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RESEARCH PAPER

Both stimulation of GLP-1 receptors and inhibition of glycogenolysis additively contribute to a protective effect of oral miglitol against ischaemia-reperfusion injury in rabbits

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BACKGROUND AND PURPOSE
We previously reported that pre-ischaemic i.v. miglitol reduces myocardial infarct size through the inhibition of glycogenolysis during ischaemia. Oral administration of miglitol has been reported to produce glucagon-like peptide 1 (GLP-1). We hypothesized that p.o. administration of miglitol, an absorbable antidiabetic drug, reduces myocardial infarct size by stimulating GLP-1 receptors and inhibiting glycogenolysis in the myocardium.

EXPERIMENTAL APPROACH
The effects of p.o. and i.v. administration of miglitol on myocardial infarct size were compared in a rabbit model of ischaemia induced by 30 min of coronary occlusion and 48 h of reperfusion. The levels of phospho(p)-PI3kinase and p-Akt were measured in cardiac tissue by use of Western blot analysis.

RESULTS
Both p.o. and i.v. administration of miglitol reduced the infarct size, and this effect was greater after p.o. than after i.v. administration under similar plasma miglitol concentrations. The reduction in infarct size induced by p.o. miglitol but not that induced by i.v. miglitol was partially inhibited by treatment with exendin(9-39), a GLP-1 receptor blocker. Both p.o. and i.v. miglitol improved ejection fraction and $\pm dP/dt$ after myocardial infarction. Miglitol administered p.o. but not i.v. up-regulated the myocardial expression of phospho(p)-PI3kinase and p-Akt following myocardial infarction; an effect that was inhibited by exendin(9-39).

CONCLUSIONS AND IMPLICATIONS
Administration of miglitol p.o. reduces myocardial infarct size through stimulation of GLP-1 receptors and activation of PI3kinase-Akt pathway in addition to the inhibition of glycogenolysis. These findings may have clinical implications for the p.o. administration of miglitol for the treatment of patients with diabetes mellitus combined with coronary artery disease.

Abbreviations
GLP-1, glucagon-like peptide 1; **LV**, left ventricle

Introduction

Patients with type 2 diabetes mellitus are at substantially increased risk of coronary artery diseases such as angina pectoris and myocardial infarction (Stamler *et al.*, 1993; UKPDS Group, 1998). The Funagata Diabetes Study reported that postprandial glucose levels, not fasting glucose levels, are associated with cardiovascular disease (Tominaga *et al.*, 1999). Therefore, α-glucosidase inhibitors, which inhibit glucose absorption from the intestine (Bischoff, 1994), thereby reducing postprandial hyperglycaemia (Satoh *et al.*, 2000), are useful for the treatment of type 2 diabetes mellitus. Among the α-glucosidase inhibitors, miglitol is a drug that can be absorbed from the intestine, while acarbose and voglibose are unabsorbable drugs. We previously reported that i.v. administration of miglitol significantly reduces myocardial infarct size by inhibiting glycogenolysis during ischaemia (Minatoguchi *et al.*, 1999) and this is associated with inhibition of hydroxyl radical production during ischaemia and reperfusion (Wang *et al.*, 2004). On the other hand, it has been reported that chronic p.o. treatment with miglitol increases plasma glucagon-like peptide 1 (GLP-1) levels in humans (Lee *et al.*, 2002; Arakawa *et al.*, 2008). GLP-1 is one of two physiological hormones that meet the criteria of 'incretin' that is released from the intestine in response to nutrients and exerts a potent insulin-releasing effect on pancreatic β-cells (Grieve *et al.*, 2009). It is now well established that GLP-1-induced insulin secretion leads to significant postprandial glucose lowering in both diabetic animal models and patients with type 2 diabetes (Grieve *et al.*, 2009). The major active form of GLP-1 is a GLP-1(7-36) amide (Orskov *et al.*, 1994) and following its release into the circulation, GLP-1(7-36) amide undergoes rapid enzymatic degradation by dipeptidyl peptidase-4 (DPP-4) (Deacon, 2004), which removes an N-terminal dipeptide to form inactive GLP-1(9-36) amide (Knudsen and Pridal, 1996). It has also been reported that stimulation of GLP-1 receptors protects the heart against ischaemia-reperfusion injury (Bose *et al.*, 2005; Ban *et al.*, 2008; Timmers *et al.*, 2009).

We therefore in the present study hypothesized that p.o. administration of miglitol may have cardioprotective effects produced not only by inhibiting glycogenolysis during ischaemia but also through the stimulation of GLP-1 receptors due to an increase in plasma GLP-1 induced by p.o. administration of miglitol. The aim of the present study was to determine whether p.o. administration of miglitol would reduce myocardial infarct size and, if so, to investigate the different molecular mechanism of action of miglitol administered p.o. from that administered i.v.

Methods

Experimental animals

All animal care and experimental procedures used in this study complied with the Guide for the Care and Use of Laboratory 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). Miglitol was provided by Sanwa Chemical Industry Company (Nagoya, Japan).

Determination of plasma miglitol levels after p.o. and i.v. administration of miglitol

To compare the effects of p.o. and i.v. administration of miglitol on myocardial infarct size, we firstly tried to select a dose of miglitol to administer p.o. that would reach a similar plasma miglitol level as that observed after i.v. administration of miglitol. We previously reported the effect of three doses (1, 5 and 10 $mg \cdot kg^{-1}$) of miglitol on plasma miglitol levels 5 min, 30 min and 60 min after its i.v. administration in rabbits, as shown in Figure 1A (Minatoguchi *et al.*, 1999). In the present study, to obtain a plasma miglitol level similar to that after i.v. administration of miglitol, as a preliminary study, three doses of miglitol were given to rabbits p.o. and plasma miglitol levels were measured, as shown in Figure 1B. On the basis of these data, we decided to select 2000 ppm miglitol for p.o. administration of miglitol and 5 $mg \cdot kg^{-1}$ of miglitol for i.v. administration of miglitol to obtain similar plasma miglitol levels between p.o. and i.v. administration of miglitol. We also measured plasma miglitol levels after i.v. administration of 5 $mg \cdot kg^{-1} \cdot day^{-1}$ of miglitol for 7 days to compare with those after a 1 day injection of 5 $mg \cdot kg^{-1}$ of miglitol.

In the present study, rabbits (2 kg in weight) ate 100 $g \cdot day^{-1}$ of chow and therefore 100 $mg \cdot kg^{-1} \cdot day^{-1}$ miglitol (2000 ppm miglitol) was orally administered for 7 days. Plasma miglitol levels were measured in the same blood samples that were used to measure the plasma glucose concentration, which were taken from the ear artery. Diet with miglitol-containing chow was stopped for 12 h on the sixth day and then, on the seventh day, re-feeding was initiated. Blood samples were taken before, 1, 2, 3 and 4 h after initiation of re-feeding. However, in the miglitol-i.v. group, blood samples were taken before, 5 min, 30 min and 60 min after i.v. injection of 5 $mg \cdot kg^{-1}$ miglitol. To measure plasma levels of miglitol, miglitol in plasma was converted to miglitol acetate derivative according to the method described by Guerrant and Moss (Guerrant and Moss, 1984). Miglitol acetate derivative was determined by HPLC (Nanospace S1-2, Shiseido, Tokyo, Japan) and using a mass spectrometer (TSQ, Thermo Fisher Scientific, Waltham, MA, USA) through Cadenza CD-C18 column (75 mm × 2.0 mm, internal diameter of 3 mm, Imtakt, Kyoto, Japan).

