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Comparison of Efficacies of a Dipeptidyl Peptidase IV Inhibitor and α-Glucosidase Inhibitors in Oral Carbohydrate and Meal Tolerance Tests and the Effects of Their Combination in Mice

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Abstract. E3024 (3-but-2-ynyl-5-methyl-2-piperazin-1-yl-3,5-dihydro-4*H*-imidazo[4,5-*d*]pyridazin-4-one tosylate) is a dipeptidyl peptidase IV (DPP-IV) inhibitor. Since the target of both DPP-IV inhibitors and α-glucosidase inhibitors is the lowering of postprandial hyperglycemia, we compared antihyperglycemic effects for E3024 and α-glucosidase inhibitors in various oral carbohydrate and meal tolerance tests using normal mice. In addition, we investigated the combination effects of E3024 and voglibose on blood glucose levels in a meal tolerance test using mice fed a high-fat diet. ER-235516-15 (the trifluoroacetate salt form of E3024, 1 mg/kg) lowered glucose excursions consistently, regardless of the kind of carbohydrate loaded. However, the efficacy of acarbose (10 mg/kg) and of voglibose (0.1 mg/kg) varied with the type of carbohydrate administered. The combination of E3024 (3 mg/kg) and voglibose (0.3 mg/kg) improved glucose tolerance additively, with the highest plasma active glucagon-like peptide-1 levels. This study shows that compared to α-glucosidase inhibitors, DPP-IV inhibitors may have more consistent efficacy to reduce postprandial hyperglycemia, independent of the types of carbohydrate contained in a meal, and that the combination of a DPP-IV inhibitor and an α-glucosidase inhibitor is expected to be a promising option for lowering postprandial hyperglycemia.

Keywords: dipeptidyl peptidase IV inhibitor, α-glucosidase inhibitor, combination effect, tolerance test

Introduction

Dipeptidyl peptidase IV (DPP-IV) degrades glucagon-like peptide-1 [GLP-1(7 – 36) amide and GLP-1(7 – 37)], which is an incretin released from L cells in the intestine after meal intake that enhances insulin secretion in a glucose-dependent manner. GLP-1 has an antidiabetic action in patients with type 2 diabetes (1, 2). DPP-IV cleaves GLP-1 rapidly so the latter's half-life is only 1 – 2 min. Therefore, the prevention of GLP-1 inactivation by DPP-IV inhibition is currently being actively explored as a novel approach to the treatment of type 2 diabetes (3). DPP-IV inhibition leads to blood glucose-lowering effects in animal models of diabetes (4 – 6), and in patients with type 2 diabetes (7, 8).

E3024 (3-but-2-ynyl-5-methyl-2-piperazin-1-yl-3,5-dihydro-4*H*-imidazo[4,5-*d*]pyridazin-4-one tosylate) is a novel, selective, and competitive DPP-IV inhibitor, discovered in our laboratories (9). E3024 has antihyperglycemic effects in Zucker *fa/fa* rats, an animal model of type 2 diabetes, with augmented plasma insulin levels and increased plasma active GLP-1 levels. Demuth et al. (10) have categorized DPP-IV inhibitors based on their mode of inhibition and structures as follows: reversible product analog inhibitors [e.g., P32/98 (11)], covalently modifying product analog inhibitors [e.g., vildagliptin (LAF237) (12)], and reversible non-peptidic heterocyclic inhibitors [e.g., sitagliptin (MK-0431) (13)]. E3024 belongs to the third group and has a novel, imidazopyridazinone structure.

α-Glucosidase inhibitors are used worldwide for the treatment of diabetes. They inhibit reversibly the enzy-

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matic cleavage of complex carbohydrates to simple absorbable sugars and hence slow the absorption of carbohydrate from the small intestine, thereby lowering postprandial hyperglycemia (14). Therefore, both DPP-IV inhibitors and α-glucosidase inhibitors are primarily effective in lowering postprandial hyperglycemia via different mechanisms of action. In this study, we compared the efficacy of E3024 and the α-glucosidase inhibitors, acarbose and voglibose, employing various oral carbohydrate tolerance tests and a meal tolerance test in normal mice. Furthermore, we assessed the effects of a combination of E3024 and voglibose on glucose tolerance in this meal tolerance test using mice fed a high-fat diet to examine the possibility that this combination may be valuable for reducing postprandial hyperglycemia efficiently in the clinic. IC_{50} values of E3024 toward mouse DPP-IV were reported to be 0.10 μmol/l using recombinant mouse DPP-IV and 0.28 μmol/l using mouse plasma (9). The high-fat-diet-fed mouse model is considered to be a robust model for impaired glucose tolerance and early type 2 diabetes (15), both of which are targets of DPP-IV inhibitors.

Materials and Methods

Chemicals

E3024 and its trifluoroacetate salt form (ER-235516-15) were synthesized in our laboratories. Acarbose (*O*-4,6-dideoxy-4-[[(1*S*,4*R*,5*S*,6*S*)-4,5,6-trihydroxy-3-(hydroxymethyl)-2-cyclohexene-1-yl]amino]-α-D-glucopyranosyl-(1→4)-*O*-α-D-glucopyranosyl-(1→4)-D-glucopyranose) was purchased from Bayer Yakuhin, Ltd. (Osaka). Voglibose ((+)-1L-[1(OH),2,4,5/3]-5-[2-hydroxy-1-(hydroxymethyl)ethyl]amino-1-*C*-(hydroxymethyl-1,2,3,4-cyclohexanetetrol) was purchased from Takeda Chemical Industries (Osaka) and Toronto Research Chemicals, Inc. (Ontario, Canada). The chemical structures of these compounds are shown in Fig. 1. Glucose, sucrose, maltose, lactose, and starch (wheat) were obtained from Wako Pure Chemical Industries (Osaka). Isomaltose was purchased from Sigma (St. Louis, MO, USA).

