European Journal of Pharmacology 761 (2015) 109–115

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Pulmonary, gastrointestinal and urogenital pharmacology

Critical role of renal dipeptidyl peptidase-4 in ameliorating kidney injury induced by saxagliptin in Dahl salt-sensitive hypertensive rats

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ARTICLE INFO

Article history:
Received 13 November 2014
Received in revised form
7 April 2015
Accepted 13 April 2015
Available online 1 May 2015

Keywords:
Saxagliptin
Kidney injury
Renal dipeptidyl peptidase-4
Dahl salt-sensitive hypertensive rat

ABSTRACT

Saxagliptin, a potent dipeptidyl peptidase-4 (DPP-4) inhibitor, is currently used to treat type 2 diabetes mellitus, and it has been reported to exhibit a slower rate of dissociation from DPP-4 compared with another DPP-4 inhibitor, sitagliptin. In this study, we compared the effects of saxagliptin and sitagliptin on hypertension-related renal injury and the plasma and renal DPP-4 activity levels in Dahl salt-sensitive hypertensive (Dahl-S) rats. The high-salt diet (8% NaCl) significantly increased the blood pressure and quantity of urinary albumin excretion and induced renal glomerular injury in the Dahl-S rats. Treatment with saxagliptin (14 mg/kg/day via drinking water) for 4 weeks significantly suppressed the increase in urinary albumin excretion and tended to ameliorate glomerular injury without altering the blood glucose levels and systolic blood pressure. On the other hand, the administration of sitagliptin (140 mg/kg/day via drinking water) did not affect urinary albumin excretion and glomerular injury in the Dahl-S rats. Meanwhile, the high-salt diet increased the renal DPP-4 activity but did not affect the plasma DPP-4 activity in the Dahl-S rats. Both saxagliptin and sitagliptin suppressed the plasma DPP-4 activity by 95% or more. Although the renal DPP-4 activity was also inhibited by both drugs, the inhibitory effect of saxagliptin was more potent than that of sitagliptin. These results indicate that saxagliptin has a potent renoprotective effect in the Dahl-S rats, independent of its glucose-lowering actions. The inhibition of the renal DPP-4 activity induced by saxagliptin may contribute to ameliorating renal injury in hypertension-related renal injury.

1. Introduction

Diabetic nephropathy is the leading cause of end stage renal disease (ESRD) worldwide. Urinary albumin is not only an important predictor of renal function deterioration in patients with diabetic nephropathy, but also an independent risk factor for cardiovascular disease (Mogensen and Poulsen, 1994). Numerous clinical studies have demonstrated that a reduction in urinary albumin is associated with a significant reduction in the rate of progression to ESRD (Ravid et al., 1996; Lebovitz et al., 1994; Burnier and Zanchi, 2006). Furthermore, the Diabetes Control and Complications Trial (DCCT) and United Kingdom Prospective Diabetes Study (UKPDS) showed that intensive diabetes therapy reduces the incidence of albuminuria and overt nephropathy in diabetic patients (DCCT Research Group, 1993; Stratton et al., 2000).

Dipeptidyl peptidase-4 (DPP-4) inhibitors improve glucose metabolism by preventing the degradation of incretin hormones, such as glucagon-like peptidase-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP). While these agents have been used in the treatment of type 2 diabetes mellitus (T2DM), clinical studies have shown that DPP-4 inhibitors reduce the level of urinary albumin excretion in patients with T2DM (Hattori, 2011; Harashima et al., 2012; Groop et al., 2013). Recent animal studies have also demonstrated that DPP-4 inhibitors ameliorate urinary albumin excretion in streptozotocin (STZ)-induced diabetic animals without altering the blood glucose levels, thus suggesting that DPP-4 inhibitors exert renoprotective effects independently of their glucose-lowering actions (Liu et al., 2012; Kodera et al., 2014; Sharkovska et al., 2014). Kanasaki et al. (2014) indicated that inhibition of the renal DPP-4 activity is associated with amelioration of kidney fibrosis in STZ-induced diabetic mice. These findings suggest that renal tissue DPP-4 inhibition is critical for the renal protective effects of DPP-4 inhibitors.