Determination of plasma glucose, insulin and GLP-1 levels

Twenty rabbits were used for measurement of plasma glucose, insulin and GLP-1 levels. The miglitol group ($n = 10$) was fed a diet containing 100 $mg \cdot kg^{-1} \cdot day^{-1}$ miglitol for 7 days, while 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 h after feeding for measurement of plasma glucose, insulin and GLP-1 levels. Moreover, in the miglitol-p.o. group, some animals ($n = 10$) were pretreated with the GLP-1 receptor blocker exendin(9-39) to examine whether blockade of GLP-1 receptors affects plasma

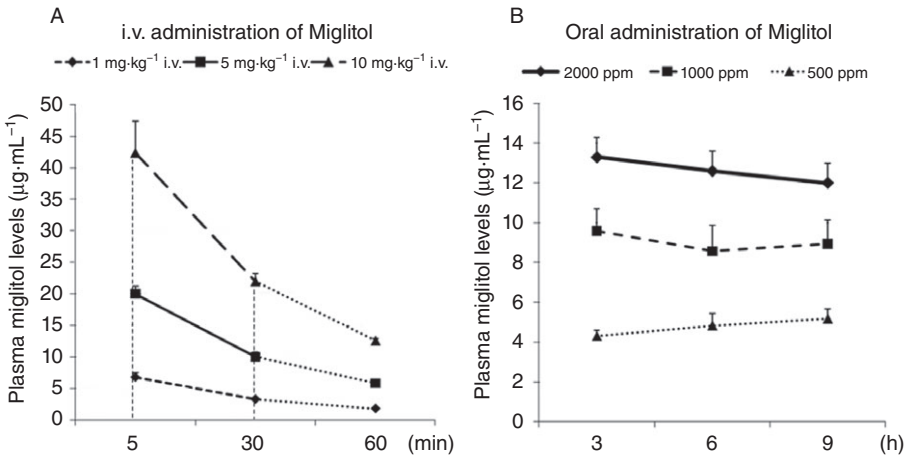

Figure 1

(A) Previously reported effect of i.v. administration of 1 mg·kg^{-1}, 5 mg·kg^{-1} and 10 mg·kg^{-1} miglitol on plasma miglitol levels (n = 3 in each). The time courses of changes of plasma miglitol levels 5 min, 30 min and 60 min after i.v. administration of miglitol are shown. (B) Effect of p.o. administration of three doses of miglitol on plasma glucose levels 3 h, 6 h and 9 h after the start of eating a diet with miglitol-containing chow (n = 3 in each).

glucose levels. The collected blood samples were put into heparin-containing ice-cold centrifuge tubes and stored at –83°C until assay. Plasma glucose levels were immediately measured using the glucose oxidation method (Glucorder MAX, A&T, Yokohama, Japan). Plasma insulin levels were measured using ARCHITECT Insulin kit (ABBOT JAPAN., CO., LTD, Matsudo, Japan). Plasma GLP-1 levels were measured using an ELISA kit (LINCO Research, Inc. St. Charles, MO, USA).

Surgical preparation

All surgical procedures were performed aseptically using male Japanese white rabbits (2.0 to 2.5 kg) anaesthetized with 30 mg·kg^{-1} sodium pentobarbital administered into the ear vein and mechanically ventilated with room air. A polyethylene catheter (0.9 mm lumen diameter) was inserted into the jugular vein and was advanced ~1 cm towards the heart for administration of drugs and saline. After a left thoracotomy was 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 (LV). Coronary arterial occlusion and reperfusion were performed by tightening and then releasing a snare made with the thread.

Experimental protocol

As shown in Figure 2, the rabbits were assigned randomly to one of seven groups (n = 10 each): control group; miglitol-p.o. group (fed a diet containing 100 mg·kg^{-1}·day^{-1} miglitol for 7 days); miglitol-p.o. + exendin(9-39) [fed the same diet as the miglitol-p.o. group along with i.v. administration of 3 nmol·L^{-1} (5 ug·kg^{-1}) exendin(9-39), a GLP-1 receptor blocker]; exendin(9-39) (3 nmol·L^{-1}); miglitol-i.v. group (5 mg·kg^{-1} miglitol was injected i.v. 5 min before ischaemia); and miglitol-i.v. + exendin(9-39) group [5 mg·kg^{-1} miglitol was injected i.v. 5 min before ischaemia along with i.v. administration of 3 nmol·L^{-1} exendin(9-39)]; miglitol-i.v. 7 days group (5 mg·kg^{-1} miglitol was injected i.v. for 7 days and the last injection was performed 5 min before ischaemia). The coronary artery was occluded for 30 min and then reperfused. Haemodynamic parameters were recorded throughout the occlusion period and for 20 min thereafter. The chest was then closed and the rabbits were allowed to recover for 48 h to quantify survival.

Determination of infarct size

Forty-eight hours after reperfusion, the rabbits were treated with heparin (500 U·kg^{-1}) and killed by an overdose of pentobarbital. The heart was then excised and mounted on a Langendorff apparatus and, after the coronary artery had been re-occluded at the site of the original ligature, Evans blue dye (4%; Sigma Chemicals, St. Louis, MO, USA) 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 LV 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 min to visualize the infarct area, and photographed. The ischaemic and infarcted regions were traced on each LV slice, and the areas were multiplied by the weight of the slice. The sizes of the infarcted/ischaemic regions were then expressed as percentages of the risk area or total LV for each heart.

Physiological studies

Before the induction of coronary occlusion (baseline) and 48 h after reperfusion, arterial blood pressure and heart rate were measured using a catheter introduced in the carotid artery while the rabbits were under light anaesthesia (10 mg·kg^{-1} sodium pentobarbital) and breathing spontane-