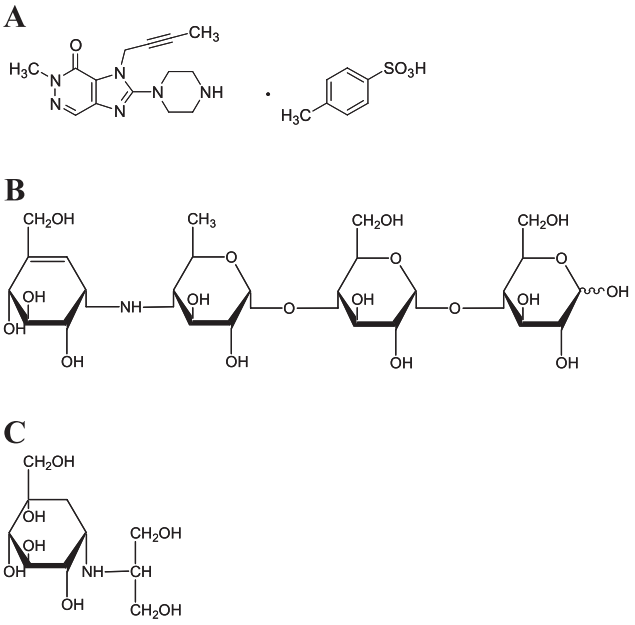

Fig. 1. Chemical structures of E3024 (A), acarbose (B), and voglibose (C).

Animals

Male C57BL/6NCrj (C57BL/6) mice were purchased from Charles River Japan (Tokyo). The mice were provided with a commercial diet (MF; Oriental Yeast, Tokyo) and water ad libitum and were kept under conventional conditions of controlled temperature, humidity, and lighting (22 ± 2°C, 55 ± 5%, and a 12-h light/dark cycle with lights on at 07:00 a.m.). All procedures were conducted according to the Eisai Animal Care Committee's guideline.

Phamacokinetics

ER-235516-15 was administered to fasted male C57BL/6 mice intravenously (i.v.) at 3 mg/kg or orally at 3 mg/kg (n = 3). Blood samples were collected from the vena cava inferior under diethyl ether anesthetization at 5 (i.v. only), 15, and 30 min and at 1, 2, 4, 6, and 8 h post-dose. Plasma was obtained after centrifugation. The concentrations of ER-235516-15 in mouse plasma were determined by a liquid chromatography tandem mass spectrometry (LC-MS/MS) method. The pharmacokinetic parameters of ER-235516-15 in mice were calculated by a model independent analysis.

Oral carbohydrate tolerance tests

Acarbose (10 mg/kg), voglibose (0.1 mg/kg), ER-235516-15 (1 mg/kg), or vehicle [0.5% methylcellulose (0.5% MC)] alone was orally administered to overnight-fasted mice via a gastric tube 0.5 h prior to carbohydrate administration. The carbohydrates loaded were as follows: glucose (2 g/kg), sucrose (2 g/kg), maltose (2 g/kg), isomaltose (2 g/kg), starch (1 g/kg), and mixed carbohydrate [2 g/kg; starch, sucrose, and lactose in a ratio of 6:3:1, which was reported to be the standard carbohydrate composition of meals (16)]. These carbohydrates were orally administered via a gastric tube. Blood samples were collected from the tail vein at 0, 0.5, 1, and 2 h after the carbohydrate administration.

Meal tolerance test

Acarbose (10 mg/kg), voglibose (0.1 mg/kg), ER-235516-15 (1 mg/kg), or vehicle (0.5% MC) alone was orally administered to overnight-fasted mice via a gastric tube 0.5 h prior to Ensure® H (Meiji Dairies Corporation, Tokyo) administration (10 ml/kg; 1.2 g carbohydrate/kg). Ensure® H was also orally administered via a gastric tube. Blood samples were collected from the tail vein 0.5 h before and at 0, 0.5, 1, and 2 h after the Ensure® H administration. Ensure® H is a common enteral nutrient (17) containing sucrose and dextrin as carbohydrate sources and consisting of 28%, 58%, and 14% of calories from fat, carbohydrate, and protein, respectively. Berthiaume and Zinker used Ensure® H in a meal tolerance test with Zucker fatty rats (18).

Effects of the combination of E3024 and voglibose on blood glucose levels in the meal tolerance test

Mice were fed a high-fat diet (D12492 Rodent Diet with 60 kcal% fat; Research Diets, Inc., NJ, USA) for four weeks from 11 weeks of age, and 32 mice were selected based on body weight and randomly divided into four groups. D12492 consisted of 60% fat, 20% carbohydrate, and 20% protein, whereas the MF diet consisted of 13% fat, 61% carbohydrate, and 27% protein, calculated as calorie ratios.

E3024 (3 mg/kg), voglibose (0.3 mg/kg), a mixture of E3024 (3 mg/kg) and voglibose (0.3 mg/kg), or vehicle (0.5% MC) alone was orally administered to overnight-fasted mice via a gastric tube 0.5 h prior to the Ensure® H administration (10 ml/kg). Blood samples were collected from the tail vein 0.5 h before and at 0, 0.5, 1, and 2 h after, the Ensure® H administration.

