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Involvement of the active metabolites in the inhibitory activity of K579 on rat plasma dipeptidyl peptidase IV

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Abstract

K579 ((*S*)-1-[4-methyl-1-(2-pyrimidinyl)-4-piperidylamino]acetyl-2-pyrrolidinecarbonitrile), which is a long-acting and a slow binding dipeptidyl peptidase IV inhibitor, preserved the endogenously secreted active forms of glucagon-like peptide-1, augmented the insulin response and ameliorated the glucose excursion during oral glucose tolerance test in rats. In this study, we measured plasma concentrations of K579 after oral administration to rats. However, K579 was eliminated rapidly from plasma after oral administration to rats. Therefore, we postulated that there are active metabolites of K579 in rat plasma. We investigated the effect of K579 on plasma dipeptidyl peptidase IV activity using bile duct-cannulated rats. The duration of inhibitory action of plasma dipeptidyl peptidase IV after the administration of K579 in bile duct-cannulated rats was shorter than that in sham-operated rats. Moreover, we investigated the effect of bile obtained from K579-treated rat on plasma dipeptidyl peptidase IV activity in normal rats. The bile collected from K579-treated rats exhibited tardive and potent inhibitory activity of normal rat plasma. These results suggest that K579 sustained the duration of inhibitory action of plasma dipeptidyl peptidase IV by the character as a slow-binding inhibitor and, as well, by the presence of metabolites of K579, which exhibit the inhibitory activity of dipeptidyl peptidase IV.

Keywords: Dipeptidyl peptidase IV; Active metabolite; Bile; (Rat)

1. Introduction

Impaired insulin secretion in type 2 diabetes is often characterized by a decreased first-phase insulin response (Polonsky et al., 1988; Porte, 1991; Taylor et al., 1994), which leads to postprandial hyperglycemia (Kosaka et al., 1994). Control of the matters such as incretins, which potentiate physiological glucose-dependent insulin release, would be preferred for the treatment of insulin secretory disorders. However, incretins have short duration of action, due to enzymatic degradation in vivo by dipeptidyl peptidase IV (Deacon et al., 1995; Hansen et al., 1999; Kieffer et al., 1995).

Dipeptidyl peptidase IV inhibitors, which protect incretins, such as glucagon-like peptide 1 or glucose-dependent insulinotropic polypeptide, from enzymatic degradation, have been noticed as new oral therapeutic tools for type 2 diabetes (Pauly et al., 1996; Drucker, 1998; Holst and Deacon, 1998; Sudre et al., 2002; Villhauer et al., 2003; Pospisilik et al., 2003; Nagakura et al., 2003; Ahren et al., 2004). Dipeptidyl peptidase IV inhibitors would correct the postprandial glucose excursion in type 2 diabetes patients by prolonging the action of postprandial incretins and insulin.

K579 ((*S*)-1-[4-methyl-1-(2-pyrimidinyl)-4-piperidylamino]acetyl-2-pyrrolidinecarbonitrile), which is a long-acting dipeptidyl peptidase IV inhibitor, inhibited plasma dipeptidyl peptidase IV activity, preserved the endogenously secreted active forms of glucagon-like peptide-1,

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augmented the insulin response, and ameliorated the glucose excursion during oral glucose tolerance test in normal and obese Zucker rats (Takasaki et al., 2004), which are the characterized model of obesity and insulin resistance (Kurtz et al., 1989; Turcotte et al., 2001). The kinetic study using cell extract revealed that K579 was a slow binding inhibitor and was slowly dissociated from the enzyme-inhibitor complex, which could in part contribute to the long-acting inhibitory activity of K579 (Takasaki et al., 2004).

In this study, we measured plasma concentrations of K579 after oral administration to rats. However, K579 was eliminated rapidly from plasma after oral administration to rats. Therefore, we postulated that there are active metabolites of K579 in rat plasma. We investigated the effect of K579 on plasma dipeptidyl peptidase IV activity using bile duct-cannulated rats. Moreover, we investigated the effect of bile obtained form K579-treated rat on plasma dipeptidyl peptidase IV activity in normal rats.

