Antidiabetic Drug Voglibose Is Protective Against Ischemia—Reperfusion Injury Through Glucagon-Like Peptide 1 Receptors and the Phosphoinositide 3-Kinase-Akt-Endothelial Nitric Oxide Synthase Pathway in Rabbits

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Abstract: Glucagon-like peptide 1 (GLP-1) reportedly exerts a protective effect against cardiac ischemia. We hypothesized that the α-glucosidase inhibitor voglibose, an unabsorbable antidiabetic drug with cardioprotective effects, may act through stimulation of GLP-1 receptors. The results of the present study suggest oral administration of voglibose reduces myocardial infarct size and mitigates cardiac dysfunction in rabbits after 30 minutes of coronary occlusion and 48 hours of reperfusion. Voglibose increased basal and postprandial plasma GLP-1 levels and reduced postprandial plasma glucose levels. The infarct size-reducing effect of voglibose was abolished by treatment with exendin(9-39), wortmannin, Nω-nitro-L-arginine methylester, or 5-hydroxydecanoate), which inhibit GLP-1 receptors, phosphoinositide 3-kinase, nitric oxide synthase, and K_{ATP} channels, respectively. Western blot analysis showed that treatment with voglibose upregulated myocardial levels of phospho-Akt, phospho-endothelial nitric oxide synthase after myocardial infarction. The upregulation of phospho-Akt was inhibited by exendin(9-39) and wortmannin. These findings suggest that voglibose reduces myocardial infarct size through stimulation of GLP-1 receptors, activation of the phosphoinositide 3-kinase-Akt-endothelial nitric oxide synthase pathways, and the opening of mitochondrial K_{ATP} channels. These findings may provide new insight into therapeutic strategies for the treatment of patients with coronary artery disease.

Key Words: voglibose, GLP-1, ischemia–reperfusion, Akt, eNOS, KATP channel

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INTRODUCTION

Patients with Type 2 diabetes mellitus are at substantially increased risk of coronary artery diseases such as angina pectoris and myocardial infarction,[1] and it is believed this is attributable in large part to the hyperglycemia itself. However, although the UK Prospective Diabetes Study reported that insulin treatment does not affect the incidence of cardiovascular events among these patients,[2] the Funagata Diabetes Study found that postprandial glucose levels, not fasting glucose levels, were associated with cardiovascular disease.[3] The α-glucosidase inhibitor voglibose, which is used as an antidiabetic drug, inhibits glucose absorption from the intestine,[4] thereby reducing postprandial hyperglycemia.[5] In addition, chronic treatment with voglibose reportedly increases plasma glucagon-like peptide 1 (GLP-1) levels in both humans and mice[6,7] and stimulation of GLP-1 receptors protects the heart against ischemia–reperfusion injury.[8–10] Moreover, a recent large-scale clinical trial, the STOP-NIDDM trial (a study of ways to prevent noninsulin-dependent diabetes mellitus), showed that another α-glucosidase inhibitor, acarbose, reduces the risk of myocardial infarction.[11] We therefore hypothesized that voglibose may have cardioprotective effects. Our aim in the present study was to determine whether oral administration of voglibose would reduce myocardial infarct size and, if so, to investigate its mechanism of action.

METHODS

Experimental Animals

All rabbits used in this study received humane care in accordance with the Guide for the Care and Use of Laboratory

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Animals published by the US National Institutes of Health (NIH publication 8523, revised 1985). The study protocol was approved by the Ethical Committee of Gifu University Graduate School of Medicine, Gifu, Japan.

Chemicals

Exendin(9-39) was purchased from BACHEM AG (Bubendorf, Switzerland). Wortmannin, NG-nitro-L-arginine methyl ester (L-NAME) and 5-hydroxydecanoate (5-HD) were purchased from Sigma Chemical Co (St. Louis, MO). Voglibose was provided by Takeda Pharmaceutical Company (Osaka, Japan).

Determination of Plasma Glucose and Glucagon-Like Peptide 1 Levels

Twenty rabbits were used for measurement of plasma glucose and GLP-1 levels. The voglibose group (n = 10) was fed a diet containing 3.5 mg/kg voglibose per day for 7 days, whereas the control group (n = 10) was fed a normal diet for the same period. Arterial blood samples were collected from the ear artery before feeding and 1, 2, and 3 hours after feeding for measurement of plasma glucose and GLP-1 levels. In the voglibose group, moreover, some animals were pretreated with the GLP-1 receptor blocker exendin(9-39) to examine whether blockade of GLP-1 receptors affects plasma glucose levels. The collected blood samples were put into heparinized ice-cold centrifuge tubes and stored at –83°C until assayed. Plasma glucose levels were immediately measured using the glucose oxidation method (Glucorder MAX, A&T, Yokohama, Japan). Plasma GLP-1 levels were measured using an enzyme-linked immunosorbent assay kit (LINCO Research, Inc, St Charles, MO).

Surgical Preparation

All surgical procedures were performed aseptically using male Japanese white rabbits (2.0–2.5 kg) anesthetized with 30 mg/kg sodium pentobarbital and mechanically ventilated with room air. A polyethylene catheter (0.9-mm lumen diameter) was inserted into the jugular vein and was advanced approximately 1 cm toward the heart for administration of drugs and saline. After a left thoracotomy performed in the third intercostal space, the heart was exposed and a 4-0 silk thread was placed beneath the large arterial branch coursing down the middle of the anterolateral surface of the left ventricle. Coronary arterial occlusion and reperfusion were performed by tightening and the releasing a snare made with the thread.