Effects of the combination of E3024 and voglibose on plasma insulin and active GLP-1 levels after the meal loading

Mice were fed a high-fat diet (D12492 Rodent Diet) for four weeks from 11 weeks of age, and 40 mice were selected based on body weight and randomly divided into four groups. E3024 (3 mg/kg), voglibose (0.3 mg/kg), a mixture of E3024 (3 mg/kg) and voglibose (0.3 mg/kg), or vehicle (0.5% MC) alone was orally administered to overnight-fasted mice via a gastric tube 0.5 h prior to the Ensure® H administration (10 ml/kg). Blood samples were collected from the tail vein 15 min after and from the orbital sinus 0.5 h after the Ensure® H administration.

Blood glucose, plasma insulin, and active GLP-1 determination

In the oral carbohydrate tolerance tests and meal tolerance test, blood samples (10 μl) were collected from the tail vein and mixed with 140 μl of 0.6 mol/l perchloric acid. After centrifugation, the supernatants were assayed for glucose using an enzymatic assay kit (Glucose CII-test WAKO; Wako Pure Chemicals Industries) with a microplate spectrophotometer (SpectraMax; Molecular Devices, CA, USA).

For plasma insulin determination, blood samples (approximately 50 μl) were drawn from the tail vein with heparinized capillary tubes. For plasma active GLP-1 determination, blood samples (approximately 250 μl) were collected from the orbital sinus with heparinized capillary tubes. Blood samples were centrifuged (7,000 rpm, 5 min, 4°C) and the obtained supernatants were used for the assay. Plasma insulin levels were determined using an enzyme-linked immunosorbent assay kit (Ultra sensitive rat insulin ELISA kit; Morinaga Institute of Biological Science, Kanagawa) and mouse insulin (Morinaga Institute of Biological Science) as a standard. Plasma immunoreactive intact GLP-1 levels were measured with a Glucagon-Like Peptide (Active) ELISA kit (Linco Research, St. Charles, MO, USA).

Statistical analyses

Data are expressed as the mean ± S.E.M. To determine the integrated glucose response to the carbohydrate or meal challenge, the area under the curve (AUC) of delta blood glucose after the carbohydrate or meal load was calculated using the trapezoidal rule. One-way analysis of variance (ANOVA) was performed on the AUC values, followed by the Tukey multiple comparison test to determine the difference between the two groups.

In the combination study, two-way ANOVA with E3024 and voglibose treatment as the two factors was performed on the AUC values of blood glucose, plasma insulin, and active GLP-1 levels. The Tukey multiple comparison test was used to examine the difference between the two groups. A probability (P) value <0.05 (two-sided) was considered statistically significant. Statistical analyses were performed by the SAS software package version 8.1 (SAS Institute Japan, Ltd., Tokyo).

Results

Table 1 indicates the pharmacokinetic profiles of ER-235516-15 as its free base estimated after a single intravenous administration at 3 mg/kg or a single oral administration at 3 mg/kg to mice. Following oral administration of ER-235516-15, the plasma levels peaked at about 0.5 h with C_{max} of 0.55 μg/ml and then decreased with $t_{1/2}$ of 2.55 h. Thus, it was judged that

Table 1. Phamacokinetic parameters of ER-235516-15 in mice

Parameter	ER-235516-15	
	3 mg/kg, i.v.	3 mg/kg, p.o.
t_{max} (h)	—	0.50
C_{max} (μg/ml)	—	0.55
$t_{1/2}$ (h)	3.25	2.55
AUC ($\mu g \cdot h/ml$)	0.92	0.88
MRT (h)	1.56	1.87
CL ($l \cdot h^{-1} \cdot kg^{-1}$)	3.246	—
V_{ss} (l/kg)	5.076	—
F_{oral} (%)	—	95.7

Each parameter represents the mean of three mice. F_{oral} was estimated by the mean are under the concentration curve (AUC) values. t_{max}, time at maximum observed concentration; C_{max}, maximum concentration; $t_{1/2}$, terminal half-life; MRT, mean residence time; CL, total plasma clearance; V_{ss}, volume of distribution at steady state; F_{oral}, oral bioavailability; —, not calculated.

procedures of the carbohydrate and meal tolerance tests in mice were appropriate to assess effects of the compound.

Figure 2 shows glucose excursions and Fig. 3 indicates the AUC values of delta blood glucose for several oral carbohydrate tolerance tests in normal mice. Both acarbose (10 mg/kg) and voglibose (0.1 mg/kg) had no effect on glucose excursion when oral glucose was loaded (Figs. 2A and 3A), as previously reported (16, 19). Unlike with the glucose load, both acarbose and voglibose almost completely inhibited the blood glucose rise after sucrose treatment (Figs. 2B and 3B). In the maltose and isomaltose tolerance tests, we observed a difference between acarbose and voglibose in efficacy of glucose tolerance improvement. Voglibose, but not acarbose, significantly reduced glucose excursion when maltose was administered (Figs. 2C and 3C). In the isomaltose tolerance test, a prominent decrease in AUC values was induced by voglibose treatment (Figs. 2D and 3D). In contrast, acarbose had no significant blood glucose-lowering effect with the maltose or isomaltose load. In addition, a significant decrease in AUC values was observed in the voglibose-treated group, but not in the acarbose-treated group, in the starch tolerance test (Figs. 2E and 3E). For ER-235516-15, a glucose-lowering effect was seen consistently in all tolerance tests (Figs. 2: A – E and 3: A – E).

The oral mixed carbohydrate tolerance test showed significant and similar glucose-lowering effects for acarbose, voglibose, and ER-235516-15 (Fig. 4: A and B). Furthermore, we compared the efficacy of glucose tolerance improvement for the three compounds in the meal tolerance test. The results of this test indicated that only ER-235516-15 improved glucose tolerance significantly at the same dose as in the mixed carbohydrate tolerance test (Fig. 5: A and B).

