Short Communication

Effects of Combination Treatment With Dipeptidyl Peptidase IV Inhibitor and Sulfonylurea on Glucose Levels in Rats

Kotaro Takasaki¹*, Takao Nakajima¹, Kimihisa Ueno¹, Yuji Nomoto¹, and Katsuya Higo¹

¹Pharmaceutical Research Institute, Kyowa Hakko Kogyo Co., Ltd.,
1188 Shimotogari, Nagaizumi-cho, Suto-gun, Shizuoka 411-8731, Japan

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Abstract. The effects of orally administered dipeptidyl peptidase IV (DPP-IV) inhibitor on the glucose-lowering effect of glibenclamide are still unknown. We evaluated the effects of combination treatment with a long-lasting DPP-IV inhibitor, K579 ([S]-1-[4-methyl-1-(2-pyrimidinyl)-4-piperidylamino]acetyl-2-pyrrolidinecarbonitrile), and glibenclamide on the glycemic responses to glucose loading in rats. Treatment with K579 inhibited the plasma DPP-IV activity even 8 h after the administration. K579 significantly suppressed the blood glucose elevation in glibenclamide-pretreated rats without excessive hypoglycemia. These profiles of K579 indicate that it could be useful agent to correct the postprandial glucose excursion in type 2 diabetes patients by combination treatment with glibenclamide.

Keywords: dipeptidyl peptidase IV, glibenclamide, glucose tolerance

Inhibitors of dipeptidyl peptidase IV (DPP-IV) activity delay the degradation of incretins, endogenous insulinotropic peptide, which in turn results in a reduction of postprandial glucose without inducing hypoglycemia (1–4). We previously reported that a long-lasting DPP-IV inhibitor, K579 ([S]-1-[4-methyl-1-(2-pyrimidinyl)-4-piperidylamino]acetyl-2-pyrrolidinecarbonitrile), attenuated the glucose excursion after glucose loading without affecting the control fasting glucose level in normal and Zucker fatty rats (5), a well-characterized model of obesity and insulin resistance. Sulfonylureas, which are widely used as potent hypoglycemic agents for type 2 diabetes, stimulate insulin secretion irrespective of blood glucose levels, and thus, cause hypoglycemia, which is a common undesirable side effect of sulfonylurea treatment (6–8). Combination therapy with sulfonylureas and other agents, mechanism of which differs from sulfonylureas such as alpha-glucosidase inhibitor or biguanide, is beneficial for type 2 diabetes compared with sulfonylureas alone (9, 10). Indeed, the glucose-lowering effect of glibenclamide was further enhanced by intravenous infusion of glucagon-like peptide 1 (GLP-1) (11); however, the effects of orally administered DPP-IV inhibitor on the glucose-lowering effect of glibenclamide are still unknown. In the present study, we evaluated the effects of combination treatment with the long-lasting DPP-IV inhibitor K579 and glibenclamide on the glycemic responses to glucose loading in normal rats.

Male Wistar rats (Charles River Japan, Kanagawa) received standard laboratory chow, FR-2 (Funabashi Farms, Chiba), and water ad libitum. They were housed in a temperature (19–25°C)-, humidity (30–70%)-, and light (diurnal time; 0700–1900)-controlled room. At the age of 9 weeks, they were fasted 24 h and were transferred from the holding room to the laboratory and were left to be acclimatized to the new conditions for at least 1 h before the experiments. The protocol was approved by the Bioethical Committee of Pharmaceutical Research Institutes, Kyowa Hakko Kogyo Co., Ltd.

K579 was synthesized at Pharmaceutical Research Institute, Kyowa Hakko Kogyo Co., Ltd. (Shizuoka). Glibenclamide was purchased from Sigma-Aldrich (St. Louis, MO, USA). Each compound was suspended in 0.5% methylcellulose 400cP (Wako Pure Chemical, Osaka) and orally administered at a volume of 5 ml/kg.

A tail blood sample was taken from each rat, and K579 (0.1, 0.3, 1.0 mg/kg) or vehicle was administered.

*Corresponding author. FAX: +81-55-986-7430
E-mail: kotaro.takasaki@kyowa.co.jp
orally. Blood samples were collected prior to and 0.5, 1, 2, 4, and 6 h after administration of K579 in chilled Eppendorf tubes. Blood was centrifuged immediately to obtain plasma and the DPP-IV activity was measured using the cleavage rate of 7-amino-4-methylcoumarin (AMC; Peptide Institute, Inc., Osaka) from the substrate Gly-Pro-AMC (Peptide Institute, Inc.), based on a modified method described previously (12, 13). Briefly, aliquots of plasma were incubated with the substrate in an assay buffer, which was composed of 25 mmol/l HEPES (Nacalai Tesque, Kyoto), 140 mmol/l NaCl (Kokusan Chemical, Tokyo) and 1% bovine serum albumin (Seikagaku Corp., Tokyo). After incubation at room temperature, free AMC generated in proportion to DPP-IV activity was determined using a spectrofluorometer (excitation at 390 nm and emission at 460 nm) (Wallac 1420 ARVOsx; Wallac Oy, Törku, Finland). Catalytic DPP-IV activity in plasma was expressed as the amount of product (nmol) per minute per ml.

