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Combination of miglitol, an anti-diabetic drug, and nicorandil markedly reduces myocardial infarct size through opening the mitochondrial K_{ATP} channels in rabbits

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1 The anti-diabetic drug miglitol, an α-glucosidase inhibitor, which is currently used clinically, reduces myocardial infarct size by reducing the glycogenolytic rate through inhibition of the α-1,6-glucosidase of glycogen-debranching enzyme in the heart. Nicorandil, a K_{ATP} channel opener with a nitrate-like effect, which is also currently used clinically, also reduces the infarct size. Therefore, we hypothesized that combination of nicorandil and submaximal dose of miglitol could markedly reduce myocardial infarct size more than miglitol or nicorandil alone, and investigated the mechanism for the infarct size-reducing effect.

2 Japanese white rabbits without collateral circulation were subjected to 30 min coronary occlusion followed by 48 h reperfusion. Pre-ischaemic treatment with submaximal dose of miglitol (5 mg kg^{-1}, i.v.) and nicorandil alone (100 μg kg^{-1} min^{-1} 5 min) moderately reduced the infarct size as a percentage of area at risk (24±4 and 25±4%, respectively), and 10 mg kg^{-1} of miglitol markedly reduced the infarct size (15±2%) compared with the controls (42±2%). Combination of 5 mg kg^{-1} of miglitol and nicorandil (100 μg kg^{-1} min^{-1} 5 min), and 10 mg kg^{-1} of miglitol and nicorandil (100 μg kg^{-1} min^{-1} 5 min) significantly reduced the infarct size (13±4 and 12±3%, respectively) more than miglitol or nicorandil alone.

3 Pretreatment with 5HD completely abolished the infarct size-reducing effect of 10 mg kg^{-1} of miglitol alone (36±7%) and that of combination of 5 mg kg^{-1} of miglitol and nicorandil (46±2%).

4 Combination of nicorandil and submaximal dose of miglitol markedly reduced the myocardial infarct size more than miglitol or nicorandil alone. This effect was suggested to be related to the opening of mitochondrial K_{ATP} channels.

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Abbreviations: ANOVA, analysis of variance; 5HD, 5-hydroxydecanoate; LV, left ventricle; Mig5, miglitol 5 mg kg^{-1}; Mig10, miglitol 10 mg kg^{-1}; NADH, nicotinamide adenine dinucleotide phosphate; Nico, nicorandil; TTC, 2,3,5-triphenyltetrazolium chloride

Introduction

Recently, we found that pharmacological inhibition of glycogenolysis by N-methyl-1-deoxynojirimycin, an α-glucosidase inhibitor, through the blockade of α-1,6-glucosidase of glycogen-debranching enzyme in the heart during ischaemia markedly reduces the myocardial infarct size (Arai *et al*., 1998). We also reported that N-hydroxyethyl-1-deoxynojirimycin (miglitol), an α-1,4-glucosidase inhibitor, which inhibits the breakdown of oligosaccharides into absorbable monosaccharides in the intestine (Yoshikuni, 1988) and is currently used clinically for the treatment of diabetes mellitus because of the anti-hyperglycaemic action, has also the effect inhibiting α-1,6-glucosidase and markedly reduces the infarct size by inhibiting glycogenolysis in the rabbit heart without collateral circulation (Minatoguchi *et al*., 1999). In addition, nicorandil, a K_{ATP} channel opener, which is also currently used clinically for the treatment of angina pectoris, has also been reported to reduce the infarct size (Ohno *et al*., 1997; Imagawa *et al*., 1998). However, the clinical dose of each drug is smaller than that used in the animal experiments, and a single clinical dose of each drug may not be effective in reducing the infarct size in the case of human myocardial infarction. We hypothesized that the simultaneous administration of an α-1,4- and α-1,6-glucosidase inhibitor, miglitol, and a K_{ATP} channel opener, nicorandil, would produce a greater additive reduction in infarct size than that observed in either treatment alone. If so, a combination of these two drugs would benefit a specific population of patients, i.e., diabetic patients already being treated with miglitol. Therefore, the aim of the present study was to evaluate whether (1) a combination of miglitol and nicorandil could produce a marked reduction in infarct size, and (2) whether the infarct size-reducing effect was related to the opening of the mitochondrial K_{ATP} channels in an *in vivo* model of rabbits without collateral circulation.