Experimental Protocol

As shown in Figure 1, the rabbits were assigned randomly to one of 10 groups (n = 10 each): control; voglibose (fed a diet containing 3.5 mg/kg voglibose per day); voglibose + exendin(9-39) (fed the same diet as the voglibose group along with intravenous administration of 3 nmol/L (5 μg/kg) exendin[9-39], a GLP-1 receptor blocker); exendin(9-39) (3 nmol/:); voglibose + wortmannin (fed the same diet as the voglibose group along with intravenous administration of 0.6 mg/kg wortmannin, a phosphoinositide 3-kinase [PI3K] inhibitor); wortmannin (0.6 mg/kg); voglibose + L-NAME (fed the same diet as the voglibose group along with intravenous administration of 10 mg/kg L-NAME, a nitric oxide synthase inhibitor); L-NAME (10 mg/kg); voglibose + 5-HD (fed the same diet as the voglibose group along with intravenous administration of 5 mg/kg 5-HD, a mitochondrial K_{ATP} channel blocker); and 5-HD (5 mg/kg). Each protocol was carried out for 7 days, at the end of which the coronary artery was occluded for 30 minutes and then reperfused. Hemodynamic parameters were recorded throughout the occlusion period and for 20 minutes thereafter. The chest was then closed, and the rabbits were allowed to recover for 48 hours to quantify survival.

Determination of Infarct Size

Forty-eight hours after reperfusion, the rabbits were heparinized (500 U/kg) and euthanized by an overdose of pentobarbital. The heart was then excised and mounted on a Langendorff apparatus and, after reoccluding the coronary artery at the site of the original ligature, Evans blue dye (4%; Sigma Chemicals Co) was infused retrogradely into the ascending aorta at 80 mmHg to determine the area at risk. Because we had left the string beneath the coronary artery at the occlusion site when we closed the chest, it was easy to identify the location of the previous coronary ligature. The left ventricle was then sectioned into seven slices cut parallel to the atrioventricular ring. Each slice was weighed, incubated in a 1% solution of triphenyl tetrazolium chloride at 37°C for 10 minutes to visualize the infarct area, and photographed. The ischemic and infarcted regions were traced on each left ventricular slice, and the areas were multiplied by the weight of the slice. The sizes of the infarcted/ischemic regions were then expressed as percentages of the risk area or total left ventricle for each heart.

Physiological Studies

Before induction coronary occlusion (baseline) and 48 hours after reperfusion, arterial blood pressure and heart rate were measured using a catheter introduced in the carotid artery while the rabbits were under light anesthesia (10 mg/kg sodium pentobarbital) and breathing spontaneously. A micromanometer-tipped catheter (SPR 407; Millar Instruments Houston, TX) was also inserted into the left ventricle to record +dP/dt (maximum), an indicator of cardiac systolic function, as well as –dP/dt (maximum), an indicator of cardiac diastolic function. All measurements were made by two persons blinded to the treatment protocol. In addition, echocardiographic studies (SSD2000; Aloka Co, Ltd, Tokyo, Japan) were carried out, and two dimensional parasternal long axis views of the left ventricle were obtained. In general, the best views were obtained with the transducer lightly applied to the middle of the upper left anterior chest wall. The transducer was then gently manipulated until desirable images were obtained. Ejection fraction and left ventricular end-diastolic dimensions were then measured. Ejection fraction was measured using Teichholz method from M-mode images by echocardiography.

Western Blot Analysis

On Day 2 postinfarction, transmural tissue samples (approximately 200 mg) were collected from the center of the infarcted region and a nonischemic area on the opposite side of the left ventricle. The samples were immediately frozen and

