Journal of Pharmacological Sciences xxx (2017) 1–5

Contents lists available at ScienceDirect

Journal of Pharmacological Sciences

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

Full paper

The persistent inhibitory properties of saxagliptin on renal dipeptidyl peptidase-4: Studies with HK-2 cells *in vitro* and normal rats *in vivo*

Masako Uchii [a], Mariko Sakai [a], Yuhei Hotta [a], Satoshi Saeki [a], Naoya Kimoto [b], Akinori Hamaguchi [c], Tetsuya Kitayama [a], Shunji Kunori [a, *]

[a] *Nephrology Research Laboratories, Nephrology R&D Unit, R&D Division, Kyowa Hakko Kirin Co., Ltd., 1188 Shimotogari, Nagaizumi-cho, Sunto-gun, Shizuoka 411-8731, Japan*
[b] *Translational Research Unit, R&D Division, Kyowa Hakko Kirin Co., Ltd., 1188 Shimotogari, Nagaizumi-cho, Sunto-gun, Shizuoka 411-8731, Japan*
[c] *Research Function Unit, R&D Division, Kyowa Hakko Kirin Co., Ltd., 3-6-6 Asahi-machi, Machida-shi, Tokyo 194-8533, Japan*

ARTICLE INFO

Article history:
Received 9 July 2017
Received in revised form
6 October 2017
Accepted 12 October 2017
Available online xxx

Keywords:
Dipeptidyl peptidase-4 inhibitor
Saxagliptin
Sitagliptin
Renal dipeptidyl peptidase-4
Renal tubular cells

ABSTRACT

Saxagliptin, a potent and selective DPP-4 inhibitor, exhibits a slow dissociation from DPP-4. We investigated the sustained effects of saxagliptin on renal DPP-4 activity in a washout study using renal tubular (HK-2) cells, and in a pharmacodynamic study using normal rats. In HK-2 cells, the inhibitory potency of saxagliptin on DPP-4 activity persisted after washout, while that of sitagliptin was clearly reduced. In normal rats, a single treatment of saxagliptin or sitagliptin inhibited the plasma DPP-4 activity to similar levels. The inhibitory action of saxagliptin on the renal DPP-4 activity was retained, even when its inhibitory effect on the plasma DPP-4 activity disappeared. However, the inhibitory action of sitagliptin on the renal DPP-4 activity was abolished in correlation with the inhibition of the plasma DPP-4 activity. *In situ* staining showed that saxagliptin suppressed the DPP-4 activity in both glomerular and tubular cells and its inhibitory effects were significantly higher than those of sitagliptin. Saxagliptin exerted a sustained inhibitory effect on the renal DPP-4 activity *in vitro* and *in vivo*. The long binding action of saxagliptin in renal tubular cells might involve the sustained inhibition of renal DPP-4.

1. Introduction

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, and are used widely for the treatment of type 2 diabetes mellitus.

DPP-4, a serine proteinase, exists in two forms, a soluble form in plasma and a cell membrane-bound forms; the soluble form is thought to arise from the shedding of the membrane-bound form, which mainly contributes to the control of the postprandial blood glucose levels through degradation of GLP-1.[1,2] DPP-4 is widely expressed in cell surface of various tissues, and is predominantly expressed in the kidney.[3] Membrane-bound DPP-4 is expressed on the proximal tubular cells, mesangial cells and podocytes in the kidney.[4] Recent animal studies have showed that DPP-4 inhibitors prevent renal damage without glycemic action, suggesting an important role of renal DPP-4 in the progression of diabetic nephropathy.[2,5–7]

Saxagliptin, a potent and selective DPP-4 inhibitor, is widely used for the treatment type 2 diabetes mellitus. Saxagliptin is reported to be linked to DPP-4 by a reversible covalent bond, and exhibits a slower rate of dissociation from DPP-4 in comparison to other DPP-4 inhibitors, such as sitagliptin and vildagliptin.[8] We have previously demonstrated that saxagliptin, but not sitagliptin, exerts a renoprotective effect in Dahl salt-sensitive hypertensive (Dahl-S) rats, and indicated the important role of renal membrane-bound DPP-4, but not circulating DPP-4, in hypertension-induced renal injury.[9] These findings suggest that the action of DPP-4 inhibitors on the kidney varies according to the compound, and saxagliptin would be expected to exert renoprotective effects through its tight covalent binding and following the long-lasting inhibition of renal membrane-bound

* Corresponding author.
E-mail address: shunji.kunori@kyowa-kirin.co.jp (S. Kunori).
Peer review under responsibility of Japanese Pharmacological Society.

https://doi.org/10.1016/j.jphs.2017.10.003

DPP-4. In the present study, we examined the duration of the effects of saxagliptin on DPP-4 activity in an *in vitro* washout study using an HK-2 human proximal tubule cell line and an *in vivo* study with normal rats. We herein report that saxagliptin exerts sustained inhibitory effects on renal DPP-4 activity both *in vitro* and *in vivo*.

2. Materials and methods

2.1. 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).

2.2. Cell culture

HK-2 cells, a primary human proximal tubular cell line (American Type Culture Collection), were grown in T175 cm^2 flasks at 37 °C in a humidified atmosphere (95% O_2/5% CO_2) in DMEM containing 10% fatal bovine serum and 1% penicillin streptomycin (Gibco, NY, USA).

