SPSS 19.0 software (IBM Corp., Armonk, NY, USA). Each experiment was repeated 3 times, and the mean and standard deviations were calculated. Measurement data are presented as the mean $\pm$ standard deviation. Comparisons of curative effects were conducted using the chi-square test (χ^2 test). Comparisons of changes in BUN, SCr, phosphorus and calcium levels were analyzed by two-factor and two-level methods. Comparisons between two samples obtained at the same time point were analyzed using *t*-tests, while comparisons among different groups were performed by one-way analysis of variance (ANOVA). $p < 0.05$ was considered statistically significant.

Results

BPS attenuates CRF in rats

Hematoxylin and eosin (HE) staining methods were performed to detect pathological changes in renal tissues in each group. As shown in Fig. 1, the rats in the sham group exhibited a normal glomerulus structure, normal proportion of the glomerulus tissue and capsular space, and normal renal tubular wall thickness and lumen size. In the CRF group, we observed an abnormal glomerulus structure, a large number of brownish black crystals in renal tubules, expanded focal renal tubules, a small glomerulus and glomerulus, enlarged capsular

Fig. 1. HE staining results indicating that BPS attenuates CRF and that the renal tissues of rats treated with BPS show a generally normal glomerulus structure and lumen size, a normal proportion of the glomerulus ratio and capsular space, and a small amount of inflammatory cell infiltration ($\times 400$). Note: HE, hematoxylin-eosin; BPS, beraprost sodium; CRF, chronic renal failure.

space, asymmetrical renal tubular wall thickness, unequal and distended lumen size, and a large amount of inflammatory cell infiltration. The renal tissues of rats treated with a low dose of BPS exhibited a slightly improved morphological structure

in the renal tubules and glomerulus; however, inflammatory cell infiltration was still observed. In the moderate-dose group, the glomerulus exhibited a rather normal structure, slightly shrunken glomerulus, slightly altered capsular space in the glomerulus (but within normal proportions), reduced renal tubule swelling, and a slight degree of inflammatory cell infiltration. However, compared to renal tissues in the low and moderate-dose groups, renal tissues in the high-dose group displayed a generally normal glomerulus structure and lumen size, normal proportions of glomerulus tissue and capsular space, and a minor degree of inflammatory cell infiltration. These findings indicated that BPS may attenuate CRF in rats.

BPS decreases the levels of BUN, SCr and phosphorus while increasing calcium levels in CRF rat

The levels of BUN, SCr, phosphorus, and calcium were determined using an automatic biochemistry analyzer. Compared with renal tissues in the sham group, renal tissues in the CRF group exhibited markedly higher levels of BUN, SCr, and phosphorus and lower levels of calcium (all $p < 0.05$). After 8 weeks of BPS treatment, the levels of BUN, SCr, and phosphorus were lower, while calcium levels were remarkably increased in the low, moderate and high-dose groups, with the high-dose group exhibiting the most notable changes (all $p < 0.05$) (Table 2). Thus, BPS may decrease the levels of BUN, SCr, and phosphorus and increase the level of calcium in CRF rats.

BPS ameliorates CRF in renal hemodynamics

A micropuncture study was conducted to evaluate renal hemodynamics, and the results are shown in Fig. 2. Increases in MAP, SNGFR, single-nephron plasma flow (Qa), glomerular capillary pressure (PGC), perfusion pressure (SFP), and the hydrostatic pressure gradient (ΔP) were observed in CRF rats, and these changes were ameliorated by BPS. BPS prevented glomerular hyperfiltration (an increase in PGC) and hyperfiltration (an increase in SNGFR). In addition, decreases in afferent resistance (AR), efferent resistance (ER), and GFR were observed in CRF rats, and these changes were ameliorated by BPS. The most relevant changes observed in the BPS-treated group were the normalization of PGC and SNGFR, which were significantly increased in preglomerular tone, as indicated by an increase in AR.

BPS reduces MDA levels and increases SOD levels in CRF rats

The chemistry colorimetric method was performed to determine the serum levels of MDA and SOD. Compared with rats in the sham group, rats in the CRF group had remarkably lower SOD levels and notably higher levels of MDA. After 8 weeks of treatment, compared with rats in the CRF group, rats in the low-, moderate- and high-dose BPS groups all exhibited notably higher SOD levels and significantly lower MDA levels, with the high-dose group showing the most significant effects ($p < 0.05$). In the blood serum, MDA levels were significantly lower, while SOD levels were markedly higher in the BPS group than in the CRF group (all $p < 0.05$) (Table 3), indicating that BPS reduced the production of oxygen free radical generation via its antiperoxidation function, resulting in a delay in CRF.