Figure 6 illustrates changes of blood glucose levels and the AUC of delta blood glucose in the meal tolerance test using mice fed a high-fat diet that were treated with E3024 (3 mg/kg) and/or voglibose (0.3 mg/kg). Two-way ANOVA indicates significant main effects of both E3024 and voglibose on the AUC values. No significant interaction was detected between the effects of E3024 and voglibose. The AUC of the combination was significantly lower than those of the other three groups.

Figure 7 shows effects of E3024 and/or voglibose on plasma insulin and active GLP-1 levels after the Ensure® H loading in mice fed a high-fat diet. E3024 treatment caused a significant increase in plasma insulin levels, compared with vehicle treatment, 15 min after the meal administration (Fig. 7A). Voglibose alone treatment and combination treatment resulted in significantly lower plasma insulin levels than in vehicle treatment. There was no significant difference of plasma insulin levels between the voglibose-treated group and the E3024 plus voglibose-treated group. E3024 alone administration significantly increased plasma active GLP-1 levels 0.5 h after the meal loading compared with the control, while voglibose alone administration did not affect the levels (Fig. 7B). The combination of E3024 and voglibose led to a drastic and significant increase of plasma active GLP-1, being the highest levels among the groups.

Discussion

Acarbose (10 mg/kg) and voglibose (0.1 mg/kg) showed similar and strong efficacy in the oral sucrose tolerance test, that is, almost complete inhibition of glucose elevation after sugar administration. In the maltose and isomaltose tolerance tests, however, only voglibose manifested a significant decrease in glucose excursion at the same doses. The inhibitory activities of voglibose towards sucrase, maltase, and isomaltase were reported to be respectively 190-, 270-, and 3,900-fold stronger than those of acarbose in an in vitro study using the sucrase-isomaltase complex in rat intestinal mucosa (19). Thus, our findings could reflect the differences between voglibose and acarbose with respect to inhibitory activities towards maltase and isomaltase. Neither acarbose nor voglibose was effective in the glucose tolerance test, which is because of the mechanisms of action of α-glucosidase inhibitors. Therefore, the efficacy of α-glucosidase inhibitors in oral carbohydrate tolerance tests is influenced by not only the kind of

