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A novel anti-diabetic drug, miglitol, markedly reduces myocardial infarct size in rabbits

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1 We examined whether N-hydroxyethyl-1-deoxynojirimycin (miglitol), a new human anti-diabetic drug with effects to inhibit α-1,6-glucosidase glycogen debranching enzyme and reduce the glycogenolytic rate as well as to inhibit α-1,4-glucosidase, could reduce infarct size in the rabbit heart. Rabbits were subjected to 30-min coronary occlusion followed by 48-h reperfusion.

2 The infarct size as a percentage of area at risk was not reduced by pre-ischaemic treatment with 1 mg kg^{-1} miglitol (42.7±4.0%, $n=10$) compared with the saline control group (41.7±2.3%, $n=10$). However, it was significantly and dose-dependently reduced by pre-ischaemic treatment with 5 or 10 mg kg^{-1} of miglitol (25.7±4.5%, $n=10$, and 14.6±2.4%, $n=10$, respectively) without altering the blood pressure, heart rate or blood glucose level. However, there was no evidence of an infarct-size reducing effect after pre-reperfusion treatment with 10 mg kg^{-1} of miglitol (35.0±3.0%, $n=10$).

3 Another 40 rabbits given 1, 5 and 10 mg kg^{-1} of miglitol or saline before ischaemia ($n=10$ in each) were sacrificed at 30 min of ischaemia for biochemical analysis. Miglitol preserved significantly the glycogen content, and attenuated significantly the lactate accumulation in a dose dependent manner in the ischaemic region at 30 min of ischaemia.

4 Pre-ischaemic treatment, but not pre-reperfusion treatment, with miglitol markedly reduced the myocardial infarct size, independently of blood pressure and heart rate. A dose-dependent effect of miglitol on infarct size, glycogenolysis and lactate formation suggests that the mechanism may be related to the inhibition of glycogenolysis. Thus, miglitol may be beneficial for coronary heart disease as well as diabetes mellitus.

Keywords: Myocardial infarction; ischaemia; α-glucosidase inhibitor; myocardium; glycogen

Abbreviations: LV, left ventricle; Mig, miglitol; NADH, nicotinamide adenine dinucleotide; NADP, nicotinamide adenine dinucleotide phosphate; TTC, triphenyl tetrazolium chloride

Introduction

Brief episodes of ischaemia and reperfusion before a subsequent prolonged period of ischaemia precondition the myocardium and reduce myocardial infarct size. This phenomenon is known as ischaemic preconditioning, and has been demonstrated in various animal species such as rats (Li *et al*., 1992), rabbits (Thornton *et al*., 1990), dogs (Murry *et al*., 1986) and pigs (Schott *et al*., 1990) and also in humans (Nakagawa *et al*., 1995). Since ischaemic preconditioning is not appropriate for clinical use, it is desirable to precondition the heart with chemicals ('pharmacological preconditioning') which exert a beneficial effect similar to ischaemic preconditioning without any side effects.

Several possible mediators by which ischaemic preconditioning protects the heart include adenosine (Liu *et al*., 1991), noradrenaline (Kariya *et al*., 1997), bradykinin (Goto *et al*., 1995), free radicals (Tanaka *et al*., 1994), the activation of protein kinase C (Ytrehus *et al*., 1994) and the opening of K_{ATP} channels (Gross & Auchampach, 1992). Clinically, noradrenaline, bradykinin, free radicals and the activation of protein kinase C are not practical because of their side effects. Adenosine potentiators dipyridamole and dilazep which have been used clinically do not reduce the infarct size (Miura *et al*., 1992; Itoya *et al*., 1994). Although the K_{ATP} channel opener nicorandil has an infarct size reducing effect in an animal model (Auchampach & Gross, 1993), the effect is not marked. Another mechanism of ischaemic preconditioning is the reduction of energy demand, as evidenced by improved preservation of ATP and the attenuation of lactate and H^+ accumulation during the subsequent sustained ischaemic insult (Kida *et al*., 1991), which have been considered to be due to pre-ischaemic glycogen depletion by ischaemic preconditioning (Jennings *et al*., 1991). Therefore, we hypothesized that the pharmacological inhibition of glycogenolysis can reduce myocardial infarct size.