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Methods

In this study, all rabbits received humane care in accordance with the Guide for the Care and Use of Laboratory Animals, published by the U.S. National Institutes of Health (NIH publication 8523, revised 1985). The study protocol was approved by the Ethical Committee of Gifu University School of Medicine, Gifu, Japan.

Surgical preparation

Male Japanese white rabbits weighing 1.9–2.5 kg were anaesthetized with 30 mg kg^{-1} sodium pentobarbital and mechanically ventilated with room air. All surgical procedures were performed aseptically. The left carotid artery and jugular vein were cannulated to monitor arterial blood pressure and to administer drugs or saline, respectively. After left thoracotomy was performed in the third intercostal space, the heart was exposed and 4-0 silk string was placed beneath the large coronary arterial branch coursing down the middle of the anterolateral surface of the left ventricle. Coronary arterial occlusion and reperfusion were performed by pulling or releasing a snare around the string. Myocardial ischaemia was confirmed by regional cyanosis and electrocardiographical change. Reperfusion was confirmed by myocardial blush over the risk area after releasing the snare. Body temperature was measured by an electric thermistor placed in the rectum, and maintained at 37°C by a heating pad placed under the rabbit.

Infarct size

Rabbits were assigned randomly to nine groups (Figure 1); Mig5 group (miglitol 5 mg kg^{-1}, $n=8$), Mig10 group (miglitol 10 mg kg^{-1}, $n=10$), nicorandil group (100 μg kg^{-1} min^{-1} 5 min, $n=7$), Mig5 + nicorandil group ($n=8$), Mig10 + nicorandil group ($n=8$), Mig10 + 5HD group ($n=5$), 5HD + Mig5 + nicorandil group ($n=5$), 5HD group ($n=7$) and saline control group ($n=10$). In the control group, 1 ml of placebo saline was injected 30 min before ischaemia. In the Mig group, 5 or 10 mg kg^{-1} of miglitol was intravenously administered 30 min before ischaemia. In the nicorandil group, nicorandil (100 μg kg^{-1} min^{-1} 5 min, i.v.) was administered 10 min before ischaemia. The Mig5 + nicorandil group and Mig10 + nicorandil group were administered 5 and 10 mg kg^{-1} of miglitol, respectively, instead of saline 30 min before ischaemia, and nicorandil (100 μg kg^{-1} min^{-1} 5 min) was infused 10 min before ischaemia. In the 5HD + Mig10 group was administered 5-hydroxydecanoate (5HD, 5 mg kg^{-1}, i.v., a mitochondrial K_{ATP} channel blocker 40 min before ischaemia and 10 mg kg^{-1} of miglitol was intravenously administered 30 min before ischaemia. In the 5HD + Mig5 + nicorandil group, 5HD (5 mg kg^{-1}) was intravenously administered 40 min before ischaemia, and 5 mg kg^{-1} of miglitol was administered 30 min before ischaemia and nicorandil (100 μg kg^{-1} min^{-1} 5 min) was infused 10 min before ischaemia. In the 5HD group, 5HD was intravenously administered 10 min before ischaemia. After the treatment, the coronary artery was occluded for 30 min and reperfused. Haemodynamic parameters were recorded throughout the experiment until 20 min after reperfusion. Then, the chest was closed and the rabbits were allowed to recover from anaesthesia for 48 h of survival. At the end of the study, the rabbits were heparinized (500 u kg^{-1}) and killed by an overdose of pentobarbital. The heart was excised and mounted on a Langendorff apparatus. The coronary branch was reoccluded and monastral blue dye (4%, Sigma Chemical Co., St. Louis, MO, USA) was injected from the aorta at 80 mmHg to determine the area at risk. The left ventricle (LV) was sectioned into seven slices parallel to the atrio-ventricular ring. Each slice was weighed, incubated in a 1% solution of triphenyl tetrazolium chloride (TTC) at 37°C to visualize the infarct area (Fishbein *et al*., 1981), and photographed. The areas of the ischaemic region and the infarcted myocardium were traced photographically on each LV slice using a personal computer, multiplied by the slice weight, then expressed as a fraction of the risk region or LV for each heart.