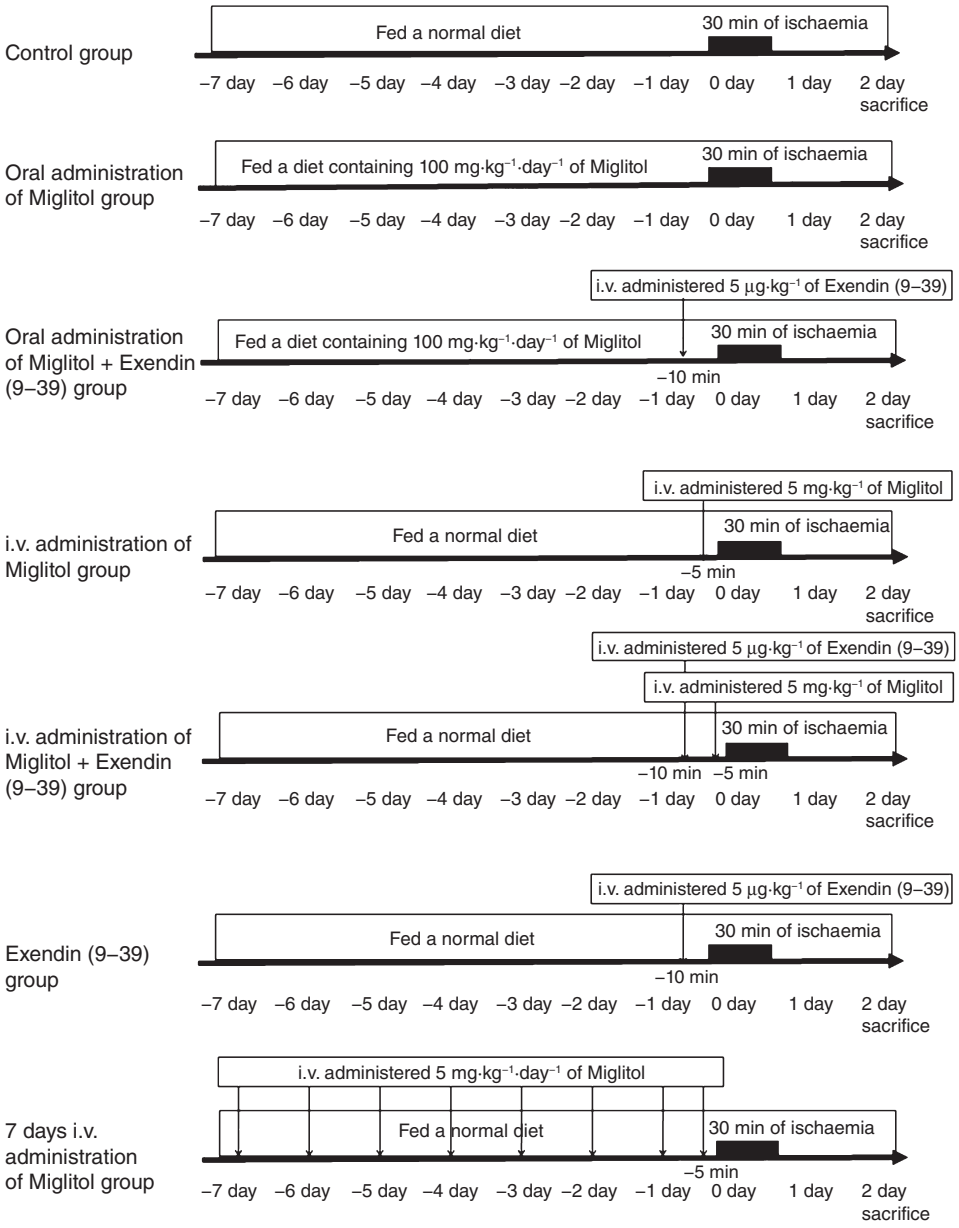

Figure 2

Experimental protocols for assessing the effects of miglitol on infarct size following myocardial infarction. To investigate the infarct size-reducing effect of miglitol, Japanese white rabbits underwent 30 min of coronary occlusion followed by 48 h of reperfusion. They were then assigned randomly to seven groups (n = 10 in each). Exendin(9-39) is a GLP-1 receptor blocker.

ously. A micromanometer-tipped catheter (SPR 407, Millar Instruments) was also inserted into the left ventricle to record +dP/dt (max), an indicator of cardiac systolic function, as well as –dP/dt (max), an indicator of cardiac diastolic function. All measurements were made by two persons unaware of the treatment protocol. In addition, echocardiographic studies (SSD2000, Aloka Co, Ltd) were carried out, and two dimensional parasternal long axis views of the LV 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 LV 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 (~200 mg) were collected from the centre of the infarcted region and a non-ischaemic area on the opposite side of the LV. The samples were immediately frozen and stored at –83°C until the assayed. Western blot analyses were carried out using lysates of the heart tissue samples. Proteins were separated and transferred to membranes using standard protocols. The

Table 1
Haemodynamic parameters

Mean blood pressure (mmHg)	Before ischaemia	10 min during ischaemia	20 min during ischaemia	30 min during ischaemia	10 min after reperfusion
Control	83.0 ± 2.9	72.2 ± 3.6	69.7 ± 3.9	68.1 ± 2.6	71.2 ± 3.4
p.o. miglitol group	77.5 ± 3.2	70.3 ± 2.4	69.6 ± 2.4	67.4 ± 2.4	72.9 ± 4.3
p.o. miglitol + exendin(9-39) group	78.6 ± 3.5	71.5 ± 4.6	70.2 ± 3.4	68.3 ± 3.1	74.2 ± 3.6
i.v. miglitol group	83.0 ± 3.9	71.6 ± 4.3	70.2 ± 3.3	69.9 ± 3.6	72.4 ± 4.3
i.v. miglitol + exendin(9-39) group	81.8 ± 3.0	73.9 ± 4.4	71.4 ± 3.4	69.0 ± 3.6	72.3 ± 2.9
Exendin(9-39) group	79.2 ± 3.3	69.2 ± 2.4	67.4 ± 1.8	67.2 ± 3.8	71.5 ± 3.3
i.v. miglitol for 7 days group	80.2 ± 2.2	72.9 ± 3.8	71.1 ± 2.5	67.6 ± 3.5	74.0 ± 4.2
Heart rate (beats.min^{-1})	**Before ischaemia**	**10 min during ischaemia**	**20 min during ischaemia**	**30 min during ischaemia**	**10 min after reperfusion**
Control	245.4 ± 5.1	236.7 ± 6.7	238.4 ± 7.1	241.8 ± 7.3	240.5 ± 8.6
p.o. miglitol group	244.2 ± 6.0	239.2 ± 4.4	239.5 ± 3.4	242.8 ± 3.6	230.4 ± 4.5
p.o. miglitol + exendin (9-39) group	250.1 ± 9.9	242.4 ± 9.8	243.3 ± 10.3	244.7 ± 9.6	240.9 ± 6.7
i.v. miglitol group	242.7 ± 5.4	246.2 ± 6.6	244.8 ± 8.6	245.3 ± 5.3	245.4 ± 7.9
i.v. miglitol + exendin (9-39) group	235.9 ± 3.2	245.7 ± 3.2	240.2 ± 7.5	239.0 ± 6.8	233.5 ± 3.6
Exendin(9-39) group	242.6 ± 4.3	246.5 ± 3.4	239.6 ± 6.8	239.5 ± 6.0	236.4 ± 7.8
i.v. miglitol for 7 days group	238.4 ± 5.2	243.3 ± 5.1	241 ± 5.2	240.2 ± 4.2	241.4 ± 5.4

phosphorylations (activations) of PI3-kinase and Akt were assessed using antibodies against PI3-kinase and phosphorylated (p)-PI3-kinase (from Cell Signaling Technology), Akt and phosphorylated (p)-Akt (from Cell Signaling Technology). The blots were visualized using chemiluminescence (ECL, Amersham), and the signals were quantified by densitometry. α-Tubulin (analysed with an antibody from Santa Cruz) served as a loading control.