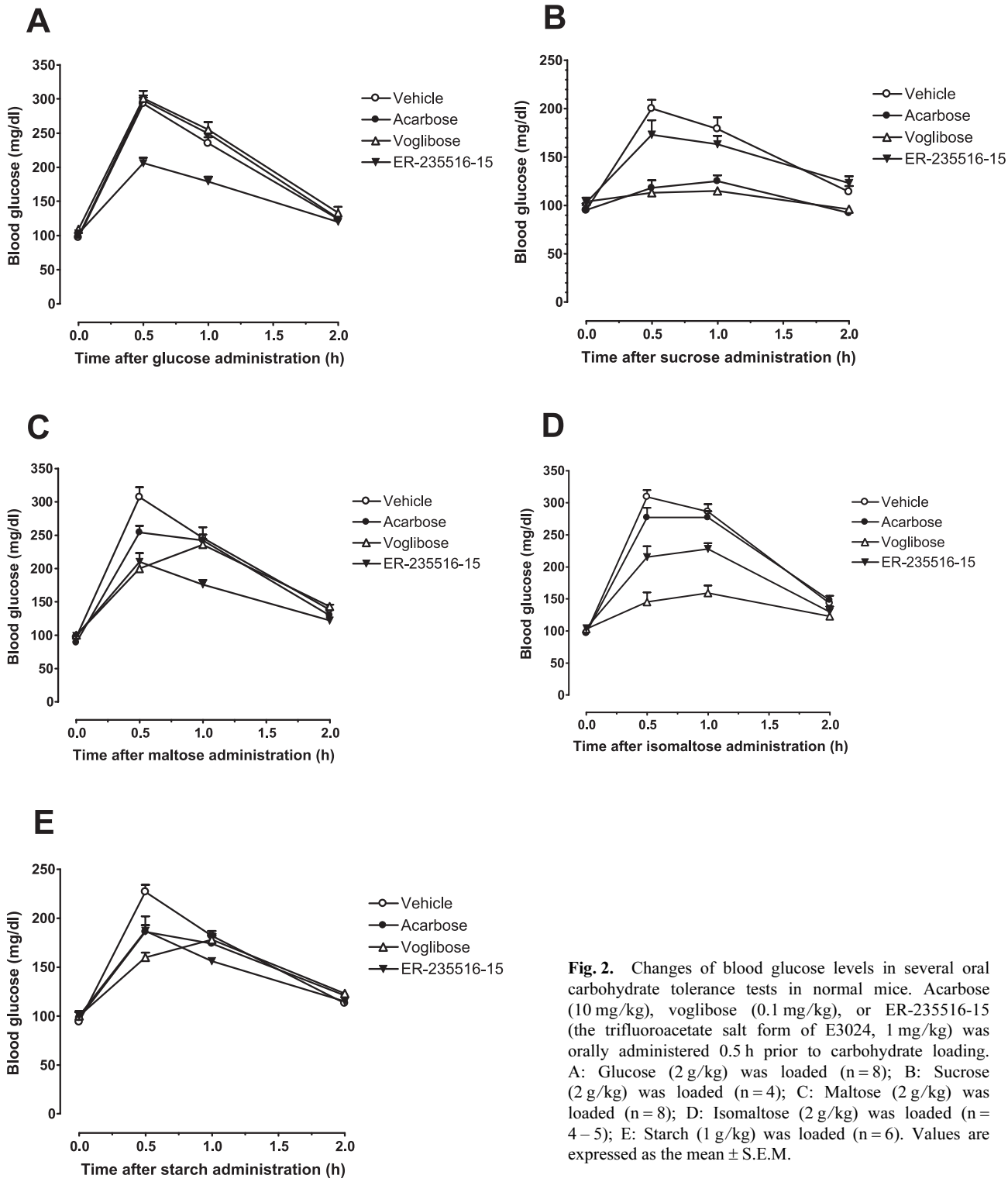

Fig. 2. Changes of blood glucose levels in several oral carbohydrate tolerance tests in normal mice. Acarbose (10 mg/kg), voglibose (0.1 mg/kg), or ER-235516-15 (the trifluoroacetate salt form of E3024, 1 mg/kg) was orally administered 0.5 h prior to carbohydrate loading. A: Glucose (2 g/kg) was loaded (n = 8); B: Sucrose (2 g/kg) was loaded (n = 4); C: Maltose (2 g/kg) was loaded (n = 8); D: Isomaltose (2 g/kg) was loaded (n = 4 – 5); E: Starch (1 g/kg) was loaded (n = 6). Values are expressed as the mean ± S.E.M.

carbohydrates loaded due to their mechanisms of action, but also their inhibitory activities towards disaccharidases. However, ER-235516-15 consistently caused a decrease in blood glucose in the carbohydrate tolerance tests, which is because of the mechanisms of action of DPP-IV inhibitors.

The meal tolerance test provides a simple, oral, complete nutrient challenge including carbohydrate, fat, and protein, and it can be used as a method to assess glucose and insulin excursions (18). Compared to a

