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Beraprost sodium improves survival rates in anti-glomerular basement membrane glomerulonephritis and 5/6 nephrectomized chronic kidney disease rats

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ABSTRACT

Beraprost sodium, a stable prostacyclin analog, was showed to improve survival rates in two different rat models, anti-glomerular basement membrane (GBM) glomerulonephritis (GN) and 5/6 nephrectomized (Nx) chronic kidney disease (CKD) rats. In the anti-GBM rat, beraprost sodium (0.2 and 0.6 mg/kg/day) improved survival rate (hazard ratio for beraprost sodium 0.6 mg/kg/day group, 0.10; 95% confidence interval, 0.01 to 0.68). Subsequently, in the 5/6 Nx CKD rat, beraprost sodium (0.6 mg/kg/day) improved survival rate (hazard ratio for beraprost sodium, 0.46; 95% confidence interval, 0.23 to 0.92), serum creatinine doubling time and the slope of the reciprocal of serum creatinine. In the anti-GBM GN rats, beraprost sodium suppressed the serum accumulation of representative uremic toxins such as indoxyl sulfate. Furthermore, beraprost sodium inhibited human aortic endothelial cell (HAEC) injury induced by indoxyl sulfate, indicating that beraprost sodium might have a protective effect against cardiovascular damage due to CKD. These results show that beraprost sodium can improve the survival rates in two rat models of anti-GBM GN and 5/6 Nx CKD rats by protecting endothelial cells and thereby ameliorating decreased renal function. Therefore, clinical studies are needed in patients with chronic kidney failure to determine whether beraprost sodium will become a useful medication in CKD.

1. Introduction

Chronic kidney disease (CKD), the most common causes of which are diabetes mellitus, hypertension and glomerulonephritis (GN), results in a decline in glomerular filtration rate and other kidney functions of over a period of months to years. The number of potential patients with CKD is increasing worldwide (EL Nahas and Bello, 2005). CKD is identified by a blood test for creatinine, and elevated creatinine levels indicate a declining glomerular filtration rate. Recent professional guidelines classify the severity of CKD into five stages (National Kidney Foundation, 2002), with stage 1 being the mildest to stage 5, end-stage renal disease being a severe illness associated with poor life expectancy if left untreated. When a patient develops end-stage renal disease, renal replacement therapy is required in the form of either dialysis or a transplant. Therefore, patients with CKD are treated so as to reduce or halt the progression of CKD to stage 5; control of blood pressure and treatment of the underlying disease, whenever feasible, are the broad principles of management, and in general, angiotensin converting enzyme inhibitors (Maschio et al., 1999; Wright et al., 2002) or angiotensin AT1 receptor blockers (Brenner et al., 2001; Lewis et al., 2001) are used since they have been confirmed to reduce the progression of CKD to stage 5. However, since patients with CKD progressively lose renal function while on such medications and as there appears to be no specific treatment that markedly inhibits the worsening of CKD, there is still interest in the development of new medications that inhibit its progression.

Beraprost sodium, which is an orally available and chemically stable prostacyclin analog, is used to treat pulmonary arterial hypertension (Nagaya et al., 1999) and atherosclerosis obliterans (Lievre et al., 2000). Yamada, et al. have so far reported the suppressive effect of beraprost sodium on serum creatinine levels with established renal dysfunction in a rat model of glomerulonephritis (GN) induced by injection of anti-glomerular basement membrane (GBM) antibodies (Yamada et al., 2002). However, since it is unknown whether the suppressive effect of the drug resulted in the prevention of the loss of renal function, that is, whether it

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improves the survival rate, in this study we evaluated the drug using the rat GBM GN model. Furthermore, we have examined whether beraprost sodium improves survival in 5/6 nephrectomized (Nx) CKD rats. Blood levels of uremic toxins, which are known to accumulate in rats with kidney disease and thus accelerate the deterioration of renal function, and are reported to be involved in the progression of CKD (Miyazaki et al., 2000; Palm et al., 2010). Indoxyl sulfate, a uremic toxin, is known to accumulate in 5/6 Nx CKD rats (Miyazaki et al., 2000); however, this finding has not yet been reported in the anti-GBM GN rats. Therefore, we have evaluated whether indoxyl sulfate accumulates in anti-GBM GN rats and whether beraprost sodium suppresses this accumulation. Additionally, in order to clarify the mechanism of action of beraprost sodium, we investigated whether beraprost sodium can inhibit human aortic endothelial cell (HAEC) injury to be induced by uremic toxins such as indoxyl sulfate (Adelibieke et al., 2012; Dou et al., 2004, 2007).