Statistical analysis

All values are presented as the mean ± s.e.mean. Risk and infarct sizes were compared among the groups by one-way analysis of variance combined with Bonferroni's *post hoc* test for multiple comparisons. The difference in haemodynamics over the time course between the control and the drug-treated groups was assessed by two-way repeated measures analysis of variance (ANOVA). Differences with $P<0.05$ were considered statistically significant.

Results

Mortality and animal exclusion

Seventy-eight rabbits were initially enrolled in the infarct size study. There was no significant difference in the incidence of ventricular fibrillation or mortality. Among these animals, one rabbit each developed ventricular fibrillation during coronary occlusion in the Mig5, Mig10, nicorandil, Mig5 + nicorandil, and the control groups, and one rabbit each developed ventricular fibrillation during reperfusion in the Mig10 + Nicodandil, Mig10 + 5HD, Mig5 + nicorandil + 5HD, 5HD and control groups, and all these rabbits died. Thus, the experiments were completed in the remaining 68 rabbits and the findings from these animals were used for the analysis.

Haemodynamic parameters

Table 1 shows the hemodynamic parameters. There was no significant difference in mean blood pressure or heart rate among the nine groups.

Infarct size

The mean percentages of area at risk (per cent of left ventricle) were 26.3 ± 3, 28 ± 3, 28 ± 2, 24 ± 2, 22 ± 2, 27 ± 2, 31 ± 3, 29 ± 4 and 31 ± 4 in the control, nicorandil, Mig5, Mig10, Mig5 + nicorandil, Mig10 + nicorandil, 5HD + Mig10, 5HD + Mig5 + nicorandil and 5HD groups, respectively (Figure 2A). No significant difference was seen among the

groups. As shown in Figure 2B, the infarct size as a percentage of area at risk was significantly but modestly reduced in the Mig5 (24±4%, $n=10$) and nicorandil (25±4%, $n=7$) groups compared with the saline control group (42±2%, $n=10$). However, combination of 5 mg kg^{-1} of miglitol and nicorandil significantly reduced the infarct size (13±4, $n=8$) compared with miglitol or nicorandil alone. Miglitol 10 mg kg^{-1} significantly and markedly reduced the infarct size (15±2%, $n=10$). However, no further reduction in infarct size was observed by a combination of miglitol