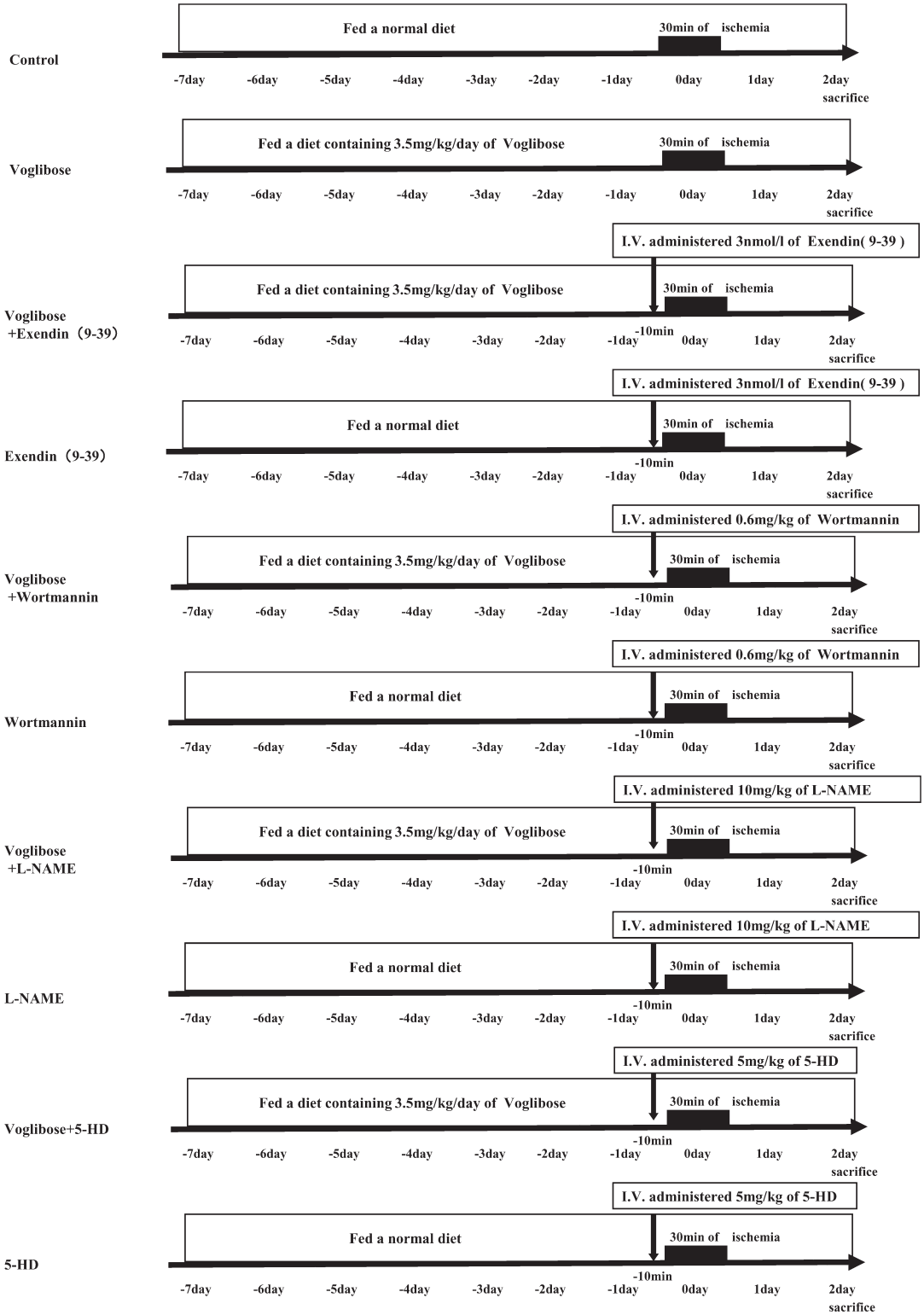

FIGURE 1. Experimental protocols for assessing the effects of voglibose on infarct size and cardiac function after myocardial infarction. To investigate the infarct size-reducing effect of voglibose, 100 Japanese white rabbits underwent 30 minutes of coronary occlusion followed by 48 hours of reperfusion. They were then assigned randomly to 10 groups (n = 10 in each). Exendin(9-39) is a glucagon-like peptide 1 (GLP-1) receptor blocker; 5-hydroxydecanoate (5-HD), a mitochondrial K_{ATP} channel blocker; wortmannin, a phosphoinositide 3-kinase inhibitor; and NG-nitro-L-arginine methyl ester (L-NAME), a nitric oxide synthase inhibitor. IV, intravenous.

stored at –83°C until assayed. Western blot analyses were carried out using lysates of the heart tissue samples. Proteins were separated and transferred to membranes using standard protocols, after which they were probed with antibodies against GLP-1 receptor (from Santa Cruz Biotechnology, Inc). The phosphorylation (activation) of Akt and endothelial nitric oxide synthase was assessed using antibodies against Akt and phosphorylated (p)-Akt (from Cell Signaling Technology) and phosphorylated endothelial nitric oxide synthase (from BD Biosciences). The blots were visualized using chemiluminescence (Amersham), and the signals were quantified by densitometry. α-Tubulin (analyzed with an antibody from Santa Cruz) served as a loading control.

Statistical Analysis

All values are expressed as means ± standard error of mean. The risk areas, infarct sizes, and Western blot data were compared among groups using one-way analysis of variance combined with Bonferroni post hoc test for multiple comparisons. Differences in the hemodynamic parameters over time between the control and the drug-treated groups were assessed by two-way repeated-measures analysis of variance. Values of $P < 0.05$ were considered significant.

RESULTS

Hemodynamic Parameters

Table 1 shows the mean blood pressures and heart rates in the 10 experimental groups studied (see "Methods" for detailed descriptions of the protocols). There were no significant differences among the groups during the experiment.

Plasma Glucose Levels

Figure 2A shows the time course the changes of plasma glucose levels in the control and voglibose groups. There were no significant differences in the fasting plasma glucose levels between these two groups; however, postprandial glucose levels, measured 2 hours and 3 hours after feeding, were significantly lower in the voglibose group than the control group. Moreover, plasma glucose levels were unaffected by intravenous administration of the GLP-1 receptor blocker exendin(9-39).

Plasma Glucagon-Like Peptide 1 Levels

Figure 2B shows the time course of changes in plasma GLP-1 levels in the control and voglibose groups. GLP-1 levels were consistently higher in the voglibose group before feeding and 1, 2, and 3 hours afterward than in the control group.

Physiological Findings

The echocardiographic findings and ±dP/dt for the 10 groups studied are summarized in Figure 3. Before coronary occlusion, there were no significant differences in left ventricular ejection fraction or ±dP/dt among the 10 groups. After 48 hours of reperfusion, however, both of these parameters were significantly higher in the voglibose group than in the control group. Moreover, the voglibose-mediated increase in left ventricular ejection fraction was abolished by