2. Materials and methods

2.1. Chemicals

K579 was synthesized at Pharmaceutical Research Institute, Kyowa Hakko Kogyo (Shizuoka, Japan). K579 was suspended in 0.5% methylcellulose 400cP (Wako, Osaka, Japan) and orally administered at a volume of 5 ml/kg.

2.2. Animals

Male Wistar rats were purchased from Charles River Japan (Kanagawa, Japan). The animals received standard laboratory chow, FR-2 (Funabashi Farms, Chiba, Japan) and water ad libitum. They were housed in a temperature (19–25 °C)-, humidity (30–70%)- and light (diurnal time: 0700–1900 h)-controlled room. The protocol was approved by the Bioethical Committee of Pharmaceutical Research Institutes, Kyowa Hakko Kogyo.

2.3. Measurement of K579 and its oxide metabolite concentration in plasma

After 24 h fasting and a period of acclimatization in the laboratory, K579 was administered orally to Wistar rat at the age of 9 weeks. Tail blood samples were collected at designated time after administration of K579. Rats were fasting until 6 h after administration of K579, although water was provided ad libitum. Blood samples were centrifuged and separated plasma was stored at −20 °C until analyses. The plasma sample was mixed with acetonitrile containing internal standard, K735, (*S*)-1-[4-methyl-1-(5-phenyl-2-pyridyl)-4-piperidylamino]acetyl-2-pyrrolidinecarbonitrile. After centrifugation at 24,000×*g* for 10 min, the supernatant was dried up and the residue was reconstituted with 10 mmol/l aqueous ammonium acetate–acetonitrile (4/1, v/v) and analysed by liquid chromatography-linked mass spectroscopy with a Sciex API365 mass spectrometer with an Agilent Technologies 1100 HPLC system. The detection was performed by multiple reactions monitoring of *m*/*z* 329→108, 345→124 and 404→183 for K579, oxide metabolite of K579 and internal standard, respectively.

2.4. Pharmacokinetic analysis

Pharmacokinetic parameters were determined with a non-compartmental analysis (Gibaldi and Perrier, 1982). The peak plasma concentration (C_{max}) and time to reach C_{max} (t_{max}) were determined through the observation of individual animal concentrations versus time curves. Elimination rate constant (k_e) was estimated by linear least-square regression on the final plasma concentrations of K579 (Fig. 1). The half-life of elimination ($t_{1/2}$) was calculated with the following equation: $t_{1/2}=0.693/k_e$. The area under the plasma concentration curve from time zero to infinity ($AUC_{0-\infty}$) was calculated by the conventional trapezoidal and extrapolation method.

2.5. Effect of K579 on plasma dipeptidyl peptidase IV activity in bile duct-cannulated rats

After 24 h fasting, Wistar rats at the age of 9 weeks were anesthetized with diethyl ether and the bile duct was exposed via a midline incision in the abdomen. One end of a polyethylene tube (PE-10, Becton Dickinson, Sparks, MD) was inserted into the bile duct. The free end of the catheter was tunneled subcutaneously and was taken to the exterior through an incision in the abdominal skin. After suturing of the abdominal skin, the rats were placed in a restraining cage (Ballman Cage, KN-326-1, Natsume Seisakusho Tokyo, Japan) and allowed to

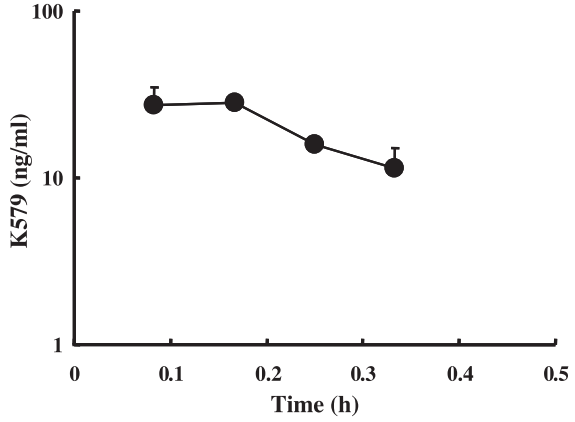

Fig. 1. Plasma concentrations of K579 after oral administration to rats at a dose of 3 mg/kg. K579 was orally administered at 0 min to Wistar rat. Tail blood samples were collected at designated times after administration of K579. Each point represents the mean+S.D. (*N*=3).