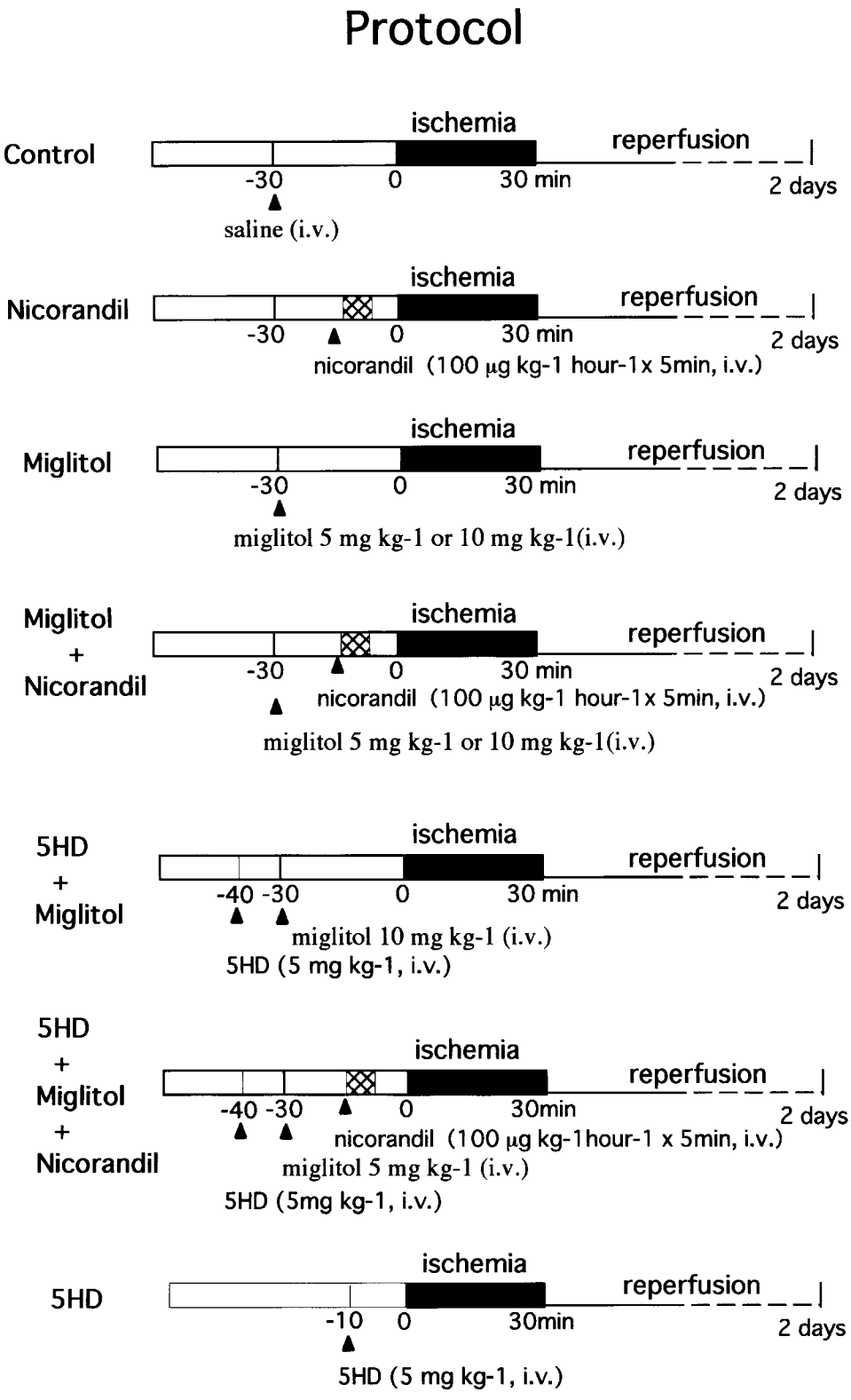

Figure 1 Experimental protocol for the measurement of infarct size. 5HD = 5-hydroxydecanoate.

Table 1 Haemodynamic parameters

	Baseline	*Before ischaemia*	*20 min ischaemia*	*20 min reperfusion*
MBP (mmHg)				
Control	101±4	97±4	89±5	79±4
Nico	91±6	90±8	80±5	79±4
Mig5	101±4	102±4	91±4	84±4
Mig10	98±4	90±4	82±6	76±5
Mig5+Nico	94±4	93±6	91±4	84±4
Mig10+Nico	96±4	94±5	92±5	87±3
Mig10+5HD	92±3	91±4	88±4	85±3
Mig5+Nico+5HD	89±4	90±6	81±3	78±2
5HD	96±3	99±4	86±2	79±2
Heart rate (beats min^{-1})				
Control	243±12	240±11	245±9	233±10
Nico	240±14	251±10	240±10	230±9
Mig5	255±11	256±8	244±8	223±10
Mig10	252±6	248±11	229±10	226±9
Mig5+Nico	280±11	288±10	277±10	268±11
Mig10+Nico	278±10	286±9	275±7	225±8
Mig10+5HD	282±9	280±7	272±7	257±6
Mig5+Nico+5HD	283±8	279±6	267±6	258±4
5HD	287±7	280±11	271±11	254±6

MBP = mean blood pressure; Mig5 = miglitol 5 mg kg^{-1}; Mig10 = miglitol 10 mg kg^{-1}; Nico = nicorandil.