Table 2. BPS decreases the levels of BUN, SCr, and phosphorus but increases the levels of calcium in CRF rats. Note: #, compared with the sham group, $p < 0.05$; *, compared with the CRF group, $p < 0.05$; BUN, blood urea nitrogen; SCr, serum creatinine; CRF, chronic renal failure

Group	Dose $\mu\text{g/d}$	BUN (mmol/l)	SCr (mmol/l)	Calcium (mmol/l)	Phosphorus (mmol/l)
Sham group	—	6.13 ± 0.93	36.49 ± 3.298	2.49 ± 0.08	1.37 ± 0.21
CRF group	—	$13.76 \pm 1.21^\#$	$72.28 \pm 7.24^\#$	$1.27 \pm 0.19^\#$	$2.74 \pm 0.23^\#$
Low-dose group	80	$10.74 \pm 0.83^{**}$	$63.65 \pm 5.32^{**}$	$1.83 \pm 0.33^{**}$	$2.24 \pm 0.17^{**}$
Moderate-dose group	100	$8.83 \pm 1.03^{**}$	$57.09 \pm 6.54^{**}$	$2.01 \pm 0.04^{**}$	$2.01 \pm 0.12^{**}$
High-dose group	120	$7.14 \pm 0.87^*$	$44.35 \pm 8.12^*$	$2.41 \pm 0.08^*$	$1.49 \pm 0.18^*$

Fig. 2. A micropuncture study was performed to determine whether BPS ameliorates CRF in rats by affecting renal hemodynamics. Note: (A) mean arterial pressure (MAP), (B) single-nephron glomerular filtration rate (SNGFR), (C) single-nephron plasma flow (Qa), (D) glomerular capillary pressure (PGC), (E) stop-flow pressure (SFP), (F) hydrostatic pressure gradient (ΔP), (G) afferent resistance (AR), (H) efferent resistance (ER), and (I) glomerular filtration rate (GFR) observed in 5/6NX rats treated with BPS. Data are shown as the mean $\pm$ standard error. Comparisons among groups were made with one-way ANOVA followed by Bonferroni posttest. #, compared with the sham group, $p < 0.05$; *, compared with the CRF group, $p < 0.05$; BPS, beraprost sodium; CRF, chronic renal failure.

BPS also decreases ACE activity in CRF rats. In a UV-spectrophotometry assay was performed to detect ACE activity. As shown in Table 4, ACE activity was much higher in the CRF group than in the sham group ($p < 0.05$). However, compared with the CRF group, the low-, moderate- and high-dose BPS groups had lower levels of ACE, although these levels remained higher than those in the sham group ($p < 0.05$). These findings indicate that BPS decreased ACE activity in the renal tissues of CRF rats.

Table 3. BPS increases SOD levels and decreases MDA levels in the blood serum of CRF rats. Note: #, compared with the sham group, $p < 0.05$; *, compared with the CRF group, $p < 0.05$; MDA, malondialdehyde; SOD, superoxide dismutase; BPS, beraprost sodium; PGI2, prostaglandin I₂; CRF, chronic renal failure

Group	Dose ($\mu\text{g/d}$)	SOD (U/l)	MDA (U/l)
Sham group	—	210.14 $\pm$ 24.13	2.26 $\pm$ 0.32
CRF group	—	109.38 $\pm$ 14.25#	6.43 $\pm$ 0.61#
Low-dose group	80	160.35 $\pm$ 13.10**	4.65 $\pm$ 0.43**
Moderate-dose group	100	185.12 $\pm$ 19.17*	3.89 $\pm$ 0.10**
High-dose group	120	208.34 $\pm$ 12.62*	2.38 $\pm$ 0.21*

BPS reduces AT1 expression in CRF rats

In situ hybridization was used to detect AT1 expression, and AT1 was mainly expressed in renal tubular epithelial cells. In the sham group, sparse brown granules were observed in renal tubular epithelial cells. AT1 expression was significantly higher in the CRF group than in the sham group. In contrast, AT1 expression was lower in the low-, moderate- and high-dose BPS groups than in the CRF group and decreased as the dose of BPS increased (all $p < 0.05$) (Fig. 3). These results show that BPS reduces AT1 expression in CRF rats.