TABLE 1. Hemodynamic Parameters

	Before Ischemia	10 min During Ischemia	20 min During Ischemia	30 min During Ischemia	10 min After Reperfusion
Mean Blood Pressure (mm Hg)					
Control	83.000 ± 2.984	72.209 ± 3.681	69.782 ± 3.941	68.182 ± 2.611	71.282 ± 3.448
Voglibose	77.598 ± 3.244	70.395 ± 2.498	69.633 ± 2.401	67.458 ± 2.461	72.967 ± 4.304
Voglibose + exendin(9-39)	78.671 ± 3.589	71.503 ± 4.613	70.255 ± 3.423	68.378 ± 3.183	74.289 ± 3.607
Exendin(9-39)	83.012 ± 3.993	71.614 ± 4.331	70.253 ± 3.394	69.927 ± 3.633	72.466 ± 4.344
Voglibose + wortmannin	81.824 ± 3.033	73.967 ± 4.463	71.420 ± 3.452	69.041 ± 3.654	72.351 ± 2.991
Wortmannin	84.423 ± 2.622	78.251 ± 3.335	77.124 ± 3.512	70.504 ± 2.344	78.259 ± 3.415
Voglibose + L-NAME	79.235 ± 3.324	69.232 ± 2.456	67.443 ± 1.838	67.277 ± 3.826	71.553 ± 3.366
L-NAME	77.589 ± 3.844	69.071 ± 4.452	70.714 ± 3.654	68.744 ± 3.257	70.662 ± 3.509
Voglibose + 5-HD	83.422 ± 2.559	79.103 ± 1.893	75.228 ± 2.483	73.990 ± 1.633	74.682 ± 2.663
5-HD	78.477 ± 3.667	77.442 ± 2.564	71.524 ± 1.927	72.348 ± 2.277	76.230 ± 2.796
Heart Rate (beats/min)					
Control	245.473 ± 5.174	236.771 ± 6.713	238.438 ± 7.198	241.899 ± 7.353	240.519 ± 8.653
Voglibose	244.267 ± 6.003	239.222 ± 4.458	239.572 ± 3.488	242.873 ± 3.668	230.463 ± 4.552
Voglibose + exendin(9-39)	250.123 ± 9.970	242.490 ± 9.827	243.351 ± 10.310	244.751 ± 9.651	240.955 ± 6.793
Exendin(9-39)	242.774 ± 5.445	246.201 ± 6.664	244.863 ± 8.609	245.309 ± 5.347	245.446 ± 7.962
Voglibose + wortmannin	235.935 ± 3.262	245.783 ± 3.252	240.283 ± 7.568	239.012 ± 6.878	233.526 ± 3.662
Wortmannin	242.636 ± 4.352	246.552 ± 3.426	239.649 ± 6.876	239.530 ± 6.066	236.447 ± 7.868
Voglibose + L-NAME	258.029 ± 3.472	248.834 ± 8.458	246.881 ± 10.430	244.643 ± 5.621	247.644 ± 5.572
L-NAME	254.259 ± 3.567	248.413 ± 7.781	249.034 ± 7.429	240.692 ± 9.637	246.890 ± 4.372
Voglibose + 5-HD	254.624 ± 9.622	242.455 ± 10.823	250.132 ± 5.561	243.648 ± 10.070	239.063 ± 7.863
5-HD	250.795 ± 5.978	239.567 ± 7.034	241.833 ± 6.372	240.257 ± 7.869	242.704 ± 9.443

L-NAME, NG-nitro-L-arginine methyl ester; 5-HD, 5-hydroxydecanoate.

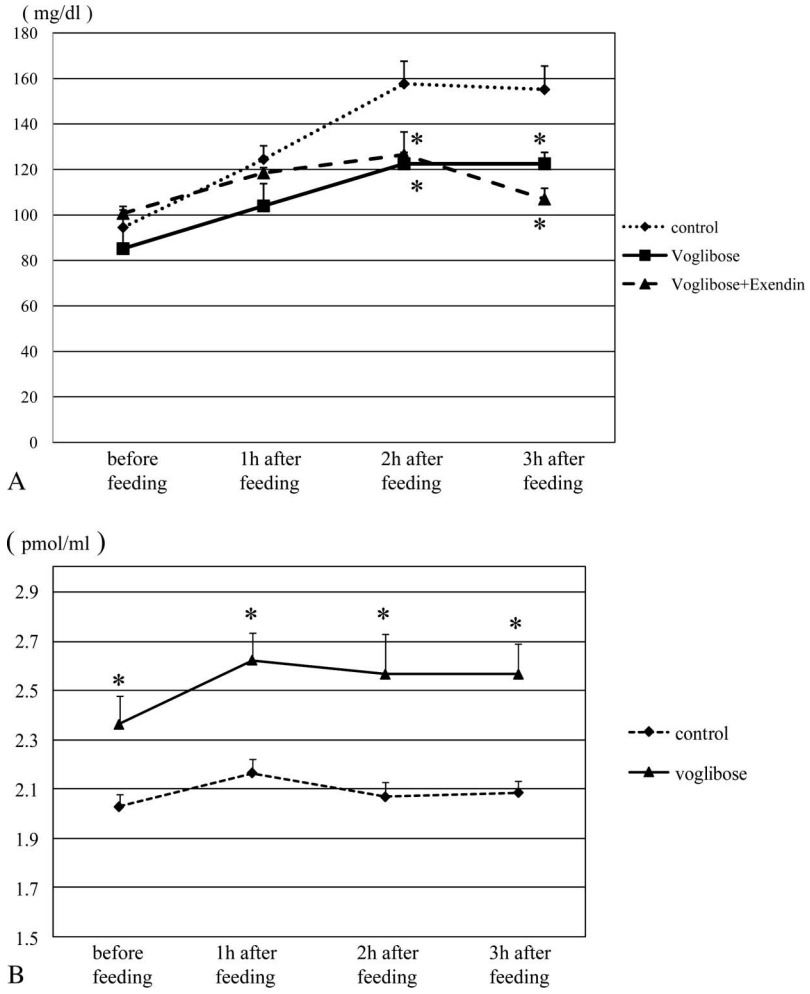

FIGURE 2. Effect of voglibose on plasma glucose (A) and glucagon-like peptide 1 (GLP-1) levels (B). Rabbits were fed a diet containing 3.5 mg/kg per day of voglibose with or without intravenous administration of 3 nmol/L exendin(9-39) for 7 days. Blood samples were collected on the seventh day in the morning before feeding and 1, 2, and 3 hours after feeding. Exendin = exendin(9-39). *$P < 0.05$ versus control.

pretreatment with exendin(9-39), wortmannin, L-NAME, or 5-HD. There was no significant difference in the left ventricular end-diastolic dimension among the 10 groups either before ischemia or after 48 hours of reperfusion.

Myocardial Infarct Size

Typical pictures of the 2,3,5-triphenyltetrazolium chloride staining and Evans blue dye staining of cross sections of the left ventricle are shown in Figure 4. The infarct size as a percentage of the area at risk looks smaller than those of other groups. On average, the sizes of the area at risk expressed as a percentage of the left ventricular were similar among the 10 groups studied (Fig. 5A). On the other hand, the infarct size was significantly smaller in the voglibose group (22.6% ± 4.2%) than in the control group (42.9% ± 3.24%). This beneficial effect of voglibose was completely abolished by the pretreatment with exendin(9-39) (36.9% ± 2.79%), wortmannin (41.7% ± 5.29%), L-NAME (37% ± 4.71%), or 5-HD (43.1% ± 1.66%) (Fig. 5B), although these agents had no effect on infarct size by themselves (37.3% ± 4.13%, 41.8% ± 3.2%, 40.6% ± 3.57%, and 42.7% ± 3.52%, respectively).