2. Materials and methods

2.1. Experimental Materials

Beraprost sodium, (sodium (±)- (1 R*, 2 R*, 3 aS*, 8bS*)- 2, 3, 3 a, 8b- tetrahydro- 2- hydroxyl- 1- [(E)- (3 S*)- 3- hydroxy- 4- methyl-1- octen- 6- ynyl]- 1 H-cyclopenta [b]benzofuran-5-butyrate) was synthesized at Toray (Tokyo, Japan). Indoxyl sulfate and 3-isobutyl-1-methylxanthine (IBMX) were purchased from Sigma-Aldrich Japan (Tokyo, Japan). Pentobarbital sodium was purchased from Tokyo Chemical Industry (Tokyo, Japan). Freund's complete adjuvant was purchased from Difco Laboratories (Detroit, USA). N-Assay CRE-L Nittobo and N-Assay BUN-L Nittobo was purchased from Nittobo (Tokyo, Japan). cAMP Biotrak Enzyme immunoassay (EIA) System was purchased from Amersham (New Jersey, USA). HAECs were purchased from Sanko Junyaku (Tokyo, Japan). Cyto-Tox 96® Non-Radioactive Cytotoxicity Assay was purchased from Promega (Tokyo, Japan). Cell Count Reagent SF was purchased from Nacalai Tesque (Kyoto, Japan).

2.2. Experimental animals

Specific pathogen-free Male Wistar-Kyoto (WKY) and Sprague-Dawley (SD) rats (International Genetic Standard, Charles River Laboratory Japan, Kanagawa, Japan) were used in the experiments. The animal experiments described in the present study were approved by the Animal Ethics Committee in Pharmaceutical Research Laboratories, Toray Industries, Inc., Japan.

2.3. Induction of glomerulonephritis

The GBM of rats was prepared by the method of Krakower et al. (Krakower et al., 1978). Five albino rabbits were subcutaneously immunized with GBM (1 mg/ml) emulsified with an equal volume of Freund's complete adjuvant. The booster was given three times at two-week intervals using the same immunogen. Four days after the final booster, the rabbits were exsanguinated from the carotid artery under anesthesia. Anti-GBM sera were heat-decomplemented for 30 min at 56 °C and absorbed with freshly harvested rat erythrocytes. The anti-GBM serum was diluted 10-fold with saline and administered intravenously to WKY rats at a dose of 0.3 ml/100 g body weight to induce GN, resulting in an increase in serum creatinine levels in the rats after14 days. The day when the GN was induced was set as Day 0.

2.4. Preparation of 5/6 nephrectomized rats

All surgical procedures were performed under pentobarbital anesthesia (50 mg/kg, intraperitoneally). SD rats underwent infarction of approximately two-thirds of the left kidney by ligation of the anterior and one or two posterior extrarenal branches of the renal artery. Seven days after the left kidney operation, the right kidney was removed after ligation of the renal pedicle. The day when the right kidney was removed and 5/6 Nx was established was set as Day 0.

2.5. Drug administration

2.5.1. (A) Anti-GBM GN rat

Beraprost sodium was dissolved in distilled water, and orally administerded at a dose of 0.1 or 0.3 mg/kg body weight twice a day, which corresponded to a daily dose of 0.2 or 0.6 mg/kg/day, respectively, in a volume of 0.2 ml/100 g body weight, starting from Day 15 until end of the experiment. Distilled water, instead of beraprost sodium, was administered to the rats in the vehicle group.

2.5.2. (B) 5/6 Nx CKD rat

Beraprost sodium was dissolved in distilled water and orally administered at a dose of 0.3 mg/kg body weight twice a day, which corresponded to a daily dose of 0.6 mg/kg/day, in a volume of 0.2 ml/100 g body weight, starting from Day 29 until end of the experiment. Distilled water, instead of beraprost sodium, was administered to the rats in vehicle group.

2.6. Determination of serum creatinine and blood urea nitrogen

Blood sampling was performed every seven days from Day 14 in the anti-GBM GN and Day 28 in the 5/6 Nx CKD, until end of the experiment, respectively; 1 ml of blood was drawn from the jugular vein of each rat under ether anesthesia. The blood was centrifuged at 1500g for 10 min at room temperature to obtain serum for the determination of creatinine and blood urea nitrogen. The levels of serum creatinine were determined by the creatinase sarcosine oxidase-POD method using a commercially available assay kit (N-Assay CRE-L Nittobo) and expressed as mg/dl serum. The blood urea nitrogen levels in the serum samples were determined by the urease–GLDH method using a commercially available assay kit (N-Assay BUN-L Nittobo) and expressed as mg/dl serum.

2.7. Evaluation of renal function in anti-GBM GN and 5/6 Nx CKD rats

The renal function of anti-GBM rats was evaluated by serum creatinine, whereas that of 5/6 Nx CKD was determined from serum creatinine doubling time and reciprocal slopes of serum creatinine. Since renal function in anti-GBM GN rats rapidly decreases, it can be evaluated as the average of serum creatinine values determined at the same time, even just prior to death. Conversely, since that of 5/6 Nx CKD rats declines very slowly and impairment of renal function is quite different in each rat, it is difficult to determine the average of serum creatinine at a given time. Thus, in 5/6 Nx CKD rats, serum creatinine doubling time and the slopes of reciprocal of serum creatinine were used to evaluate renal function.

2.8. Endpoints

In order to evaluate the deaths caused by the progression of CKD, when rats died with a serum creatinine level higher than that at the start of drug administration, such as a death was considered to be due to the progression of CKD (an endpoint).