Statistical analysis

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

Results

Haemodynamic parameters

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

Plasma miglitol levels

Figure 3A shows plasma miglitol levels 1, 2, 3 and 4 h after the start of p.o. administration of 2000 ppm (0.2%) miglitol-containing chow and Figure 3B shows the levels 5 min, 30 min and 60 min after i.v. administration of 5 mg·kg^{-1} miglitol. Plasma levels of miglitol 5 min to 30 min after i.v. injection were comparable to that at 3 h after p.o. administration of miglitol. Therefore, 30 min of coronary occlusion was applied 5 min after i.v. administration of miglitol for the miglitol-i.v. group and 3 h after the start of the diet of chow for the miglitol-p.o. group on the day of the experiment.

Plasma glucose levels

Figure 4 shows the time course of the changes in plasma glucose levels in the control, miglitol-i.v., miglitol-p.o and miglitol-p.o. + exendin groups. There were no significant differences in the fasting plasma glucose levels between these four groups; however, postprandial glucose levels, measured 1 h, 2 h and 3 h after feeding, were significantly lower in the miglitol-p.o. group than those in the control group. Moreover, plasma glucose levels were unaffected by i.v. administration of the GLP-1 receptor blocker exendin(9-39). Intravenous injection of miglitol did not affect the plasma glucose levels at all.

Plasma insulin levels

As shown in Figure 5, orally administered miglitol did not affect plasma insulin levels.

Plasma GLP-1 levels

Figure 6 shows the time course of changes in plasma GLP-1 levels in the control, miglitol-p.o and miglitol-i.v. groups. Plasma GLP-1 levels were consistently higher in the miglitol-

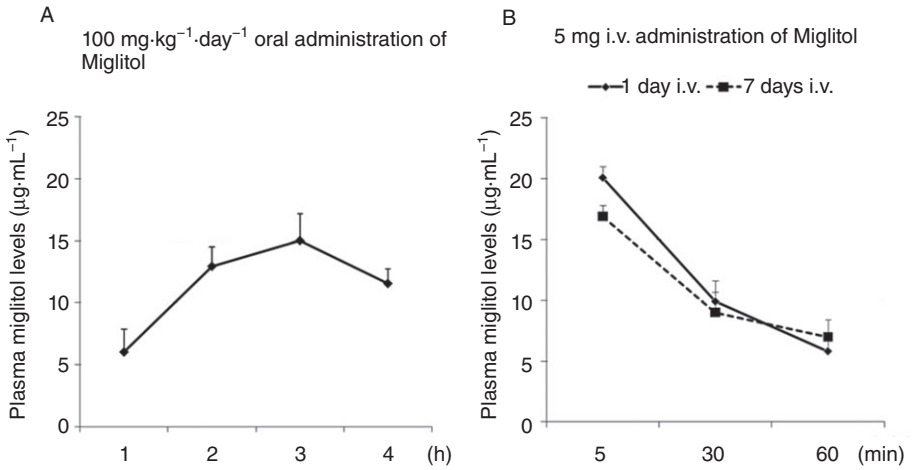

Figure 3

(A) Effect of p.o. administration of miglitol 100 mg·kg^{-1}·day^{-1} on plasma levels of miglitol (n = 10). (B) Effect of 1 day i.v and 7 days i.v. administration of miglitol 5 mg·kg^{-1} on plasma levels of miglitol (n = 10 in each).

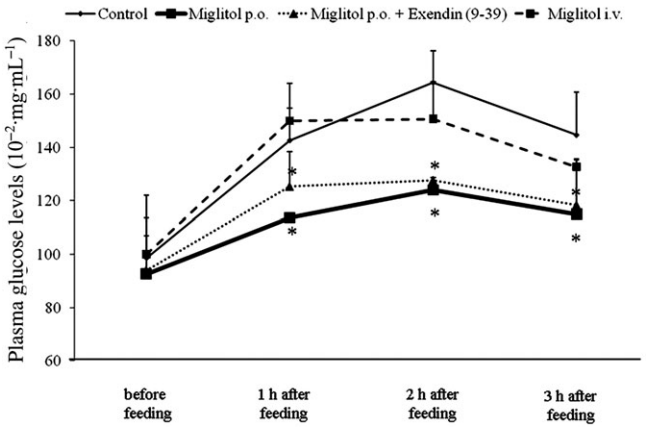

Figure 4

Time courses of changes in plasma glucose levels in the control, miglitol-p.o., miglitol-i.v and miglitol-p.o. + exendin groups (n = 10 in each). *P < 0.05 versus control.

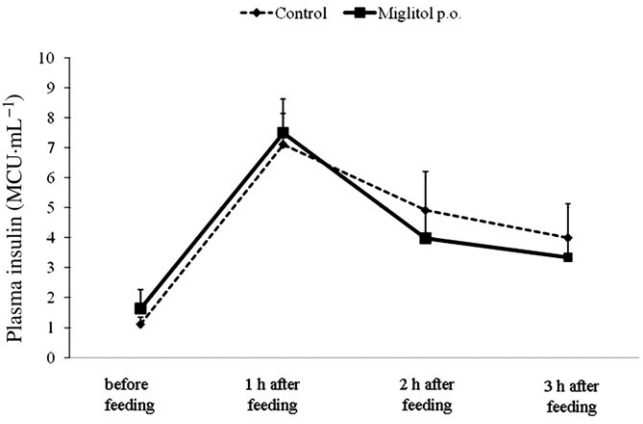

Figure 5

Plasma basal and postprandial insulin levels in rabbits treated orally with miglitol and in the control group (n = 10 in each).

p.o. group before feeding, 1, 2 and 3 h afterward than in the control group or the miglitol-i.v. group.

Myocardial infarct size

On average the sizes of the area at risk expressed as a percentage of the LV were similar among the seven groups studied (Figure 7A). On the other hand, as shown in Figure 7B, the infarct size as a percentage of area at risk was significantly smaller in the miglitol-p.o. group (25.5 ± 1.4%), miglitol-i.v. group (34.8 ± 3.1%) and miglitol-i.v. 7 days group (35.6 ± 2.7%) than in the control group (44.4 ± 2.4%). There was no significant difference in the infarct size as a percentage of area at risk between the miglitol-i.v. group and the miglitol-i.v. 7 days group. The infarct size when expressed as a percentage of left ventricle showed a similar behaviour as that observed when it was expressed as a percentage of area at risk, as shown in Figure 7C.

The infarct size was significantly smaller in the miglitol-p.o. group than in the miglitol-i.v. group. The infarct size-reducing effect of p.o. administration of miglitol was partially abolished by the pretreatment with exendin(9-39) (34.8 ± 3.3%), and the infarct size after the pretreatment with exendin(9-39) was similar to that of the miglitol-i.v. group. However, the infarct size-reducing effect of miglitol-i.v. was not affected by the pretreatment with exendin(9-39) (Figure 7B).