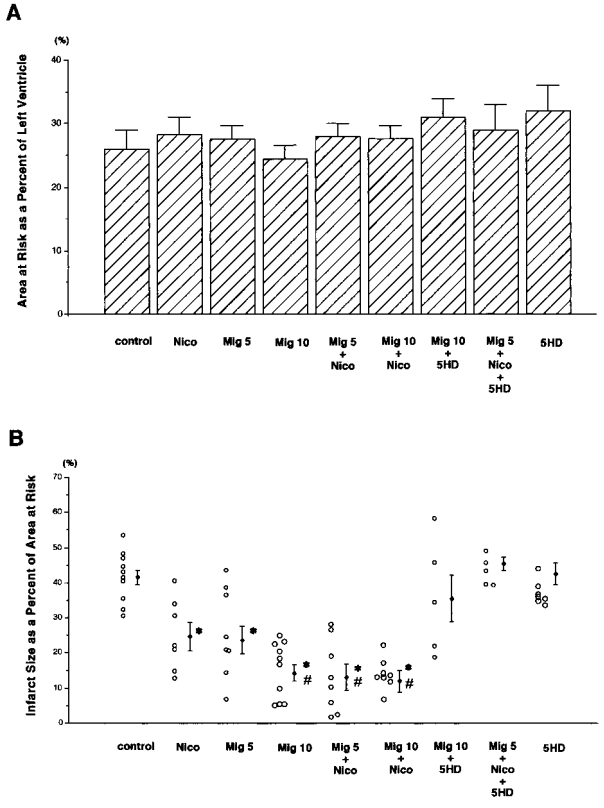

Figure 2 Comparisons of area at risk as a percentage of left ventricle (A), and of infarct size as a percentage of area at risk (B). 5HD = 5-hydroxydecanoate, Nico = nicorandil, Mig5 = miglitol 5 mg kg^{-1}, Mig10 = miglitol 10 mg kg^{-1}, $^*P<0.05$ compared with the control group. $\#P<0.05$ compared with the nicorandil group and Mig5 group. Bars represent s.e.mean.

10 mg kg^{-1} + nicorandil (12±3%, $n=8$). Pretreatment with 5HD completely abolished the infarct size-reducing effect of miglitol 10 mg kg^{-1} (36±7%, $n=5$) and combination of miglitol 5 mg kg^{-1} and nicorandil (46±2%, $n=5$). 5HD by itself did not affect the infarct size (43±3%, $n=7$).

Discussion

The present study demonstrated that (1) a combination of nicorandil and submaximal dose of miglitol markedly reduced the myocardial infarct size more than miglitol or nicorandil alone, and (2) the blockade of the infarct size-reducing effect of a combination by 5HD suggested that the mechanism for the infarct size-reducing effect was related to the opening of mitochondrial K_{ATP} channels. This is the first study showig a marked myocardial infarct size-reducing effect using a combination of miglitol and nicorandil.

In the controls, miglitol, nicorandil, miglitol + nicorandil, 5HD + miglitol + nicorandildid and 5HD groups, there was no change in blood pressure or heart rate which might influence the infarct size. In addition, the infarct size-reducing effect was independent of the regional blood flow, because the rabbit heart has no collateral circulation (Harken *et al*., 1981).

Accumulated anaerobic glycolytic products such as lactate, NADH, and H^+, contribute to intracellular acidosis and osmotic load (Wolfe *et al*., 1988) and cause irreversible cellular damage (Neely & Grotyohann, 1984). It has been reported that the time course of glycogen depletion after a brief ischaemic episode paralleled the loss of the protection from ischaemic injury (Wolfe *et al*., 1993). These findings suggest that the attenuation of glycolytic flux and lactate accumulation protect the heart against ischaemic cellular damage. Miglitol is an inhibitor of α-1,6-glucosidase as well as α-1,4-glucosidase, and reduces the glycogenolytic rate (Bollen & Stalmans, 1989). In fact, we previously reported that miglitol preserved the glycogen content and attenuated the lactate accumulation in the ischaemic myocardium during 30 min of ischaemia and reduced the infarct size in a dose-dependent manner in rabbits (Minatoguchi *et al*., 1999). This

suggests that miglitol reduced the infarct size through inhibition of α-1,6-glucosidase glycogen debranching enzyme during ischaemia.