2.3. Animals

Six-week-old male Crl:CD (SD) rats (CHARLES RIVER LABORATORIES JAPAN, INC., Yokohama, Japan) were used. The rats were kept at 19–25 °C and 30–70% humidity under a 12-h light–dark cycle and were given *ad libitum* access to tap water and commercial chow (FR-2; Funabashi Farm, Chiba, Japan) prior to the experiments. All animals received humane 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.

2.4. The effects of DPP-4 inhibitors in HK-2 cells

2.4.1. The measurement of the DPP-4 activity in HK-2 cells

HK-2 cells were harvested and grown to confluency in tissue culture treated, black, 96-well clear bottomed plates (Perkin Elmer Japan, Tokyo, Japan). The growth medium was aspirated and the cells were gently washed with assay buffer (25 mmol/L HEPES, 140 mmol/L NaCl, 80 mmol/L $MgCl_2/6H_2O$, 1 w/v% BSA, pH 7.8). The assay buffer was aspirated from the cells and 50 μL of test solution containing saxagliptin or sitagliptin was added, and the cells were incubated at room temperature for 30 min. The test solutions were aspirated and the cells were washed twice with assay buffer. Fifty microliters of substrate buffer (final concentration: 50 μmol/L Gly-Pro-7-AMIDO-4-METHYLCOUMARIN (Gly-Pro-AMC)) was added and incubated at room temperature for 20 min. The fluorescence intensity was measured using ARVO-Sx (Perkin Elmer Japan, Tokyo, Japan) at an excitation wavelength of 460 nm and an emission wavelength of 390 nm. The DPP-4 activity was expressed as the amount of AMC generated after 20 min of incubation. The following formula was used to calculate the rate of inhibition of DPP-4 activity: rate of inhibition (%) = {([no drug] − [drug])/([no drug] − [blk])} × 100, where [blk] is the fluorescence intensity of the well without cells or the drug, [drug] and [no drug] is the fluorescence intensity of the well with or without the drug, respectively. The IC_{50} value is the concentration of DPP-4 inhibitor that was required to reduce the rate of an enzymatic reaction by 50%. The IC_{50} values were calculated by a logistic regression analysis using the XLfit software program (ver 5.3.1, Guildford, ID Business Solutions Ltd.).

2.5. The effects of DPP-4 inhibitors in rats

2.5.1. The experimental procedure

In the first series of experiments to measure the plasma DPP-4 activity, the rats were divided into three groups and (1) control (vehicle, n = 8); (2) saxagliptin (10 mg/kg, n = 8); or (3) sitagliptin (100 mg/kg, n = 8) was orally administered. Blood samples were collected from the tail vein at 0, 1, 18, 24, 40 and 48 h after the administration of the respective drugs and were used for the measurement of the plasma DPP-4 activity.

In the second series of experiment to measure the tissue DPP-4 activity, the rats were anesthetized with isoflurane at 18 and 40 h after drug administration, and their kidneys were removed. Kidney samples were used for the measurement of the DPP-4 activity in tissue homogenate and for *in situ* staining of DPP-4 activity.

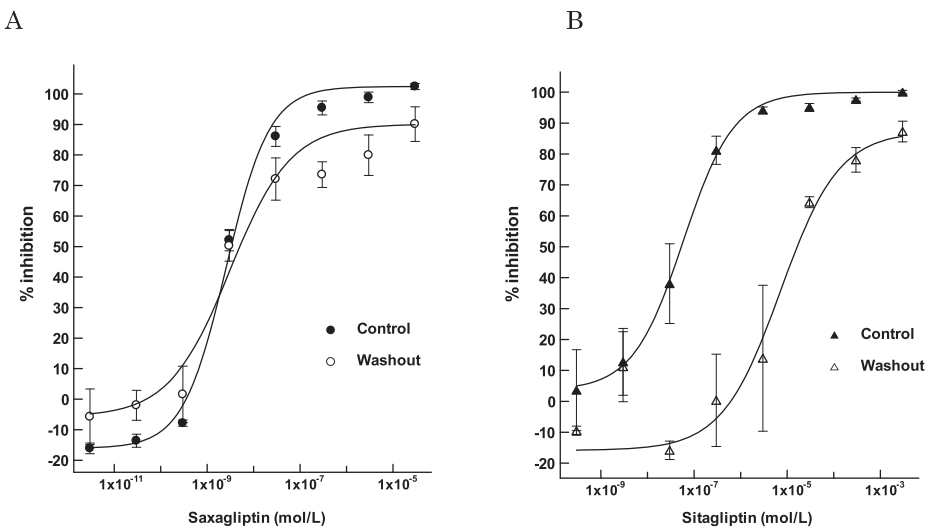

Fig. 1. The influence of cell washing on the inhibitory activities of saxagliptin (A) and sitagliptin (B) in HK-2 cells. HK-2 cells were exposed to various concentrations of saxagliptin or sitagliptin and then a substrate solution was added to start an enzymatic reaction. To investigate the persistence of the inhibitory effect, before adding the substrate solution, the cells were washed twice after exposure to the inhibitor. The data are shown as the mean ± S.D. of triplicate points.

Please cite this article in press as: Uchii M, et al., The persistent inhibitory properties of saxagliptin on renal dipeptidyl peptidase-4: Studies with HK-2 cells *in vitro* and normal rats *in vivo*, Journal of Pharmacological Sciences (2017), https://doi.org/10.1016/j.jphs.2017.10.003