Physiological findings

The echocardiographic findings and ±dP/dt after i.v and p.o. miglitol treatment are shown in Figure 8. Prior to coronary occlusion, there were no significant differences in LV ejection fraction or ±dP/dt among the groups. After 48 h of reperfusion, however, both of these parameters were significantly higher in the miglitol-p.o and miglitol-i.v. groups than in the

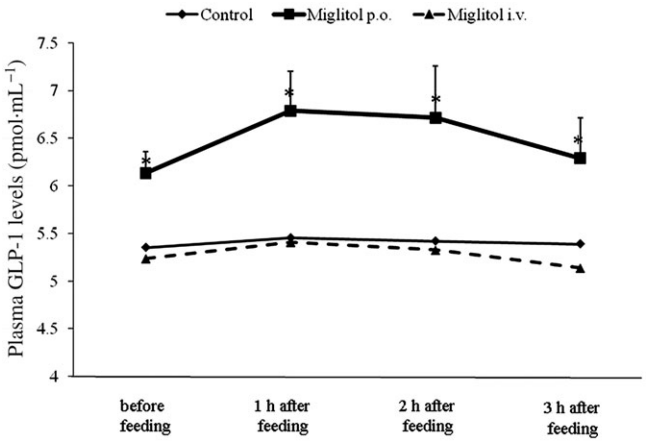

Figure 6

Time courses of changes in plasma GLP-1 levels in the control, miglitol-p.o. and miglitol-i.v. groups (n = 10 in each). *P < 0.05 versus the other groups.

control group, and there was no difference between the miglitol-p.o and miglitol-i.v. groups. There was no significant difference in the LV end-diastolic dimension among the groups, either before ischaemia or after 48 h of reperfusion.

Western blot analysis

There were also no significant differences in the expression of PI3kinase and Akt between the ischaemic and non-ischaemic areas in any of the groups tested (Figure 9). However, the expression of phosphorylated (p)-PI3kinase and p-Akt was significantly up-regulated compared with that of the control in both ischaemic and non-ischaemic areas of hearts in the miglitol-p.o. group, although the up-regulation of p-Akt was larger in the ischaemic area than in the non-ischaemic area. This up-regulation of p-PI3kinase and p-Akt in the miglitol-p.o. group was inhibited by pretreatment with exendin(9-39) in both ischaemic and non-ischaemic areas of the myocardium.

Discussion

The results of the present study demonstrated that (i) p.o. but not i.v. administration of miglitol prevented postprandial hyperglycaemia; (ii) p.o. but not i.v. administration of miglitol increased plasma GLP-1 levels; (iii) both p.o. and i.v. administration of miglitol reduced the myocardial infarct size. This reduction in infarct size was greater in the miglitol-p.o. group than in the miglitol-i.v. group, and was partially blocked by exendin(9-39), a GLP-1 receptor blocker, in the miglitol-p.o. group. (iv) Administration of miglitol p.o., but not i.v., up-regulated the expression of p-PI3K, p-Akt in the myocardium, and this up-regulation was inhibited by the pretreatment with exendin(9-39).

Haemodynamics during experiments

Among the seven groups studied, there were no significant differences in systolic and diastolic blood pressure or heart rate, suggesting that miglitol does not act to reduce infarct size by reducing oxygen consumption. In the present study, p.o. miglitol increased plasma GLP-1 levels. It has been reported that both GLP-1 significantly increases blood pressure and heart rate in rats in a dose-dependent manner within the picomolar to nanomolar range (Barragan *et al.*, 1994; Bojanowska and Stempniak, 2002; Yamamoto *et al.*, 2002). However, GLP-1 infusion in pigs (Kavianipour *et al.*, 2003) and in humans has been found to have no detectable chronotropic or pressor effects (Thrainsdottir *et al.*, 2004; Sokos *et al.*, 2006). These findings may indicate a species-specific effect of GLP-1. In the present study, the increase in plasma GLP-1 levels were within the low picomolar range, which may have had no effect on blood pressure or heart rate in rabbits.

Plasma glucose levels

In the present study, p.o. administration of miglitol to rabbits significantly inhibited the rise in plasma glucose levels otherwise seen 1, 2 and 3 h after feeding and significantly reduced the myocardial infarct size associated with the ischaemia induced by coronary ligation for 30 min and 48 h of reperfusion. In contrast, i.v. injection of miglitol did not affect the plasma glucose levels 1 h, 2 h and 3 h after miglitol injection but significantly reduced the myocardial infarct size. These results indicate that postprandial hyperglycaemia was only prevented when miglitol was administered orally (through the inhibition of glucose absorption from the intestine) and that the ability of miglitol to reduce infarct size is independent of postprandial plasma glucose levels. Since oral miglitol treatment increased the circulating concentration of GLP-1 in the present study, increased plasma GLP-1 may also be involved in decreasing plasma glucose levels although to what extent GLP-1 participates in this effect is not known.

Plasma insulin levels

In the present study, orally administered miglitol did not affect plasma insulin levels, suggesting that insulin hardly contributed to the increase in phosphorylation of PI3K and Akt. Instead, the increase in the phosphorylation of PI3K and Akt is more likely to be mainly mediated via stimulation of GLP-1 receptors, induced by increased GLP-1, as treatment with exendin(9-39) attenuated the increased phosphorylation of PI3K and Akt to similar levels as those of the control.

Plasma GLP-1 levels, GLP-1 receptors, signal transduction and infarct size

GLP-1 is a 30-amino acid intestinal hormone secreted in a nutrient-dependent manner, which stimulates insulin secretion, thereby reducing postprandial hyperglycaemia (Drucker, 2006). GLP-1 also reportedly mitigates post-ischaemic myocardial dysfunction and reduces myocardial infarct size in mouse (Ban *et al.*, 2008), rats (Bose *et al.*, 2005) and swine (Timmers *et al.*, 2009). In the present study, p.o. treatment with miglitol increased basal plasma GLP-1 levels, as well as levels 1, 2 and 3 h after feeding. This is consistent with the earlier finding that p.o. treatment with miglitol increases plasma levels of GLP-1 in humans (Lee *et al.*, 2002).

We propose two possible mechanisms for the increased plasma GLP-1 levels observed after the p.o. treatment with