α-Glucosidases are classified into α-1,1-, α-1,2-, α-1,4- and α-1,6-glucosidases. As α-1,4-glucosidase induces breakdown of oligosaccharides into absorbable monosaccharides, α-1,4-glucosidase inhibitors such as acarbose, voglibose and miglitol (N-hydroxyethyl-1-deoxynojirimycin) have an antihyperglycemic effect and have been used for the treatment of patients with diabetes mellitus (Hulin, 1994; Pagano *et al*., 1995). Meanwhile, α-1,6-glucosidase is a glycogen debranching enzyme which reduces the glycogenolytic rate.

Recently, we found that N-methyl-1-deoxynojirimycin, an α-1,6-glucosidase inhibitor, markedly reduced infarct size by inhibiting glycogenolysis in rabbit hearts (Arai *et al*., 1998).

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However, this compound is not used clinically, nor is it under trials for clinical use at present. Meanwhile, miglitol is a new human anti-diabetic agent which exerts an antihyperglycemic effect by blocking α-1,4-glucosidase in intestine (Bollen & Stalmans, 1989), and is already used clinically in the treatment of non-insulin dependent diabetes mellitus. In addition, miglitol can also reduce the glycogenolytic rate of liver and skeletal muscle by inhibiting the α-1,6-glucosidase glycogen debranching enzyme, as does N-methyl-1-deoxynojirimycin (Bollen & Stalmans, 1989; Bollen *et al*., 1988). Therefore, miglitol may be protective against lactic acidosis in the myocardium during ischaemia, as well as against diabetes mellitus. If this is the case, this compound will be beneficial in the treatment of patients with non-insulin dependent diabetes mellitus that is associated with an increased risk of coronary artery disease. Thus, in the present study, we examined whether miglitol could reduce infarct size in the rabbit heart.

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 No. 85 to 231, 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. For rabbits with 48 h reperfusion, 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 a left thoracotomy was performed at the third intercostal space, the heart was exposed and 4-0 silk ligature 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 ligature. Myocardial ischaemia was confirmed by regional cyanosis and electrocardiographic change. Reperfusion was confirmed by myocardial blush over the risk area after releasing the snare. The rabbit hearts in which the coronary vein was ligated along with the artery were excluded, because this may increase the magnitude of ischaemic lactic acidosis.

Plasma miglitol and plasma glucose levels

Twelve rabbits of the above model were used for the measurement of plasma miglitol and glucose levels. Arterial blood samples were taken slowly from the carotid artery at time points before, and 5, 30 (immediately before ischaemia) and 60 min (30 min after ischaemia) after jugular vein administration of miglitol (four rabbits each at 1, 5 and 10 mg kg^{-1}). The samples were immediately placed in heparinized ice-cold centrifuge tubes and stored at −83°C until assays were performed to measure plasma miglitol and glucose levels.

Infarct size

To investigate the infarct size-reducing effect of miglitol, 59 rabbits were assigned randomly into drug treatment or saline control groups ($n=12$). There were four drug treatment groups, i.e., three pre-ischaemic treatment groups given 1 mg kg^{-1} (Mig 1 group, $n=12$), 5 mg kg^{-1} (Mig 5 group, $n=12$), or 10 mg kg^{-1} (Mig 10 group, $n=11$) of miglitol 30 min before ischaemia, and one pre-reperfusion treatment group given 10 mg kg^{-1} of the drug 10 min before reperfusion (Mig 10-post group, $n=12$). For all treatments, the injected volume was less than 1 ml. In the control group, an equivalent volume of saline was injected 30 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, U.S.A.) 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 on each LV slice and multiplied by the slice's weight, then expressed as a fraction of the risk region or LV for each heart.