Western Blot Analysis

On Day 2 postinfarction, Western blot analysis revealed that myocardial expression of the GLP-1 receptor was markedly stronger in both ischemic and nonischemic areas of hearts in the control, voglibose, voglibose + exendin(9-39). and voglibose + wortmannin groups than in a sham-operated group (Fig. 6). There were no significant differences in the myocardial expression of GLP-1 receptors among the four groups subjected to coronary occlusion.

There were also no significant differences in the expression of Akt between the infarcted and nonischemic areas in any of the groups tested (Fig. 6). However, level of activated (phosphorylated) Akt (p-Akt) was significantly upregulated as compared with controls in both nonischemic and infarcted areas of hearts in the voglibose group, although the upregulation of p-Akt was larger in the infarcted area than in the nonischemic region. This upregulation of p-Akt was abolished by pretreatment with exendin(9-39) or wortmannin. Similarly, expression of phosphorylated endothelial nitric oxide synthase was significantly upregulated in the infarcted area and tended to be upregulated in the nonischemic areas in the voglibose group, and this upregulation of phosphorylated

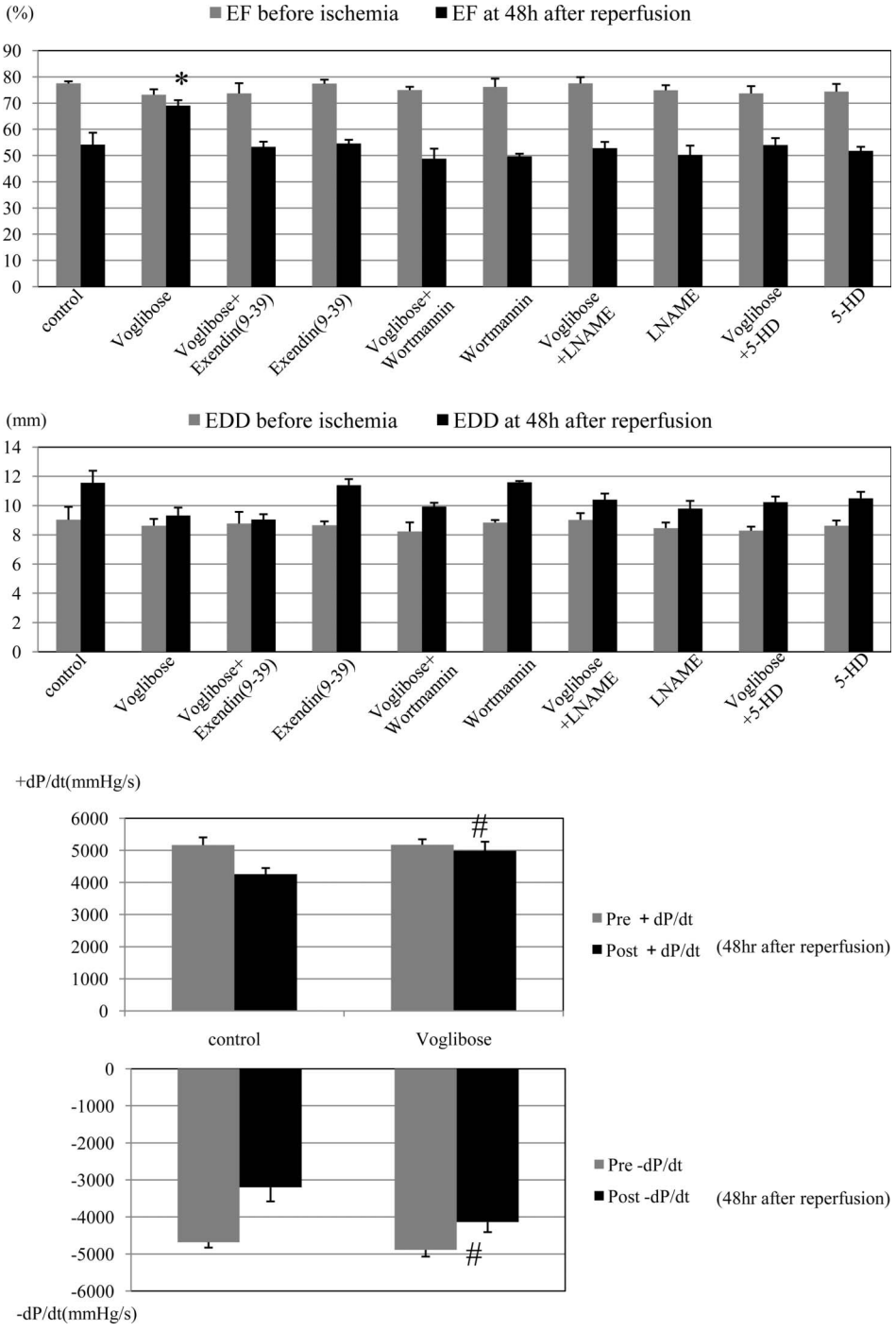

FIGURE 3. Echocardiographic analysis and ±dP/dt measurements illustrating the effects of voglibose on left ventricular ejection fraction (EF) and end-diastolic dimension (EDD) in the 10 groups studied. Data were collected before coronary occlusion (gray bars) and after 48 hours of reperfusion (black bars). *$P < 0.05$ versus EF after 48 hours of reperfusion in the other groups. #$P < 0.05$ versus control after 48 hours of reperfusion. L-NAME, NG-nitro-L-arginine methyl ester; 5-HD, 5-hydroxydecanoate.

endothelial nitric oxide synthase was also blocked by pretreatment with exendin(9-39) or wortmannin.