In another experiment, glibenclamide (10 mg/kg) was orally administered to rats 4 h before the oral glucose loading (2 g/kg). K579 (1 mg/kg) was administered orally 0.5 h before the oral glucose loading. Blood samples were obtained via the tail vain before (−4, −0.5, and 0) and 0.5, 1, and 2 h after the glucose loading, and the blood glucose concentrations were analyzed immediately according to standard glucose oxidase method (Katayama Kagaku Kogyo, Osaka).

Data were expressed as the means ± S.E.M. Statistical analyses were performed with SAS (Release 8.2; SAS Institute Inc., Cary, NC, USA) for the Windows system. Statistical significance within each group was estimated using the F-test followed by Student’s t-test or the Aspin-Welch test. When experimental series involved more than two groups, statistical analysis was done by one-way analysis of variance and further post hoc Dunnett test.

In Wistar rats, K579 inhibited the plasma DPP-IV activity dose dependently (Fig. 1). Treatment with K579 at a dose of 1 mg/kg notably inhibited the plasma DPP-IV activity even 8 h after the administration. K579 (1 mg/kg) significantly suppressed the blood glucose elevation after glucose loading in glibenclamide-non-treated rats (Fig. 2). As compared with glibenclamide-non-treated group, the blood glucose levels in 10 mg/kg of glibenclamide-pretreated group were significantly lower just before compound administration (0.5 h before glucose-loading) and 1 and 2 h after the glucose loading. K579 (1 mg/kg) significantly suppressed the blood glucose elevation after glucose loading in glibenclamide-pretreated rats.

K579 suppressed the blood glucose elevation after glucose loading during the time when the glucose-lowering effect of glibenclamide is present in normal rats. These results suggest that the combination treatment of the DPP-IV inhibitor and sulfonylureas is more useful than glibenclamide alone treatment for the glucose excursion after glucose loading.

Glibenclamide strongly inhibits the ATP-sensitive K+ channel activity by binding to the high-affinity receptor protein (sulfonylurea receptor) in the pancreatic β cell (14). In fact, in this study, glibenclamide suppressed markedly the blood glucose levels before and after

![Fig. 1.](image1.png) Effects of K579 on plasma dipeptidyl peptidase IV activity in normal rats. K579 was orally administered to rats at 0 h. Data are expressed as the mean ± S.E.M. (n = 4 or 5). *P < 0.05, **P < 0.01, ***P < 0.001: significantly different from the control by one-way analysis of variance and further post hoc Dunnett test.

![Fig. 2.](image2.png) Effects of K579 on glucose levels during oral glucose tolerance test in glibenclamide-treated Wistar rats. Oral glucose tolerance test (2 g/kg) was performed 0.5 h after administration of K579 (1 mg/kg). Glibenclamide or vehicle was orally administered to rats at 4 h before glucose loading. Data represent the mean ± S.E.M. (n = 4 or 5). *P < 0.05, ***P < 0.001: significantly different from the vehicle-vehicle group; #P < 0.05: significantly different from the glibenclamide-vehicle group, by Student’s t-test or the Aspin-Welch test, respectively.
glucose loading. On the other hand, K579 inhibited plasma DPP-IV activity, preserved the endogenously secreted active forms of GLP-1, augmented the insulin response, and ameliorated the glucose excursion during the oral glucose tolerance test in normal rats (5); thus, the mechanism(s) of insulin potentiation of K579 is essentially different from that of glibenclamide. The insulin concentration was not measured in this study, but, Gutniak et al. (11) demonstrated that the insulinotropic effect of glibenclamide was further enhanced by intravenous infusion of GLP-1 in isolated perfused rat pancreas. Therefore, K579 augmented the glucose-lowering effect of glibenclamide after glucose loading (Fig 2), which might suggest that the combination treatment with K579 and glibenclamide exhibits a synergistic effect on the insulin secretion after glucose loading.

In this study, we administered 10 mg/kg of glibenclamide, which was a sufficient dose to induce hypoglycemia and to suppress the glucose excursion after glucose loading based on the preliminary study. Under the present experiment condition, although the combination treatment of the K579 and glibenclamide did not elicit significant hypoglycemia before the glucose loading, there was no additional deterioration of the hypoglycemia inducing by glibenclamide alone treatment.

These profiles of K579 could be a useful agent to correct the postprandial glucose excursion in type 2 diabetes patients by combination treatment with glibenclamide.

References