Table 1
Pharmacokinetic parameters of K579 after oral administration to rats at a dose of 3 mg/kg

	t_{max} (h)	C_{max} (ng/ml)	$t_{1/2}$ (h)	$AUC_{0-\infty}$ (ng h/ml)
Mean	0.139	30.0	0.144	8.94
S.D.	0.048	5.2	0.055	1.27

recover from diethyl ether anesthesia. K579 was administered orally to rats and tail blood samples were collected at designated time after administration of K579. Blood samples were centrifuged and separated plasma was stored at −20 °C until analyses. The bile flowing out from the catheter was collected up to 2 h just after administration of K579.

2.6. Estimation of plasma dipeptidyl peptidase IV activity in bile-treated rats

After 24 h fasting and a period of acclimatization in the laboratory, the bile (1.4 ml/rat) obtained from the K579-treated rats (see Section 2.4.) was administered orally to Wistar rat at the age of 9 weeks. Tail blood samples were collected at designated times after administration of K579. Blood samples were centrifuged and separated plasma was stored at −20 °C until analyses.

2.7. Measurement of dipeptidyl peptidase IV activity in plasma

Plasma dipeptidyl peptidase IV activity was measured using the cleavage rate of 7-amino-4-methylcoumarin (AMC; Peptide Institute, Osaka, Japan) from a substrate, Gly-Pro-AMC (Peptide Institute), based on a modified method described previously (Deacon et al., 1998; Kubota et al., 1992). Briefly, aliquots of plasma were incubated with the substrate in an assay buffer, which was composed of 25 mmol/l HEPES (Nacalai tesque, Kyoto, Japan), 140 mmol/l NaCl (Seikagaku Corporation, Tokyo, Japan) and 1% bovine serum albumin (Seikagaku, Tokyo, Japan). After incubation at room temperature, free AMC generated in proportion to dipeptidyl peptidase IV activity was determined using a spectrofluorometer (excitation at 390 nm and emission at 460 nm) (Wallac 1420 ARVOsx, Wallac Oy, Türku, Finland). Catalytic dipeptidyl peptidase IV activity in plasma was expressed as mean±S.E.M. from the amount of product (nmol) per minute per milliliter.

2.8. Statistical analyses

Statistical analyses were performed using SAS (Release 8.2, SAS Institute Cary, NC, USA) for Windows. Statistical significance within each group was estimated using F-test followed by Student's t-test or Aspin-Welch test. P-values of less than 0.05 were considered to be statistically significant.

3. Results

3.1. Plasma concentrations of K579 after oral administration to rats

The plasma concentration–time profile of K579 after oral administration to rats is shown in Fig. 1. The pharmacokinetic parameters for K579 are shown in Table 1. After oral administration of K579 to fasted Wistar rats at a dose of 3 mg/kg, the t_{max} was 0.139 h. The absorption of K579 was rapid. The C_{max} and $AUC_{0-\infty}$ were 30.0 ng/ml and 8.94 ng h/ml, respectively.

3.2. Effects of K579 on plasma dipeptidyl peptidase IV activity in bile duct-cannulated rats

Plasma dipeptidyl peptidase IV activity after oral administration of 1 mg/kg of K579 to sham-operated and bile duct-cannulated rats was shown in Fig. 2. After vehicle treatment, the plasma dipeptidyl peptidase IV activities in sham-operated and bile duct-cannulated rats kept the baseline value although the levels of activity were different from each other. Treatment with K579 notably inhibited the plasma dipeptidyl peptidase IV activity even 6 h after the administration in sham-operated rats. In bile duct cannulated rats, plasma dipeptidyl peptidase IV activity was also totally suppressed by K579 at 0.5 h. However, the activity gradually recovered and returned to the baseline value 4 h after the administration.