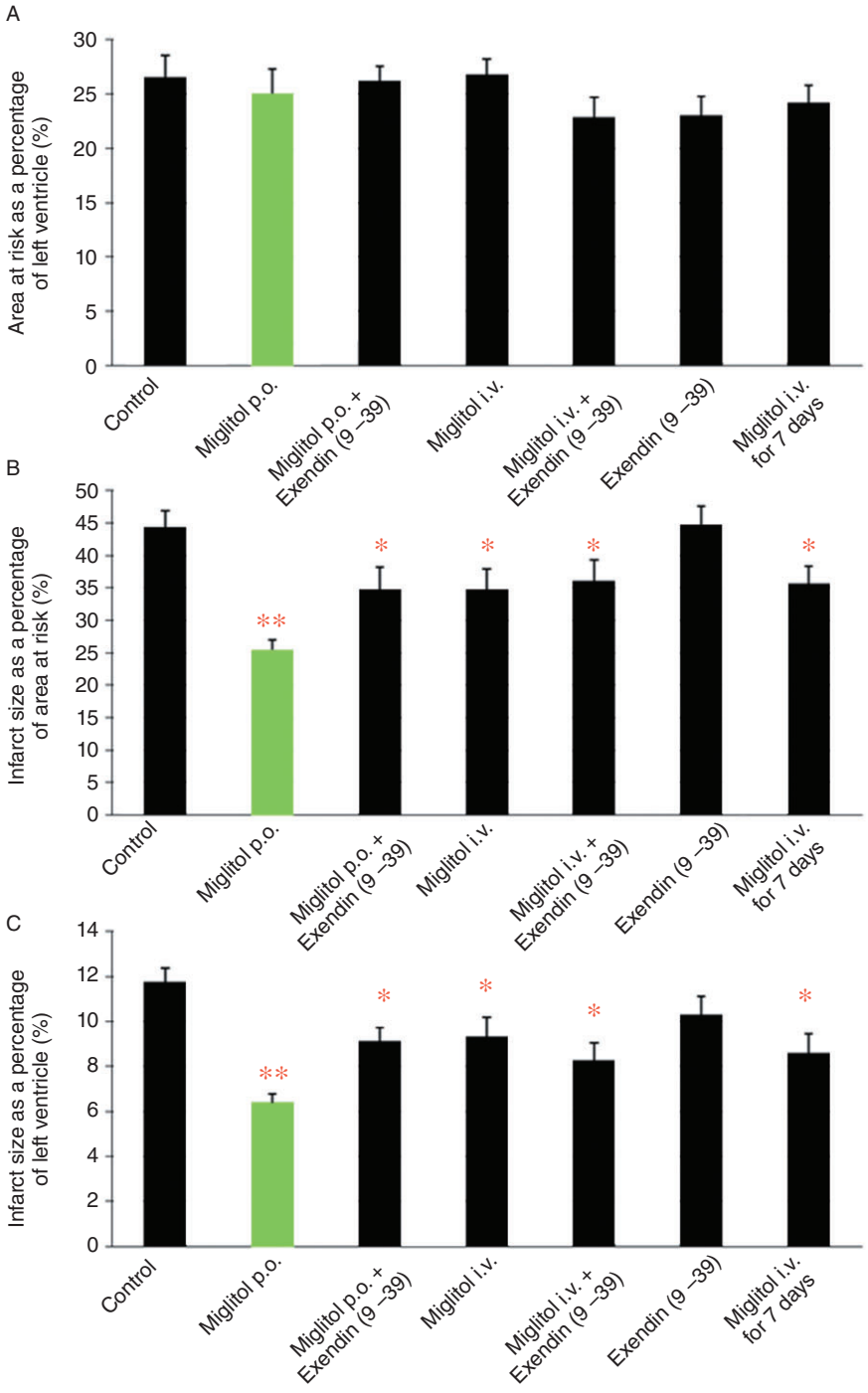

Figure 7

Effect of the indicated treatment protocol on area at risk expressed as a percentage of total left ventricle (A) and infarct size expressed as a percentage of the area at risk (B) and infarct size as a percentage of left ventricle (C) ($n = 10$ in each). There were no significant differences in risk area among the groups. Oral and i.v. administration of miglitol significantly reduced the infarct size, expressed as a percentage of the area at risk and that expressed as a percentage of left ventricle. The reduction in infarct size induced by p.o. miglitol was partially blocked by pretreatment with exendin(9-39), to levels similar to those observed after i.v. administration of miglitol. *$P < 0.05$ versus the control group, **$P < 0.05$ versus the other groups.

miglitol. (1) Miglitol is a substrate for the α-glucosidase enzyme which is present throughout the brush-border side of the small intestine. This enzyme is responsible for conversion of disaccharides such as sucrose into monosaccharides. Inhibition of this enzyme delays the digestion of starch and sucrose, thus leading to a reduction of postprandial glucose levels. Miglitol binds to this enzyme and inhibits the enzyme reversibly in the proximal parts of the intestine (duodenum

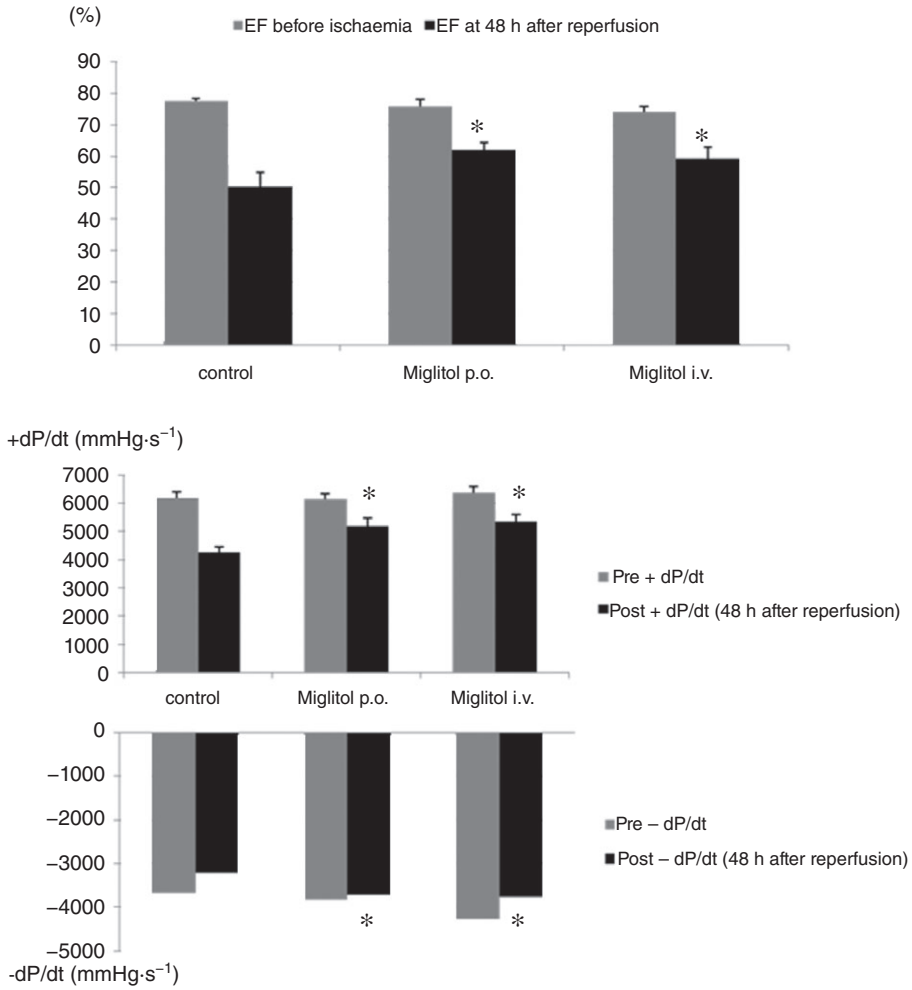

Figure 8

Effect of p.o. and i.v. administration of miglitol on left ventricular ejection fraction (EF) and ±dP/dt after myocardial infarction. Data were collected before coronary occlusion and after 48 h of reperfusion. *$P < 0.05$ versus control.

and jejunum). Thus, carbohydrates not digested in the upper part of the small intestine are transported to the lower parts of the intestine where they are digested finally. Therefore, significantly greater amounts of carbohydrates reaching the lower parts of intestine stimulate the endocrinal L cells to secrete GLP-1 (Hira *et al.*, 2009). Hence, there is a net increase in the plasma levels of GLP-1. Therefore, the production of GLP-1 must be stimulated by the presence of miglitol in the intestine, which then makes its way into the blood. Consistent with this concept, p.o. administration of another α-glucosidase inhibitor voglibose, which is not absorbed from the intestine and currently being used clinically as an antidiabetic therapy, has recently been reported to increase plasma levels of GLP-1 and to stimulate GLP-1 receptors in the myocardium which results in the reduction of the myocardial infarct size (Iwasa *et al.*, 2010). (2) Miglitol inhibits the activity of DPP-4; it has been reported that chronic administration of voglibose for 20 days, another α-glucosidase inhibitor, significantly decreased plasma DPP-4 levels (Moritoh *et al.*, 2009). However, whether p.o. administration of miglitol really inhibits DPP-4 activity remains to be elucidated.