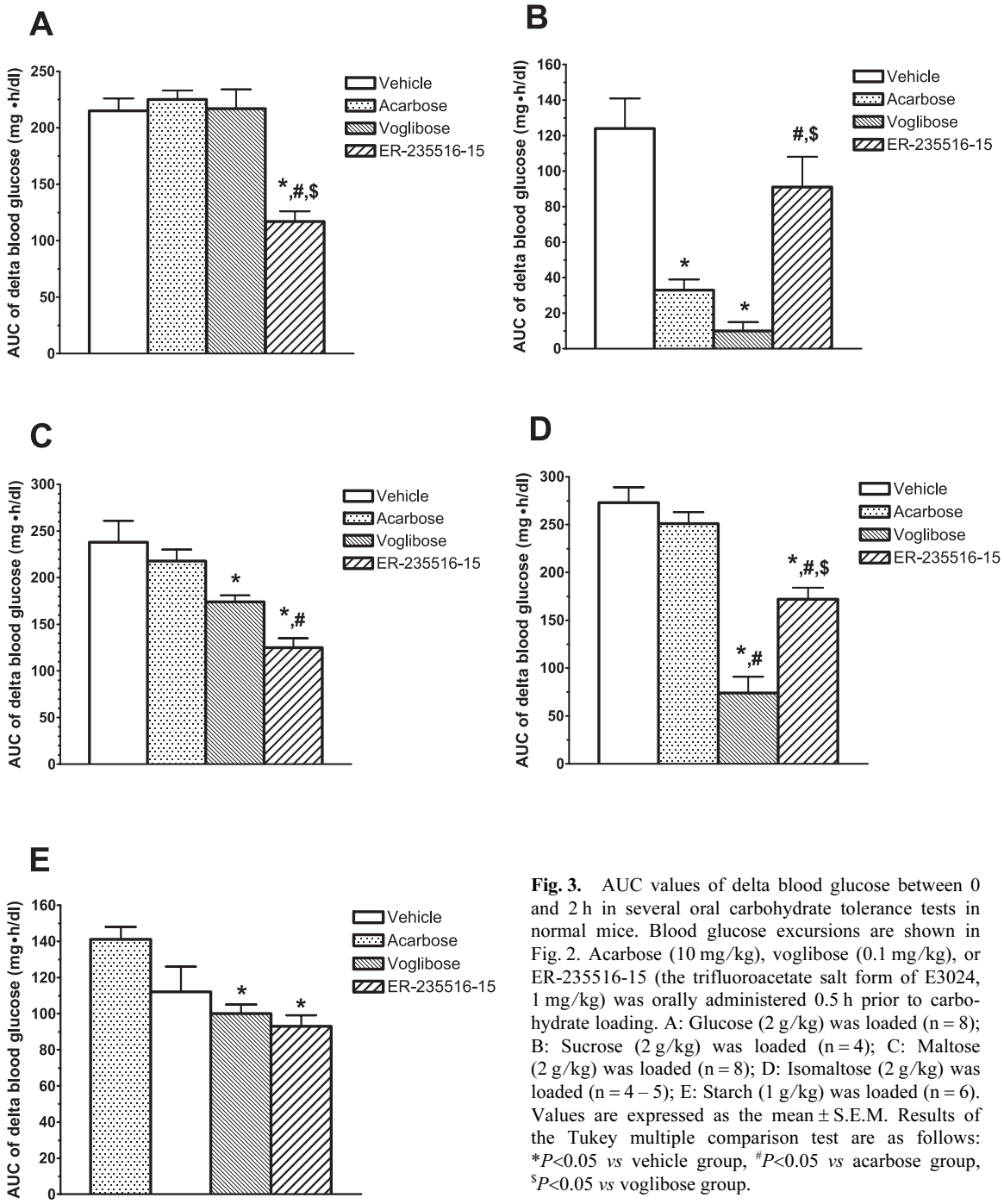

Fig. 3. AUC values of delta blood glucose between 0 and 2 h in several oral carbohydrate tolerance tests in normal mice. Blood glucose excursions are shown in Fig. 2. Acarbose (10 mg/kg), voglibose (0.1 mg/kg), or ER-235516-15 (the trifluoroacetate salt form of E3024, 1 mg/kg) was orally administered 0.5 h prior to carbohydrate loading. A: Glucose (2 g/kg) was loaded (n = 8); B: Sucrose (2 g/kg) was loaded (n = 4); C: Maltose (2 g/kg) was loaded (n = 8); D: Isomaltose (2 g/kg) was loaded (n = 4 – 5); E: Starch (1 g/kg) was loaded (n = 6). Values are expressed as the mean ± S.E.M. Results of the Tukey multiple comparison test are as follows: $^{*}P<0.05$ *vs* vehicle group, $^{\#}P<0.05$ *vs* acarbose group, $^{\$}P<0.05$ *vs* voglibose group.

challenge with glucose alone, amplified insulin and reduced glucose responses are predicted with the meal tolerance test because the fat contained in a mixed meal slowed gastric emptying (18). Thus, the meal tolerance test may be a useful test to reflect results in clinical practice. Acarbose (10 mg/kg) and voglibose (0.1 mg /kg) did not reduce the AUC values significantly in the meal tolerance test, although these doses were effective in the oral mixed carbohydrate tolerance test. On the other hand, ER-235516-15 (1 mg/kg) showed a significant improvement of glucose tolerance in the meal tolerance test. Fat intake induced glucose-dependent insulinotropic polypeptide (GIP) from K cells in the intestine (20). As its name indicates, GIP enhances insulin release from β-cells in a glucose-dependent manner as GLP-1 does. Furthermore, after secretion, GIP is also rapidly inactivated by DPP-IV (20), and GIP and GLP-1 stimulate insulin release synergistically

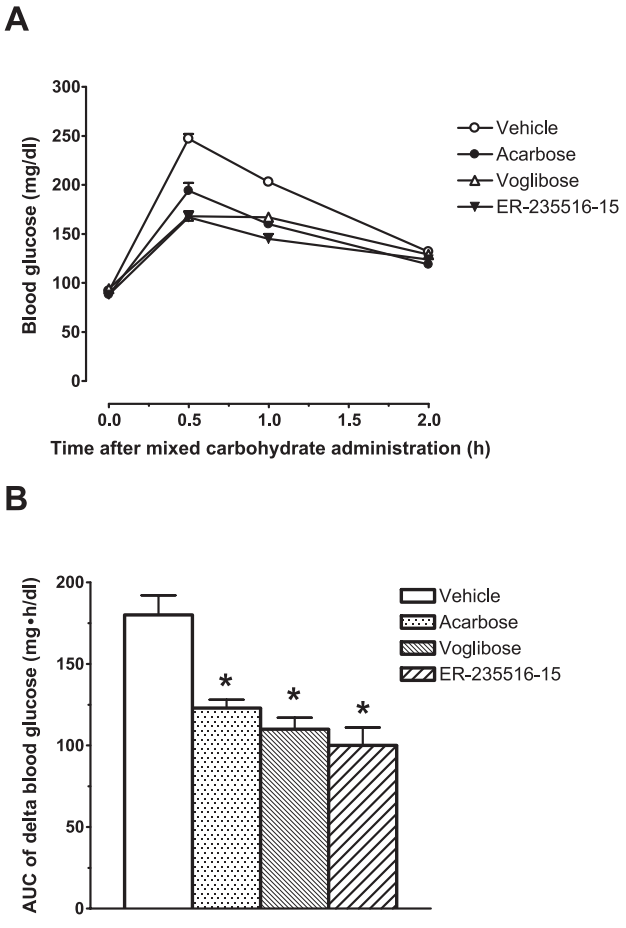

Fig. 4. Changes of blood glucose levels (A) and AUC values of delta blood glucose between 0 and 2 h (B) in an oral mixed carbohydrate tolerance test (2 g/kg; starch, sucrose, and lactose at a ratio of 6:3:1) in normal mice. Acarbose (10 mg/kg), voglibose (0.1 mg/kg), or ER-235516-15 (the trifluoroacetate salt form of E3024, 1 mg/kg) was orally administered 0.5 h prior to the carbohydrate loading. Values are expressed as the mean ± S.E.M. of six mice. Results of the Tukey multiple comparison test are as follows: **P*<0.05 *vs* vehicle group.