BPS elevates the mRNA and protein levels of AKT in CRF rats

RT-qPCR, immunohistochemistry, and western blot analyses were applied to determine the mRNA and protein levels of Bax, Bcl-2, and AKT. In the sham, CRF and low-, moderate- and high-dose BPS groups, there was no change in the expression levels of Bcl-2 and Bax in renal tissues (Fig. 4). Compared with the sham group, the CRF group exhibited decreased AKT levels. Moreover, compared with the CRF group, the low-dose BPS group showed higher AKT expression. As the BPS dose increased, the moderate- and high-dose groups showed significantly higher AKT levels closer to those observed in the sham group and exhibited alleviated renal injury (Fig. 5). These results show that BPS treatment elevated AKT levels, resulting in the alleviation of renal injury in rats with CRF.

BPS decreases the mRNA and protein levels of AT1, AGT, and ACE and increases AT2 mRNA and protein levels in CRF rats

RT-qPCR and western blot analyses were applied to determine the expression levels of key RAS factors, namely, AT1, AT2, AGT, and ACE. As shown in Fig. 6, the CRF group had higher expression levels of AT1, AGT and ACE and lower expression levels of AT2 ($p < 0.05$). Compared with the CRF group, the low-, moderate- and high-dose BPS groups displayed decreased expression levels of AT1, AGT and ACE and increased expression levels of AT2. Additionally, in the high-dose group, the AT2 expression level was similar to that observed in the sham group. Although lower ACE expression levels were detected in the high-dose group, these levels remained higher than those observed in the sham group (all $p < 0.05$). These findings indicate that BPS decreased the mRNA and protein levels of AT1, AGT, and ACE and increased the mRNA and protein levels of AT2.

Table 4. BPS decreases ACE activity in renal tissues in CRF rats. Note: #, compared with the sham group, $p < 0.05$; *, compared with the CRF group, $p < 0.05$; ACE, angiotensin-converting enzyme; BPS, beraprost sodium; CRF, chronic renal failure

Group	Dose (μg/d)	ACE (U)
Sham group	—	17.35 ± 2.63
CRF group	—	36.86 ± 4.10#
Low-dose group	80	29.37 ± 2.46**
Moderate-dose group	100	27.94 ± 2.25**
High-dose group	120	26.32 ± 1.16**

Fig. 3. In situ hybridization illustrating that BPS reduces AT1 expression in CRF rats. Note: *, $p < 0.05$, compared with the sham group; #, $p < 0.05$, compared with the CRF group; AT1, angiotensin II type 1; BPS, beraprost sodium; CRF, chronic renal failure.

Fig. 4. RT-qPCR and immunohistochemistry illustrating that BPS has no effect on the mRNA and protein levels of Bax and Bcl-2 in each group. Note: A, mRNA levels of Bax and Bcl-2 in renal tissues among the five groups were detected by RT-qPCR; B, the protein levels of Bax and Bcl-2 in renal tissues among the five groups were detected by immunohistochemistry; C, the results of immunohistochemistry staining to determine the expression profiles of Bax and Bcl-2 in renal tissues in the five groups; Bax, Bcl-2-associated X protein; Bcl-2, B cell lymphoma/leukemia-2; RT-qPCR, reverse transcription quantitative polymerase chain reaction; BPS, beraprost sodium; CRF, chronic renal failure.

Fig. 5. RT-qPCR and western blot analysis results indicating that BPS elevates the mRNA and protein levels of AKT in CRF rats. Note: A, mRNA levels of AKT detected by RT-qPCR; B-C, protein level of AKT detected by western blot analysis; *, compared with the sham group, $p < 0.05$; #, compared with the CRF group, $p < 0.05$; AKT, Protein kinase B or PKB; RT-qPCR, reverse transcription quantitative polymerase chain reaction; BPS, beraprost sodium; CRF, chronic renal failure.

Fig. 6. RT-qPCR and western blot analysis results revealing that BPS decreases the mRNA and protein levels of AT1, AGT, and ACE and increases the mRNA and protein levels of AT2 in CRF rats. No significant change in mRNA levels of key factors in the RAS detected by RT-qPCR; B-C, protein levels of key RAS factors determined by western blot analysis; *, compared with the sham group, $p < 0.05$; #, compared with the CRF group, $p < 0.05$; AT1, angiotensin II type 1; AT2, angiotensin II type 2; ACE, angiotensin-converting enzyme; AGT, angiotensinogen; RAS, renin-angiotensin system; RT-qPCR, reverse transcription quantitative polymerase chain reaction; BPS, beraprost sodium; CRF, chronic renal failure.