DISCUSSION

The results of the present study suggest that oral administration of voglibose prevents postprandial hyperglycemia, reduces myocardial infarct size, and increases plasma levels of GLP-1 as well as myocardial levels of p-Akt and phosphorylated endothelial nitric oxide synthase. The reduction in infarct size by voglibose was completely blocked by exendin(9-39), wortmannin, L-NAME, or 5-HD, whereas the upregulation of p-Akt was inhibited by exendin(9-39) and wortmannin.

Hemodynamics During Experiments

Among the 10 groups studied, there were no significant differences in blood pressure or heart rate, suggesting

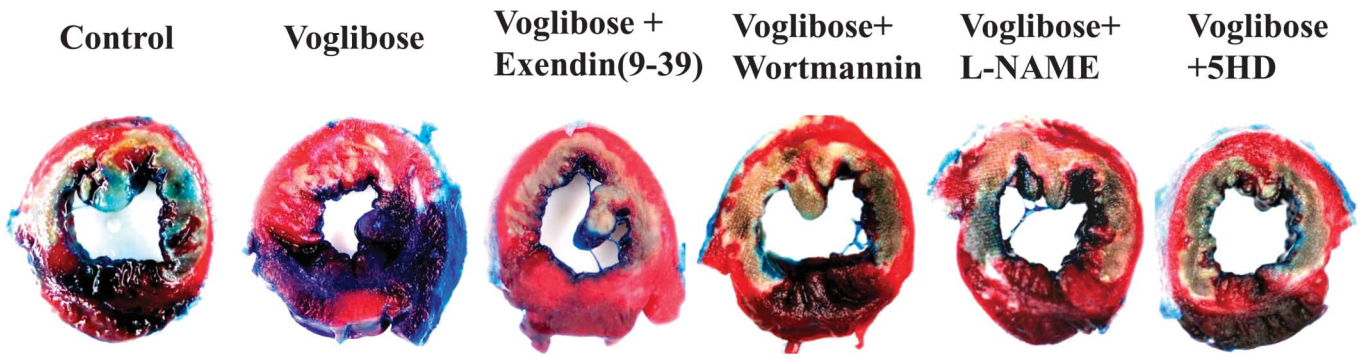

FIGURE 4. Typical pictures of the 2,3,5-triphenyltetrazolium chloride staining and Evans blue dye staining of cross-sections of the left ventricle. As can be seen, the infarct size as a percentage of area at risk is significantly smaller than those of other groups. Blue, nonrisk area; gray, infarct area; gray + red, risk area. L-NAME, NG-nitro-L-arginine methyl ester.

voglibose does not act to reduce infarct size by reducing oxygen consumption. The ±dP/dt at 48 hours of reperfusion was improved in the voglibose group as compared with the control group. The improvement of left ventricle function in the voglibose group may be the result of the reduction in the infarct size because the improvement of left ventricle ejection fraction and ±dP/dt was correlated with the reduction in the infarct size.

Plasma Glucose Levels

It is now well established that patients with diabetes mellitus are at substantially increased risk of coronary artery

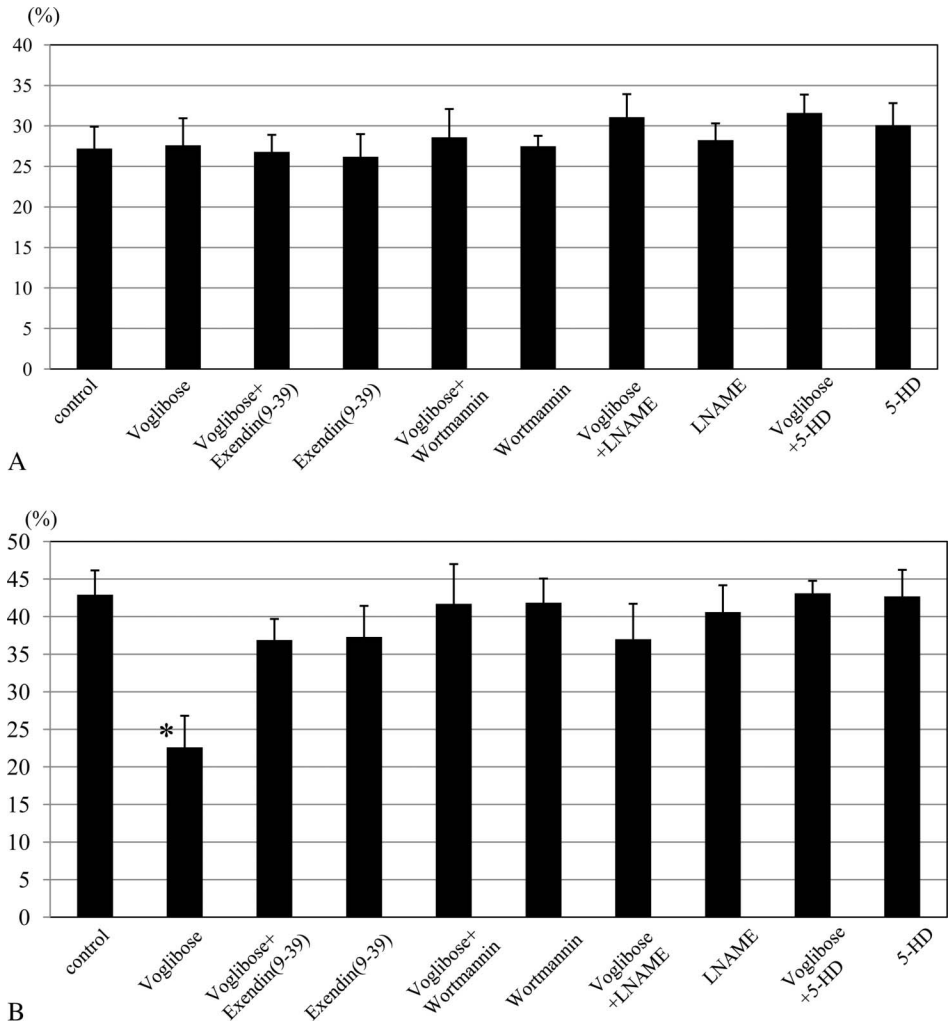

FIGURE 5. Effect of the indicated treatment protocol on area at risk expressed as a percentage of total left ventricle (A) and infarct size expressed a percentage of the area at risk (B). There were no significant differences in risk area among the groups. Voglibose significantly reduced infarct size, however. The infarct size-reducing effect of voglibose was blocked by pretreatment with exendin(9-39), wortmannin, NG-nitro-L-arginine methyl ester (L-NAME), or 5-hydroxydecanoate (5-HD). *$P <$ 0.05 versus the other groups.