As shown in Figure 7, the reduction in infarct size induced by miglitol that was mediated through stimulation of GLP-1 receptors was the difference between the infarct size of miglitol-p.o. group and that of miglitol-p.o. + exendin(9-39) group, that is, 9.3% (from 34.8% to 25.5%), and the circulating plasma level of GLP-1 in the miglitol-p.o. group in the present study was approximately 7 nmol·L^{-1}. Several studies have demonstrated the effect of GLP-1 and the GLP-1 agonist exenatide on the myocardial infarct size. Exogenously administered GLP-1 0.3 nmol·L^{-1} immediately after reperfusion to a rat isolated heart with 35 min global ischaemia and 120 min reperfusion reduced infarct size from 23.2% to 14.1%, expressed as a percentage of area at risk (Ossum *et al.*, 2009). In another *in vitro* study in a rat isolated heart with 35 min global ischaemia and 120 min reperfusion, 0.3 nmol·L^{-1} GLP-1 significantly reduced the infarct size from 58.7% to 26.7%, again expressed as a percentage of area at risk (Bose *et al.*, 2005). In an *in vivo* rat model of myocardial infarction with 30 min coronary occlusion and 120 min reperfusion, an i.v. infusion of GLP-1 (4.8 pmol·kg^{-1}·min^{-1}) commencing during stabilization and continuing throughout the

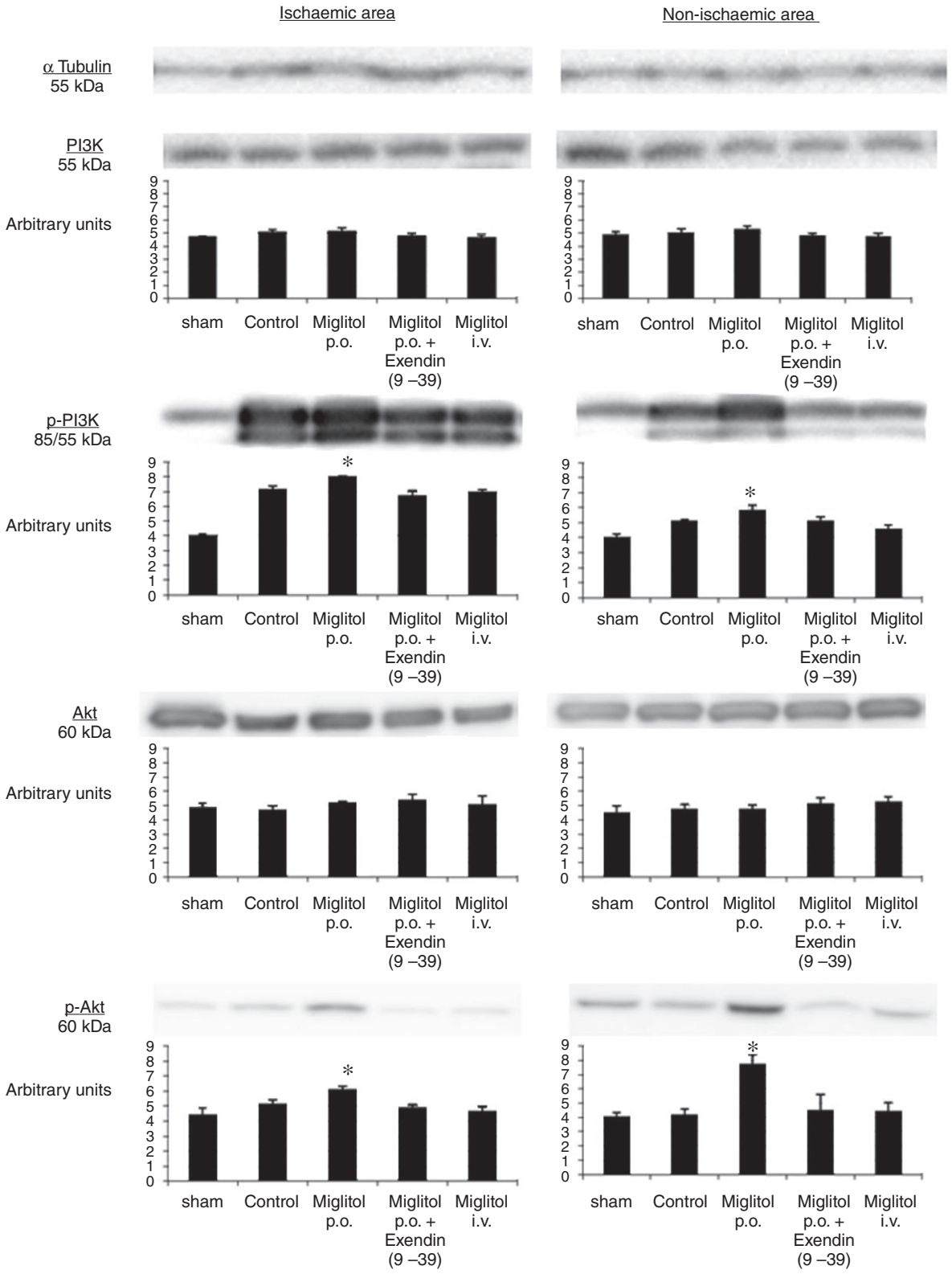

Figure 9

Western blot analysis of myocardial levels of PI3K and phosphorylated (p)-PI3K, Akt and p-Akt in the indicated groups on day 2 postinfarction ($n = 7$ in each). In the miglitol-p.o. group, expression of p-PI3K and p-Akt was significantly up-regulated in both ischaemic and non-ischaemic areas. The up-regulation of p-PI3K and p-Akt was attenuated by pretreatment with exendin(9-39). *$P < 0.05$ versus the other groups.

procedure significantly reduced the infarct size from 44.3% to 20.0% (Bose *et al.*, 2005). In a rat isolated heart with 45 min global ischaemia and 120 min reperfusion, the GLP-1 agonist exenatide 0.03 nmol·L^{-1} and 0.3 nmol·L^{-1} reduced the infarct size, expressed as a percentage of area at risk, from 33.2% to 15.6% and 14.5%, respectively (Sonne *et al.*, 2008). In a porcine *in vivo* model of 75 min coronary occlusion and 3 days reperfusion, exenatide (10 μg s.c. and 10 μg i.v. 5 min before the onset of reperfusion) significantly reduced the infarct size, expressed as a percentage of area at risk, from 53.6% to 32.7% (Timmers *et al.*, 2009). These results suggest that the plasma GLP-1 concentrations of ~7 pmol·mL^{-1} observed in the present study may well be sufficient to indicate a role for GLP-1 in the reduction in infarct size induced by miglitol.