Saxagliptin, a potent and selective DPP-4 inhibitor, is currently used to treat T2DM (Deacon and Holst, 2009). In a large-scale prospective clinical study, the SAVOR–TIMI53 trial, saxagliptin was found to reduce the proportion of diabetic patients with microalbuminuria (albumin/creatinine ratio 30–300 mg/g) and

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http://dx.doi.org/10.1016/j.ejphar.2015.04.023
0014-2999/

macroalbuminuria (albumin/creatinine ratio > 300 mg/g) (Scirica et al., 2013). However, it remains unclear whether renoprotective effects of saxagliptin are mediating its glucose-lowering action. In vitro study has shown that saxagliptin strongly inhibits DPP-4 and exhibits a slower rate of dissociation from its active site compared with other DPP-4 inhibitors, such as vildagliptin and sitagliptin (Wang et al., 2012). The differences in effects of DPP-4 inhibitors on renal injury also remain to be determined.

Dahl salt-sensitive (Dahl-S) rat is a well-established model of salt-induced hypertension and kidney injury. Dahl-S rats develop progressive kidney injury after feeding with a high-salt diet without change in the blood glucose level. To investigate the renoprotective effects independent of their glucose-lowering actions, we studied the effects of treatment with saxagliptin on kidney injury in Dahl-S rats. In addition, to elucidate the role of renal DPP-4 in the development of renal injury, we compared the effects on the renal DPP-4 activity of saxagliptin and sitagliptin in Dahl-S rats. We obtained evidence that saxagliptin had renoprotective effects without glycemic action and an intrarenal DPP-4 was involved in the development of renal injury in Dahl-S rats.

2. Materials and methods

2.1. Animals

Male Dahl salt-sensitive (Dahl-S) rats at 6 weeks of age (Japan Shizuoka Laboratory Animal Center, Inc., Hamamatsu, Japan) were used. The rats were kept at 19–25 °C and 30–70% humidity under a 12-h light–dark cycle and they given free access to tap water and commercial chow (FR-2; Funabashi Farm, Chiba, Japan) prior to the experiments. All animals received human care in compliance with the "Guiding Principles for the Care and Use of Laboratory Animals" formulated by the Japanese Pharmacological Society, and all animal experiments were approved by the Committee for Animal Experiments of Kyowa Hakko Kirin Co., Ltd.

2.2. Drugs

Saxagliptin monohydrate (saxagliptin) was obtained from Bristol Myers Squibb (Pennington, NJ, USA). Sitagliptin phosphate monohydrate (sitagliptin) was purchased from Shanghai Sunway Pharmaceutical Technology Co., Ltd (Shanghai, China). These drugs were dissolved in distilled water. Saxagliptin and sitagliptin were administered with drinking water. The dose of saxagliptin and sitagliptin in drinking water were calculated from daily water consumption. The doses of saxagliptin 0.01 and 0.04 mg/ml in drinking water were almost equal to those of 3.5 and 14 mg/kg/day, respectively. The sitagliptin dosage of 0.1 and 0.4 mg/ml were almost equal to 35 and 140 mg/kg/day, respectively. The doses of saxagliptin and sitagliptin used in the experiments were determined based on the finding of preliminary studies indicating that the inhibitory effect of 14 mg/kg/day of saxagliptin on the plasma DPP-4 activity was equal to that of 140 mg/kg/day of sitagliptin in Dahl-S rats.