3.3. Estimation of plasma dipeptidyl peptidase IV activity in bile-treated rats

Plasma dipeptidyl peptidase IV activity after oral administration of the bile obtained from 1 mg/kg of K579-treated

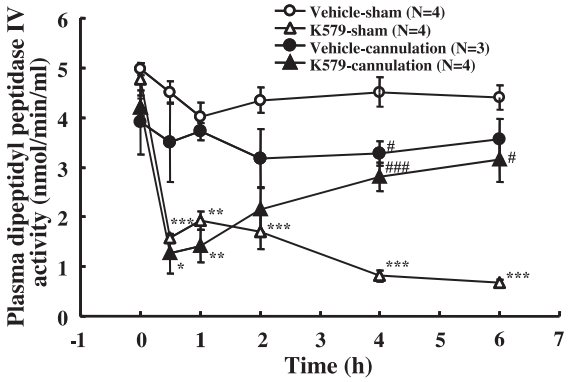

Fig. 2. Effects of cannulation into the bile duct on the inhibitory activity against plasma dipeptidyl peptidase IV by K579 in normal rats. K579 was orally administered at 0 min to sham-operated (open symbol) or bile duct-cannulated (closed symbol) rats. All rats were fasted for 24 h before the test. Data are expressed as means±S.E.M. (N=3 or 4). *P<0.05, **P<0.01, ***P<0.001; significantly different from the corresponding vehicle-treated group by Student's t-test or the Aspin-Welch test. #P<0.05, ###P<0.001; significantly different from the corresponding sham-operated group by Student's t-test or the Aspin-Welch test.

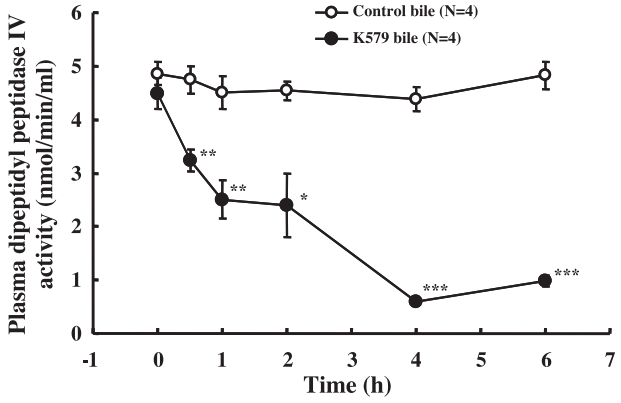

Fig. 3. Effects of the bile collected from K579-treated rats on the plasma dipeptidyl peptidase IV activity in normal rats. The bile collected from K579-treated rats was orally administered at 0 min to Wistar rats. All rats were fasted for 24 h before the test. Data represent means±S.E.M. (N=4). *P<0.05, **P<0.01, ***P<0.001; significantly different from the control by one-way analysis of variance and the Dunnett test.

rats was shown in Fig. 3. After administration of the bile, plasma dipeptidyl peptidase IV activity gradually decreased and reached the minimum at 4 h, then kept the activity low even 6 h after the administration. The bile collected from K579-treated rats exhibited tardive and potent inhibitory activity of plasma dipeptidyl peptidase IV in Wistar rats.

3.4. Plasma concentrations of oxide metabolite of K579 after oral administration of K579 to rats

Plasma concentrations of oxide metabolite of K579 after oral administration of K579 to rats at a dose of 3 mg/kg were expressed as the LC-MS area ratios to internal standard and shown in Fig. 4. The plasma concentration–time profile of oxide metabolite of K579 exhibited two peaks at 15 min and 4 h. The plasma concentration of the first peak was about five times lower than that of the second peak.

4. Discussion

K579, which is a long-acting dipeptidyl peptidase IV inhibitor, could correct the glucose excursion during oral glucose tolerance test in rats. We previously reported that K579 was a slow binding inhibitor and was slowly dissociated from the enzyme-inhibitor complex, which could in part contribute to the long-acting inhibitory activity of K579 (Takasaki et al., 2004). When the duration of dipeptidyl peptidase IV inhibitory action of K579 and the half-life of enzyme-inhibitor complex of K579 compared with those of NVP-DPP728 (1-[[[2-[(5-cyanopyridin-2-yl)amino]ethyl]amino]acetyl]-2-cyano-(*S*)-pyrrolidine), which is also a cyanopyrrolidine derivative inhibiting dipeptidyl peptidase IV by a slow-binding mechanism (Hughes et al., 1999), the long-duration of plasma dipeptidyl peptidase IV inhibitory activity of K579 could not be explained only by slow-binding profile of K579.