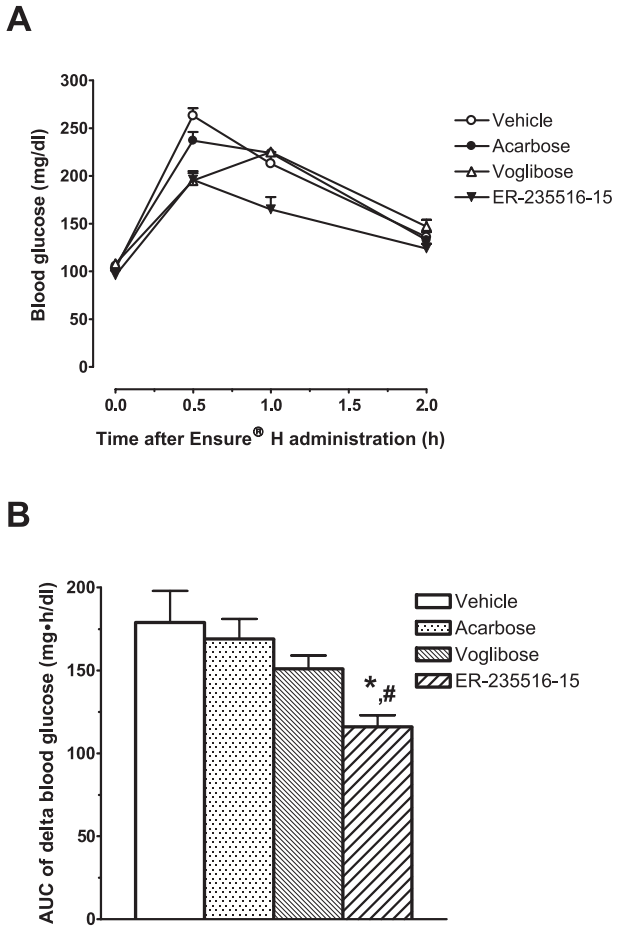

Fig. 5. Changes of blood glucose levels (A) and AUC values of delta blood glucose between 0 and 2 h (B) in a meal tolerance test (Ensure® H, 10 ml/kg) in normal mice. Acarbose (10 mg/kg), voglibose (0.1 mg/kg), or ER-235516-15 (the trifluoroacetate salt form of E3024, 1 mg/kg) was orally administered 0.5 h prior to the Ensure® H loading. Values are expressed as the mean ± S.E.M. of six mice. Results of the Tukey multiple comparison test are as follows: **P*<0.05 *vs* vehicle group, #*P*<0.05 *vs* acarbose group.

(21, 22). Accordingly, in the meal tolerance test with ER-235516-15, it is likely that insulin secretion is enhanced by inhibition of inactivation of both GLP-1 and GIP, due to DPP-IV inhibition, resulting in good efficacy.

Recently, the importance of reducing not only fasting hyperglycemia, but also postprandial hyperglycemia, has been proposed. Postprandial hyperglycemia has shown to be an independent risk factor for the development of macrovascular complications (23 – 25). The development of new agents to control postprandial glucose excursions is considered to be an essential objective for the management of type 2 diabetes. Nateglinide, repaglinide, and mitiglinide are rapid-onset /short-duration insulin secretagogues, and they have been used to manage postprandial hyperglycemia. Now, combining an insulin secretion enhancer with an α-glucosidase inhibitor is considered to increase effectiveness of postprandial hyperglycemia control. It has been reported that studies on the following combinations are currently in progress: nateglinide plus acarbose (26), repaglinide plus voglibose (26), and mitiglinide and an α-glucosidase inhibitor (27).

Given these circumstances, it is reasonable to consider the combination of a DPP-IV inhibitor and an α-glucosidase inhibitor as a candidate to control postprandial hyperglycemia effectively. In our combination study using E3024 (3 mg/kg) and voglibose (0.3 mg/kg), this combination improved glucose tolerance additively in the meal tolerance test in mice fed a high-fat diet. This is

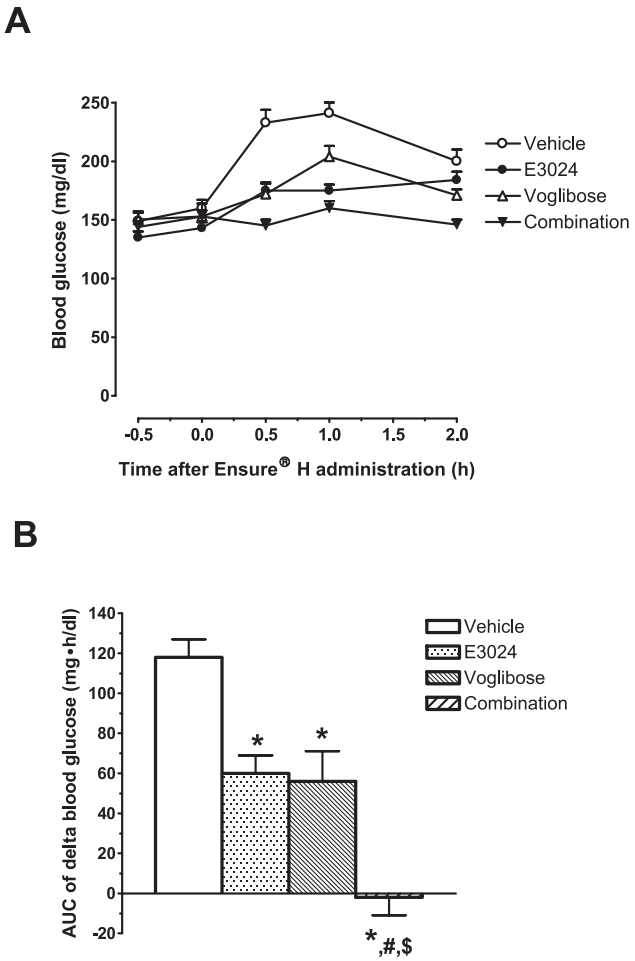

Fig. 6. Effects of a combination of E3024 (3 mg/kg) and voglibose (0.3 mg/kg) on blood glucose levels in a meal tolerance test (Ensure® H, 10 ml/kg) using mice fed a high-fat diet for four weeks. E3024 and/or voglibose was orally administered 0.5 h prior to the Ensure® H loading. Changes of blood glucose levels (A) and AUC values of delta blood glucose values between 0 and 2 h (B) are indicated. Values are expressed as the mean ± S.E.M. of eight mice. Results of two-way ANOVA for AUC values of delta blood glucose were as follows: main effect of E3024, $P<0.05$; main effect of voglibose, $P<0.05$; interaction of E3024 and voglibose, $P>0.05$. Results of the Tukey multiple comparison test are as follows: $^{*}P<0.05$ *vs* vehicle group, $^{\#}P<0.05$ *vs* E3024 group, $^{\$}P<0.05$ *vs* voglibose group.