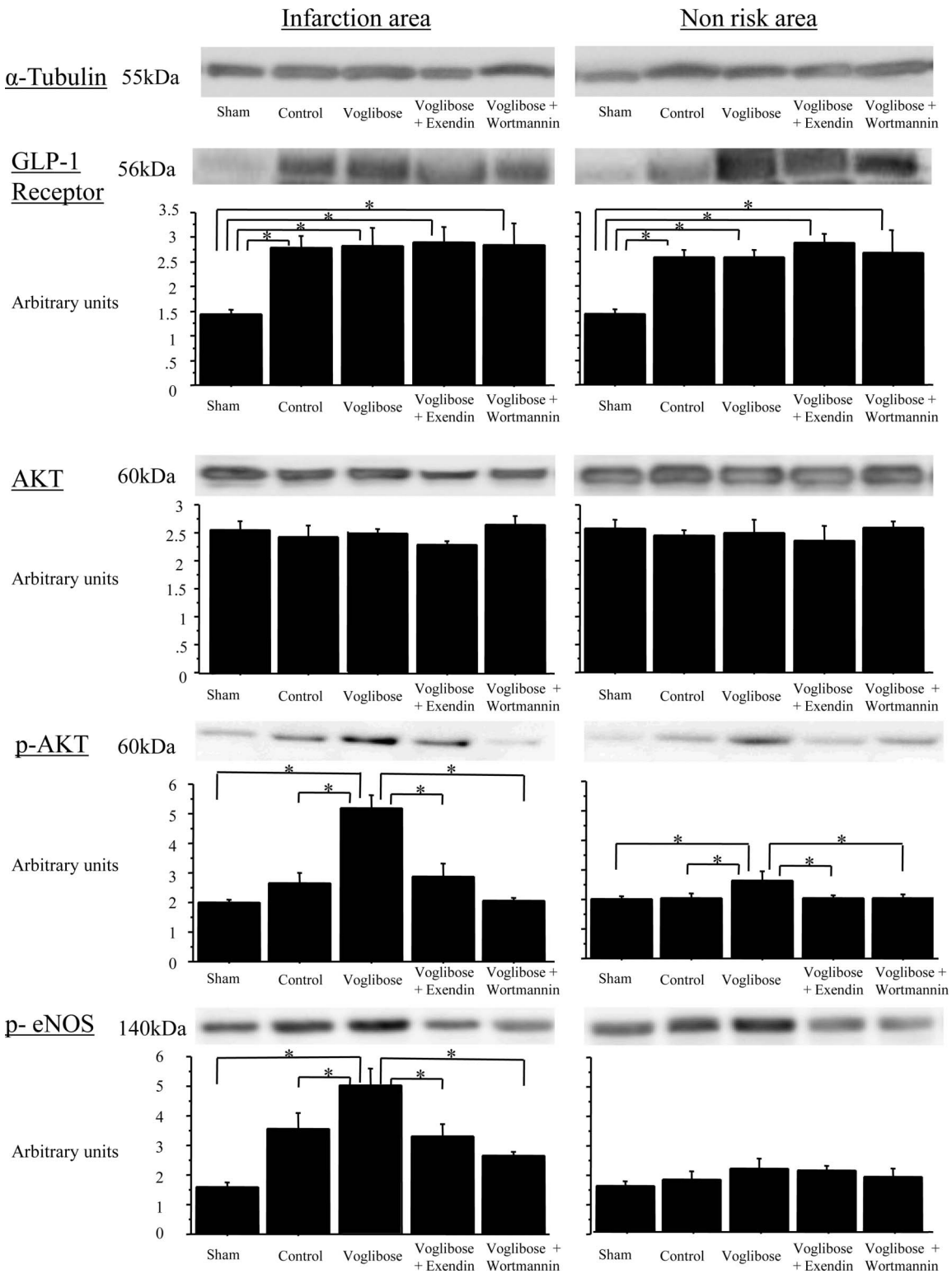

FIGURE 6. Western blot analysis of myocardial glucagon-like peptide 1 (GLP-1) receptor, Akt, (phosphorylated) p-Akt, and phosphorylated endothelial nitric oxide synthase expression in the indicated groups on Day 2 postinfarction. In the voglibose group, expression of p-Akt was significantly upregulated in both the ischemic and nonischemic areas. Similarly, expression of phosphorylated endothelial nitric oxide synthase was significantly upregulated in the infarcted area and tended to be upregulated in the nonischemic areas in the voglibose group. This upregulation was abolished by pretreatment with exendin(9-39) or wortmannin. *$P < 0.05$ versus control. p-eNOS, phosphorylated endothelial nitric oxide synthase.

diseases such as angina pectoris and myocardial infarction.[1] The effect of hyperglycemia on ischemia–reperfusion injury remains controversial. However, in the present study, oral administration of voglibose to rabbits significantly inhibited the rise of plasma glucose levels otherwise seen 1, 2, and 3 hours after feeding and significantly reduced the size of myocardial infarcts induced by coronary ligation for 30 minutes. This raises the possibility that infarct size is related in part to postprandial plasma glucose levels. Consistent with that idea, Ebel et al reported that hyperglycemia induced in rabbits by continuous dextrose infusion during ischemia and reperfusion tended to increase myocardial infarct size, although the result was not statistically significant,[12] whereas Franz et al found that hyperglycemia induced by oral administration of sucrose increased myocardial infarct size in mice.[13] Earlier studies also showed that ischemic preconditioning leads to the opening of mitochondrial K_{ATP} channels[14] and that blood glucose levels are involved in regulating the channel activity. For example, hyperglycemia abolishes ischemic preconditioning in vivo[15] and impairs activation of mitochondrial K_{ATP} channels.[16] In those studies, however, the difference in blood glucose levels was approximately 200 mg/dL, whereas the difference in the present study was only approximately 40 mg/dL, making it difficult to relate our findings to those earlier studies. Nonetheless, the observation that pretreatment with 5-HD, a K_{ATP} channel blocker, completely blocked the effects of voglibose suggests mitochondrial K_{ATP} channels play a key role in reducing myocardial infarct size.