The infarct size-reducing effect of miglitol 10 mg kg^{-1} was completely blocked by the pretreatment with a mitochondrial K_{ATP} channel blocker 5HD, suggesting that the infarct size-reducing effect of miglitol was related to the opening of the mitochondrial K_{ATP} channels. This suggests that the inhibition of glycogenolysis by miglitol is related to the opening of mitochondrial K_{ATP} channels during ischaemia. The precise mechanism how miglitol treatment leads to the opening of mitochondrial K_{ATP} channel remains to be investigated. However, one possible explanation may be that any modality which preserves glycogen content may open the mitochondrial K_{ATP} channel and show a cardioprotective effect as evidenced by a study by Gan *et al*. (1996), who reported that preservation of glycogen content induced by a combination of transient ischaemia and nucleosid transport inhibitor, adenosine A1 agonist or insulin was protective against hydrogen peroxide cardiotoxicity, and this effect was related to the opening of K_{ATP} channels.

Nicorandil, a K_{ATP} channel opener, is known to reduce the infarct size following ischaemia and reperfusion in rabbits (Ohno *et al*., 1997; Imagawa *et al*., 1998). We previously demonstrated that pre-ischaemic treatment with nicorandil reduces the infarct size and the infarct size was inversely correlated with the plasma nicorandil concentrations (Ohno *et al*., 1997). The infarct size-reducing effect was blocked by pretreatment with glibenclamide, a K_{ATP} channel blocker, suggesting the involvement of K_{ATP} channels in the infarct size-reducing effect of nicorandil (Ohno *et al*., 1997). In the present study, pre-ischaemic infusion of nicorandil at a rate of 100 μg kg^{-1} min^{-1} 5 min moderately reduced the infarct size as in the previous study (Ohno *et al*., 1997). Miglitol 5 mg kg^{-1} moderately reduced the infarct size. The combination of miglitol 5 mg kg^{-1} and nicorandil (100 μg kg^{-1} min^{-1} 5 min) markedly reduced the infarct size, and this effect was completely blocked by pretreatment with 5 mg kg^{-1} of 5HD. This suggests that the mechanism for the infarct size-reducing effect by combination of miglitol and nicorandil is mediated through the opening of mitochondrial K_{ATP} channels. The combination of miglitol 10 mg kg^{-1} and nicorandil markedly reduced the infarct size similar to that of a combination of miglitol 5 mg kg^{-1} and nicorandil or miglitol 10 mg kg^{-1} alone, and no further reduction in infarct size was observed. This suggests that a combination of miglitol 5 mg kg^{-1} and nicorandil (100 μg kg^{-1} min^{-1} 5 min) maximally reduced the infarct size in this setting of the experiment.

Diabetes mellitus is one of the most important risk factors of coronary artery disease and there are many patients with combined diabetes mellitus and coronary artery disease. Miglitol, like other N-substituted derivatives of 1-deoxynojirimycin, is rapidly and completely absorbed from the intestine (Ahr *et al*., 1997). Plasma concentration of miglitol 30 min (when coronary occlusion started) and 60 min (when reperfusion started) after i.v. dosing with 5 mg kg^{-1} of miglitol in rabbits was 10 μg ml^{-1} and 5.8 μg ml^{-1}, respectively (Minatoguchi *et al*., 1999). Plasma concentration following a single oral dose of 200 mg of miglitol in healthy human volunteers is reported to be 2.61 μg ml^{-1} (Scott & Spencer, 2000). Furthermore, 300 mg day^{-1} of miglitol did not induce any side effect in routine laboratory examination in diabetic patients (Schnack *et al*., 1989). Therefore, the dose of miglitol at 5 mg kg^{-1} used in rabbits in the present study is not so much high compared to that in the clinical dose. However, the relationship between the dose of drugs and the effect generally varies from species to species. Therefore, whether combination of miglitol and nicorandil is beneficial for diabetic patients with coronary heart disease already being treated with miglitol should be examined. Further clinical investigations are warranted.

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