Discussion

CRF is a complex disease that affects millions of people worldwide. Patients suffering from CRF could be more susceptible to other diseases such as coronary artery diseases, ultimately leading to a decrease in quality of life and death [15]. Recently, Koyama et al. revealed that BPS served as a therapeutic treatment for chronic kidney disease (CKD) in several animal models [16]. Hence, based on this evidence, we hypothesized that BPS, as a stable analogue of PGI₂, could influence the local RAS in rats with CRF.

The results of the present study demonstrated that rats with CRF had significantly higher levels of BUN, SCr, and phosphorus but lower levels of calcium. Following BPS treatment, the levels of BUN, SCr and phosphorus were lower, while the level of calcium increased. Zou et al. reported results that were in line with those reported in the current study, indicating that the levels of BUN and SCr were higher in patients with CRF [17]. Elevated serum phosphorus levels were observed among patients with CRF [18], and decreased calcium release was also detected in CRF rats [19]. BPS, a new, stable, orally active PGI₂ analogue, possesses antiplatelet and vasodilating properties [20]. Inada et al. demonstrated that BPS improved survival in patients with CRD [21], while Shimamura et al. suggested that certain dosages of BPS provided therapeutic efficacy in patients suffering from CRF [22]. In addition, BPS significantly inhibits glomerular hypertension and hyperfiltration, which may limit the progression of renal injury, while renal ablation causes hemodynamic changes in remnant nephrons, which engage in proinflammatory processes and profibrotic mechanisms, thereby amplifying nephron loss. In addition, lower levels of MDA and higher SOD levels were observed after BPS treatment, indicating that BPS decreased the production of oxygen free radical generation through its antiperoxidation function, ultimately delaying renal failure. OS indicates an imbalance between the production and detoxification of ROS [23]. MDA is the most important biomarker when evaluating OS and lipid peroxidation [24]. SOD is the main endogenous antioxidant enzyme that counteracts the deleterious effects of ROS [23]. Pan et al. revealed that BPS administration significantly ameliorated aluminum overload-induced decreases in SOD activity and increases in MDA content in rats [25], consistent with the findings of the present study.

Furthermore, our results indicated that rats in the CRF group had higher AT1, AGT, and ACE levels but lower AT2 and AKT levels, while a different trend was observed among the rats in the BPS-treated groups. These findings highlight the central role of the RAS in the occurrence of CRF and indicate that BPS could be used to treat renal failure because it

downregulates key RAS factors. The RAS plays a crucial regulatory role in the modulation of blood pressure and electrolyte metabolism and may affect the overexpression of key RAS factors that have distinct effects on the pathophysiology of cardiovascular, renal, and metabolic conditions [26]. Diet et al. asserted that the RAS significantly contributes to the regulation of myocardial growth regardless of whether a loading condition exists [27]. Abnormal stimulation of the RAS may be responsible for increased blood pressure observed in autosomal-dominant polycystic kidney disease prior to the development of renal failure [28]. Inducing RAS blockade by affecting ACE or AT1 receptors has been emphasized as an approach in landmark clinical trials to dramatically attenuate the decline in renal function associated with CKD [29]. ACE inhibitor renography is widely regarded as a safe, noninvasive, sensitive, specific, cost-effective test for excluding renovascular hypertension in patients with normal renal function [30]. Feng et al. demonstrated that under normal physiological conditions, sodium intake intervention could influence the response of the RAS [31]. BPS may inhibit apoptosis by down-regulating ACE, thus benefiting cultured retinal endothelial cells [32]. In addition, BPS can induce AKT expression [33]. AKT, also known as protein kinase B (PKB), is fully activated when phosphorylated at threonine 308 and serine 473, and promotes certain cellular processes, such as cell growth, cell cycle, cell survival, and glucose metabolism [34]. Eur et al. demonstrated that BPS could induce the expression of AKT and inhibit mesangial cell proliferation [33]. All the aforementioned studies support the results obtained in the present study, which indicate that BPS influences CRF via the RAS.

Conclusion

The results of our study provide evidence showing that BPS inhibits the expression of key factors in the local RAS, inhibiting the progression of CRF. Therefore, these results could provide a basis for the development of potential therapeutic strategies or more effective treatment methods for CRF. However, because the RAS is composed of a complicated array of components, more prospective studies need to be performed in this field to further confirm these results.

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Disclosure Statement

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