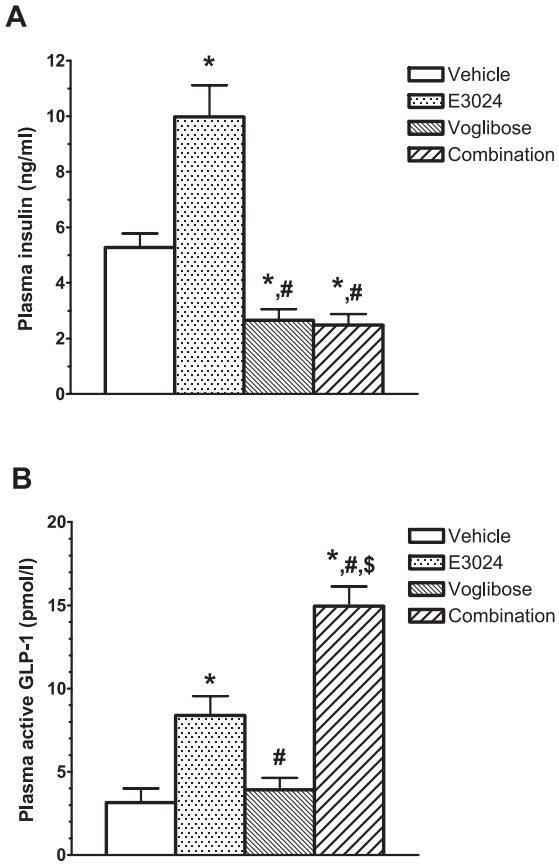

Fig. 7. Effects of a combination of E3024 (3 mg/kg) and voglibose (0.3 mg/kg) on plasma insulin (A) and active GLP-1 levels (B) in mice fed a high-fat diet for four weeks. E3024 and/or voglibose was orally administered 0.5 h prior to the Ensure® H loading, and blood was collected from the tail vein 15 min after and from the orbital sinus 0.5 h after the Ensure® H loading. A: Plasma insulin levels 15 min after the meal loading. B: Plasma active GLP-1 levels after 0.5 h after the meal loading. Values are expressed as the mean ± S.E.M. of ten mice. Results of two-way ANOVA were as follows for both insulin and GLP-1 cases: main effect of E3024, $P<0.05$; main effect of voglibose, $P<0.05$; interaction of E3024 and voglibose, $P<0.05$. Results of the Tukey multiple comparison test are as follows: $^{*}P<0.05$ *vs* vehicle group, $^{\#}P<0.05$ *vs* E3024 group, $^{\$}P<0.05$ *vs* voglibose group.

the first report suggesting benefits for the combination of a DPP-IV inhibitor and an α-glucosidase inhibitor for the efficient improvement of postprandial hyperglycemia.

The highest levels of plasma GLP-1 were in the combination group, but treatment with voglibose alone did not increase the GLP-1 level. Acarbose (28, 29), voglibose (30), and miglitol (31) have been shown to enhance the GLP-1 response in healthy volunteers and type 2 diabetic patients. It is possible that the difference of GLP-1 levels between our study and these papers is due to the difference of the determination methods of GLP-1. For example, antiserum 89390 used in the determination of GLP-1 in two groups (28, 29) cross-reacts 100% with GLP-1(7 – 36) amide and its metabolite GLP-1(9 – 36) amide (29), indicating that this method measures both active and inactive GLP-1 together. On the other hand, we determined active GLP-1 levels only using the Linco's kit specific for active GLP-1. Accordingly, we speculate that GLP-1 was released, but active GLP-1 was rapidly degraded by DPP-IV in the voglibose-treated mice. In the presence of the DPP-IV inhibitor, however, we observed that voglibose gave rise to enhancement of plasma active

GLP-1 levels.

Despite the significantly high active GLP-1 levels, plasma insulin levels in the combination group were significantly lower than those in the vehicle-treatment group, which were almost the same as the levels in the voglibose-treatment group. We postulate that it is because blood glucose levels were profoundly lowered by voglibose-induced glucose malabsorption and GLP-1-induced slower gastric emptying. It has been documented that GLP-1 induces delay of gastric emptying (32), which contributes to postprandial glucose-lowering effects. Then, insulin secretion was not enhanced due to a glucose-dependent insulin secretion mechanism of GLP-1. On the contrary, in E3024-treated mice, plasma insulin release was enhanced, responding to increases in plasma active GLP-1 levels and blood glucose levels.

The additive improvement of glucose tolerance in the combination treatment may be caused by inhibition of gastric emptying. Enç et al. (29) reported that acarbose delays gastric emptying and augments GLP-1 release: acarbose-induced alteration of carbohydrate absorption modifies the intestinal afferent neural transmission and the release of gut peptides, which in turn mediate the inhibitory actions of acarbose on gastric emptying. They thought that augmented GLP-1 release by acarbose plays a major role in the inhibition of gastric emptying.

In summary, this comparative study shows that in contrast to α-glucosidase inhibitors, E3024 is anticipated to exhibit stable glucose-lowering effects, independent of the kinds of carbohydrates in meals. Moreover, the combination of E3024 and voglibose improved glucose tolerance in the meal tolerance test additively, suggesting that a combination of a DPP-IV inhibitor and an α-glucosidase inhibitor may be very useful in clinical practice for the effective improvement of postprandial hyperglycemia.

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