2.3. Experimental procedure

In the first series of experiment, we studied the effect of saxagliptin and sitagliptin on albuminuria and glomerular injury in Dahl-S rats. Six-week-old male Dahl-S rats were used in this study. To evaluate the effect of saxagliptin, Dahl-S rats fed a high-salt diet (FR-2 containing 8% NaCl) were divided into three groups and given (1) Control (vehicle, $n=10$); (2) saxagliptin (3.5 mg/kg/day, $n=10$); or (3) saxagliptin (14 mg/kg/day, $n=10$) for 4 weeks. In the normal group, six-week-old male Dahl-S rats fed a normal-salt diet (FR-2 containing 0.19% NaCl) were given water for same period ($n=6$). The drug treatment was started at the same time as the initiation of feeding with the high-salt diet. In addition, we also performed the evaluation of sitagliptin in another experiment with the same protocol: high-salt diet fed Dahl-S rats were divided into three groups and given (1) Control (vehicle, $n=10$); (2) sitagliptin (35 mg/kg/day, $n=10$); or (3) sitagliptin (140 mg/kg/day/rat, $n=10$) for 4 weeks. Dahl-S fed a normal-salt diet (FR-2 containing 0.19% NaCl) also served as the normal group and were given water for same period ($n=6$). At 2 and 4 weeks after the start of dosing, 24-h urine samples were collected, and the urinary albumin concentration was measured using a rat urinary albumin assay kit (AKRAL-020S, Shibayagi Co., Ltd, Gunma, Japan). The systolic blood pressure (SBP) values were measured using the tail-cuff method at 4 weeks, and thereafter the kidneys and hearts were collected under anesthetized with isoflurane. Kidney samples were also used for the histological examination.

The second series of experiment was performed to compare the effects of saxagliptin and sitagliptin on plasma and renal DPP-4 activities in Dahl-S rats. Saxagliptin (14 mg/kg/day, $n=10$), sitagliptin (140 mg/kg/day, $n=10$) or control (vehicle, $n=10$) were administered to high-salt diet fed Dahl-S rat for one week. In the normal group, Dahl-S fed a normal-salt diet were given water for same period ($n=6$). After 1 week of treatment, rats were anesthetized with isoflurane, and then blood samples were collected and the kidneys were removed. Blood and kidney samples were used for the measurement of DPP-4 activity.

2.4. Measurement of the plasma and renal DPP-4 activity

The plasma samples were separated via centrifugation ($1800 \times g$ for 20 min at 4 °C) and used to measure the DPP-4 activity. The kidney tissue (80–120 mg) was subsequently homogenized in 300 μl of ice-cold phosphate buffer solution, and the supernatant was obtained via centrifugation ($20{,}000 \times g$ for 30 min at 4 °C) and used to measure the DPP-4 activity. The protein concentration of the supernatant was measured using a BCA protein assay kit (PIERCE, Rockford, IL, USA).

The plasma and renal DPP-4 activity levels in the Dahl-S rats were measured using a fluorometric assay with the substrate, Gly-Pro-7-AMIDO-4-METHYLCOUMARIN (Gly-Pro-AMC) (PEPTIDE INSTITUTE, Osaka, Japan). A 25 μl volume of plasma or supernatant of the kidney homogenate diluted two-fold with distilled water was mixed with 25 μl of assay buffer (25 mmol/l of HEPES, 140 mmol/l of NaCl, 80 mmol/l of $MgCl_2/6H_2O$, 1 w/v % BSA, pH 7.8). To exclude the non-specific aminopeptidase activity, plasma and renal supernatant samples diluted two-fold with DPP-4 inhibitor-containing solution (10 μmol/l saxagliptin) were also used. The enzyme reaction was initiated by adding 50 μl of substrate solution (final concentration: 50 μmol/l Gly-Pro-AMC) and subsequently incubated for 20 min at room temperature, at which time the reaction was terminated by adding 50 μl of 10% acetate solution. The fluorescence intensity was measured using SpectraMax M2e (Molecular Devices, Sunnyvale, USA) at an excitation wavelength of 460 nm and an emission wavelength of 390 nm. The DPP-4 activity was expressed as the AMC amount generated after 20 min of incubation.

2.5. Histological examination

The kidneys from rats in the first series of experiment were fixed in 10 vol% neutral buffered formalin solution and embedded in paraffin, and the paraffin sections were then stained with periodic acid-Schiff (PAS) for light microscopic observation. The specimens were observed under a light microscope, and histopathological