The disappearance of K579 from plasma was rapid. After intravenous administration of K579 to rats at a dose of 3 mg/kg, the urinary and biliary excretions were very low (0.17% and 0.06%, respectively, data not shown), suggest that the rapid disappearance of K579 from plasma was mainly due to the rapid metabolism.

In this study, we have revealed that the duration of inhibitory action of plasma dipeptidyl peptidase IV after the administration of K579 in bile duct-cannulated rats, which undergo no enterohepatic circulation, was shorter than that in sham-operated rats and that the bile collected from K579-treated rats exhibited tardive and potent inhibitory activity of plasma dipeptidyl peptidase IV. In preliminary study, we found at least five metabolites in plasma after oral administration of K579. From analysis of structure activity relationship, only oxide metabolite of K579 exhibits inhibitory activity of dipeptidyl peptidase IV. The glucuronide of oxide metabolite of K579 was found in the bile from K579-treated rats, suggest that K579 was oxidized in liver and excretion as the glucuronide of oxide metabolite of K579, and the glucuronide of oxide metabolite of K579 was deconjugated in intestine and was reabsorbed by intestine as the oxide metabolite of K579. Therefore, the plasma concentration–time profile of oxide metabolite of K579 exhibited a biphasic manner.

The time course of plasma dipeptidyl peptidase IV activities in sham-operated rats after K579 treatment reached the two peaks at 0.5 and 4 h, which consisted with the previous data in normal rats. The plasma dipeptidyl peptidase activity in bile duct-cannulated rats after K579 treatment showed the single minimum peak at 0.5 h after the treatment. On the other hands, the plasma dipeptidyl peptidase activity in normal rats after K579-primed bile treatment showed the single minimum peak at 4 h. These results suggest that the character as a slow-binding inhibitor mainly contribute to the early phase of the long duration of dipeptidyl peptidase inhibitory activity, and suggest that oxide metabolite of K579 which exhibit the tardive

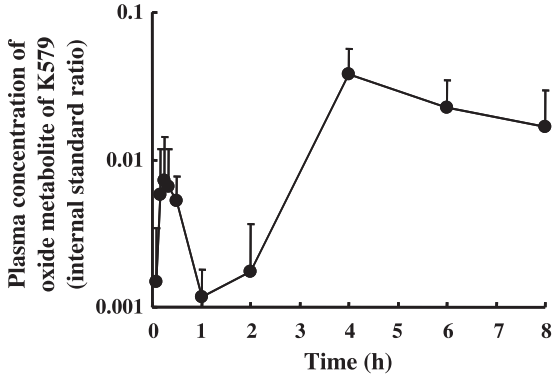

Fig. 4. Plasma concentrations of oxide metabolite of K579 after oral administration to rats at a dose of 3 mg/kg of K579. K579 was orally administered at 0 min to Wistar rat. Tail blood samples were collected at designated time after administration of K579. Data are expressed as means+S.D. of the area ratios to internal standard. (N=3).

dipeptidyl peptidase inhibitory activity contributes mainly to the late phase of the long duration of dipeptidyl peptidase inhibitory activity.

In conclusion, we revealed that the disappearance of K579 from plasma was rapid. Moreover, the duration of inhibitory action of plasma dipeptidyl peptidase IV after the administration of K579 in bile duct-cannulated rats was shorter than that in sham-operated rats. The bile collected from K579-treated rats exhibited tardive and potent inhibitory activity of plasma. These results suggest that K579 sustained the duration of inhibitory action of plasma dipeptidyl peptidase IV by the character as a slow-binding inhibitor, and, as well, by the presence of metabolites of K579 which exhibit the inhibitory activity of dipeptidyl peptidase IV.

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