Plasma Glucagon-Like Peptide 1 Levels, Glucagon-Like Peptide 1 Receptors, Signal Transduction, and Infarct Size

GLP-1 is a 30-amino acid intestinal hormone secreted in a nutrient-dependent manner that stimulates insulin secretion, thereby reducing postprandial glycemia.[17]. GLP-1 also reportedly mitigates postischemic myocardial dysfunction and reduces myocardial infarct size in rats[8,9] and swine.[10] In the present study, treatment with voglibose increased basal plasma GLP-1 levels as well as levels 1, 2, and 3 hours after feeding, which is consistent with the earlier finding that 23 days of a diet containing 1.4 mg/kg per day or 6.5 mg/kg per day of voglibose increased plasma levels of the active form of GLP-1.[7]

In the present study, voglibose significantly reduced myocardial infarct size, and this beneficial effect was completely abolished by pretreatment with exendin(9-39), a GLP-1 receptor antagonist, which suggests this effect of voglibose is mediated through myocardial GLP-1 receptors. Consistent with that idea, we observed that plasma GLP-1 levels were significantly upregulated in the voglibose group and that myocardial expression of the GLP-1 receptor was upregulated in both ischemic and nonischemic areas of hearts from rabbits in the control, voglibose, voglibose + exendin, and voglibose + wortmannin groups after coronary occlusion. Because there was no significant difference in the levels of myocardial expression of GLP-1 receptors among these four groups, we cannot rule out the possibility that infarction itself leads to upregulated expression of GLP-1 receptors. Perhaps upregulation of myocardial GLP-1 receptors is an intrinsic mechanism that protects the heart from ischemia–reperfusion injury,[8–10] If so, the voglibose-induced reduction in myocardial infarct size may be mediated through the enhanced stimulation of GLP-1 receptors reflecting the voglibose-induced increase in plasma GLP-1.

Stimulation of GLP-1 receptors reportedly activates PI3K in β-cells.[18] In the present study, the infarct size-reducing effect of voglibose was completely abolished by pretreatment with wortmannin, a PI3K inhibitor, suggesting voglibose also acts through activation of myocardial PI3K. The PI3K-Akt pathway is reported to be involved in the infarct size-reducing effect of ischemic preconditioning and in transduction of prosurvival signals.[19] In the present study, expression of p-Akt was significantly upregulated in both ischemic and nonischemic areas in the voglibose group, although the effect was larger in the ischemic areas of hearts. The fact that upregulation of p-Akt was abolished by pretreatment with exendin(9-39) or wortmannin suggests that voglibose activates a PI3K-Akt pathway through stimulation of GLP-1 receptors. Similarly, phosphorylated endothelial nitric oxide synthase expression was also significantly upregulated in the ischemic and tended to be upregulated in the nonischemic areas of hearts in the voglibose group, and this effect was too blocked by pretreatment with exendin(9-39) or wortmannin, suggesting that the upregulation of phosphorylated endothelial nitric oxide synthase by voglibose was also through activation of GLP-1 receptors and PI3K. Interestingly, nitric oxide reportedly mediates early and late-phase beneficial effects of ischemic preconditioning in rabbits.[20,21] Our finding that the infarct size-reducing effect of voglibose was completely blocked by pretreatment with L-NAME suggests that this effect of voglibose was mediated through production of nitric oxide. Moreover, endothelial nitric oxide synthase expression is reportedly situated downstream of Akt

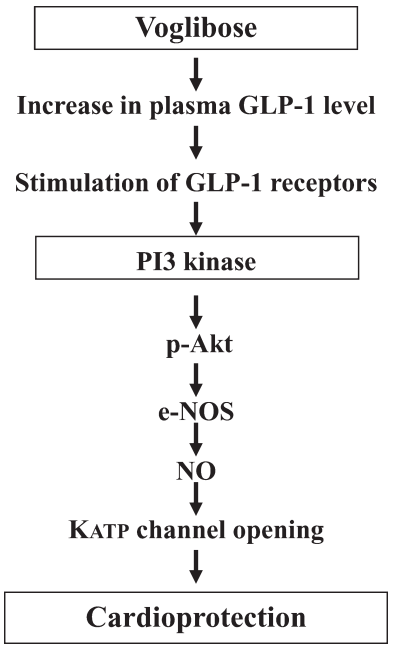

FIGURE 7. Proposed mechanism of cardioprotection by voglibose. GLP-1, glucagon-like peptide 1.

activation.[22] Thus, the infarct size-reducing effect of voglibose appears to be mediated through stimulation of GLP-1 receptors and activation of a prosurvival PI3K-Akt-endothelial nitric oxide synthase pathway. Therefore, the effects of voglibose on the heart are indirect ones because voglibose is an unabsorbable drug.

From the findings obtained in the present study, we propose a possible mechanism by which voglibose protects the heart as shown in Figure 7.

CONCLUSION

We found that orally administered voglibose protects the myocardium against ischemia–reperfusion injury through stimulation of GLP-1 receptors, activation of PI3K-Akt-endothelial nitric oxide synthase pathways, and the opening of mitochondrial K_{ATP} channels. Our findings may thus provide new insight into therapeutic strategies for the treatment of patients with coronary artery disease.

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