In the present study, both p.o. and i.v. administration of miglitol significantly reduced the myocardial infarct size compared with that in the control group. The infarct size in the miglitol-i.v. group was similar to that in the miglitol-i.v. 7 days group, suggesting that long-term i.v. administration of miglitol did not provide further cardioprotection or a preconditioning effect. The infarct size was significantly smaller in the miglitol-p.o. group than in the miglitol-i.v. group. The reduction in infarct size induced by p.o. treatment with miglitol was partially abolished by pretreatment with exendin(9-39), a GLP-1 receptor blocker, suggesting that this effect of p.o. miglitol is partially mediated via stimulation of myocardial GLP-1 receptors. The infarct size of the miglitol-p.o. group after the pretreatment with exendin(9-39) was similar to that of the miglitol-i.v. group. Furthermore, the reduction in infarct size induced by miglitol-i.v. was not affected by the pretreatment with exendin(9-39). These results suggest that the reduction in infarct size induced by i.v. administration of miglitol was not mediated by the stimulation of myocardial GLP-1 receptors and but that induced by p.o. treatment with miglitol was mediated through two mechanisms: one of which is due to stimulation of GLP-1 receptors. Miglitol has been reported to inhibit α-1,6-glucosidase glycogen debranching enzyme, which reduces the glycogenolytic rate (Bollen *et al.*, 1988; Bollen and Stalmans, 1989), as well as to inhibit α-1,4-glucosidase in the intestine, which blocks the breakdown of oligosaccharides into absorbable monosaccharides resulting in the prevention of postprandial hyperglycaemia (Pagano *et al.*, 1995). Hence, the other mechanism through which miglitol reduces myocardial infarct size may involve its ability to inhibit α-1,6-glucosidase glycogen debranching enzyme. We previously reported that the reduction in myocardial infarct size induced by i.v. administration of miglitol was associated with a decreased accumulation of myocardial lactate content and preserved myocardial glycogen content caused by the inhibition of glycogenolysis during ischaemia (Minatoguchi *et al.*, 1999). Furthermore, this effect of i.v. miglitol was also associated with decreased myocardial interstitial levels of hydroxyl radicals during ischaemia and reperfusion, which may damage the myocardium (Wang *et al.*, 2004).

Concerning the mechanism by which miglitol reduces the infarct size mediated through GLP-1 receptors, we observed that plasma GLP-1 levels were significantly increased in the miglitol-p.o. group but not in the miglitol-i.v. group. Therefore, it is suggested that increased plasma GLP-1 levels induced by p.o. treatment with miglitol stimulate GLP-1 receptors in the myocardium and reduce the myocardial infarct size since the blockade of GLP-1 receptors by exendin(9-39) partially blocked the reduction in infarct size induced by p.o. miglitol. Stimulation of GLP-1 receptors reportedly activates PI3kinase in β-cells (Buteau *et al.*, 1999). We have recently reported that the α-glucosidase inhibitor voglibose, which differs from miglitol in that it is not absorbed from the intestine, reduces myocardial infarct size via stimulation of GLP-1 receptors and activation of the PI3kinase-Akt pathway (Iwasa *et al.*, 2010). In the present study, the expression of phospho-PI3kinase was up-regulated in both ischaemic and non-ischemic areas in the miglitol-p.o. group but not in the miglitol-i.v. group (Figure 9). The up-regulation of p-PI3kinase was inhibited by the pretreatment with exendin(9-39), suggesting that up-regulation of p-PI3kinase was due to the stimulation of GLP-1 receptors. Similarly, in the present study, the expression of p-Akt was significantly up-regulated in both ischaemic and non-ischaemic areas in the miglitol-p.o. group but not in the miglitol-i.v. group (Figure 9). The fact that up-regulation of p-Akt in the miglitol-p.o. group was inhibited by pretreatment with exendin(9-39), a GLP-1 receptor blocker, suggests that p.o. administration of miglitol activates Akt via stimulation of GLP-1 receptors. We have no evidence to show that miglitol is a GLP-1 agonist. However, it is not likely that miglitol is a direct stimulator of GLP-1 receptors because p.o. but not i.v. administration of miglitol significantly up-regulated the levels of phosphorylation of PI3-kinase and Akt, a downstream signal of GLP-1 receptors in the present study. The PI3kinase-Akt pathway has been reported to be involved in the reduction in infarct size induced by ischaemic preconditioning and in the transduction of prosurvival signals (Hausenloy *et al.*, 2005). It has also been reported that a GLP-1 receptor agonist was cardioprotective through cytoprotective pathways such as PI3K-Akt pathway (Noyan-Ashraf *et al.*, 2009). This is consistent with our results. Thus, the reduction in infarct size induced by p.o. treatment with miglitol appears to be mediated at least in part through stimulation of GLP-1 receptors and activation of a prosurvival PI3kinase-Akt pathway.

In addition to a GLP-1 receptor-dependent cardioprotective action, a GLP-1 receptor-independent cardioprotective action has also been reported after acute treatment with GLP-1(9–36) upon reperfusion (but not before the onset of ischaemia), which resulted in an improvement in functional recovery (Ban *et al.*, 2008; Sonne *et al.*, 2008). In the present study, the extent to which the GLP-1 receptor-independent effect contributes to the cardioprotective action was not elucidated .

With regard to the effect of miglitol on cardiac function, both i.v. miglitol and p.o. miglitol significantly improved the systolic and diastolic left ventricular function after myocardial infarction, as shown in Figure 8. However, to what extent GLP-1 is involved in these effects remains to be elucidated. From the findings obtained in the present study, we propose a possible mechanism by which p.o. treatment with miglitol protects the heart, as shown in Figure 10.

In conclusion, we found that orally administered miglitol protects the myocardium against ischaemia-reperfusion injury through stimulation of GLP-1 receptors and activation

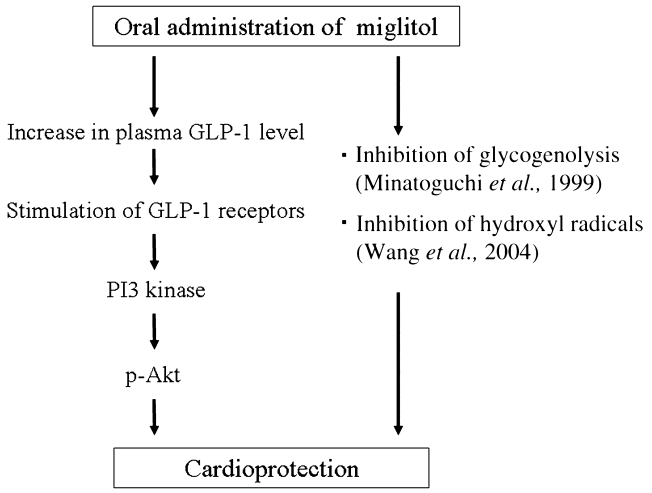

Figure 10
Proposed mechanism of cardioprotection by p.o. treatment with miglitol.

of the PI3kinase-Akt pathway, as well as through the inhibition of glycogenolysis as previously reported. Our findings thus provide new insights into therapeutic strategies for the treatment of patients with diabetes mellitus combined with coronary artery disease.

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Conflict of interest

There is no conflict of interest.

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