Biochemical determinations

Forty additional rabbits were used to assess the *in vivo* effect of miglitol on myocardial glycogen metabolism and lactate accumulation in the ischaemic myocardium. The rabbits were randomized to receive either 1, 5 or 10 mg kg^{-1} of miglitol or saline 30 min before ischaemia, and were killed at 30 min of ischaemia. Hearts were excised and transmural samples, each weighing approximately 200 mg, were taken from the centre of the left ventricular ischaemic region and the opposite non-ischaemic region. The border of the ischaemic region was defined by the distribution of cyanosis and marked on the epicardium in ink. The samples were frozen immediately and stored at −83°C until the assays were performed. Samples were weighed, homogenized, and used for the following measurements. For myocardial glycogen measurement, a supernatant of the homogenate was subsequently hydrolyzed with amyloglucosidase. The resulting glucose residue was then measured by an NADP-linked spectrophotometric method using hexokinase and glucose-6-phosphate dehydrogenase (Keppler & Decker, 1984). Lactate in the extracts was measured spectrophotometrically by monitoring hydrogen peroxide formation resulting from the enzymatic reaction with lactate oxidase (Bergmyer, 1974).

Statistical analysis

All data are presented as the mean ± s.e. Risk and infarct sizes, myocardial glycogen and lactate levels 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.

Table 1 Haemodynamic parameters

	Baseline	*Before occlusion*	*20 min occlusion*	*20 min reperfusion*
SBP (mmHg)				
Control	107±4	105±6	94±5	86±5
Mig 10	105±4	104±4	95±6	85±6
Mig 5	108±4	108±4	91±4	84±4
Mig 1	103±6	105±6	92±5	86±4
Mig 10-post	99±4	99±5	92±5	84±6
DBP (mmHg)				
Control	88±5	88±3	75±5	70±5
Mig 10	84±4	83±4	76±7	71±6
Mig 5	88±5	87±4	70±5	64±4
Mig 1	82±4	83±4	72±5	69±5
Mig 10-post	81±4	80±4	70±6	68±5
Heart rate (beats min^{-1})				
Control	263±6	257±8	244±6	237±6
Mig 10	252±10	248±11	229±10	226±9
Mig 5	255±11	256±12	244±8	223±10
Mig 1	262±8	260±8	238±9	225±7
Mig 10-post	262±11	261±11	254±10	247±10

SBP = systolic blood pressure, DBP = diastolic blood pressure, Mig 10 = miglitol 10 mg kg^{-1}, Mig 5 = miglitol 5 mg kg^{-1}, Mig 1 = miglitol 1 mg kg^{-1}, Mig 10-post = miglitol 10 mg kg^{-1} pre-reperfusion administration. The number of rabbits was 10 in each control, Mig 10, Mig 5, Mig 1 and Mig 10-post groups.

Results

Plasma miglitol and glucose levels

Plasma miglitol levels 5, 30 (immediately before ischaemia) and 60 min (30 min after ischaemia) after the administration of miglitol were 6.8±0.7, 3.3±0.2 and 1.8±0.1 μg ml^{-1} in the Mig 1 group, and 20.0±1.2, 10.0±0.8 and 5.8±0.5 μg ml^{-1} in the Mig 5 group, and 42.4±5.0, 22.0±1.2 and 12.6±0.3 μg ml^{-1} in the Mig 10 group, respectively. The plasma glucose level did not change after the administration of miglitol at 1, 5 or 10 mg kg^{-1}.

Mortality and animal exclusion

Fifty-nine rabbits were initially enrolled in the infarct size study. There was no significant difference in the number of animals assigned to each of the five groups, or in the incidence of ventricular fibrillation and mortality. Among these animals, one rabbit each developed ventricular fibrillation during coronary occlusion in the Mig 1 and Mig 5 groups, and one rabbit each developed ventricular fibrillation during reperfusion in the Mig 10, Mig 10-post, and control groups, and all these rabbits died. One rabbit died after the first day of the experiment in each of the control, Mig 1, Mig 5 and Mig 10-post groups. Thus, the experiments were completed using the remaining 50 rabbits and the data from these animals were used for the analysis.

Haemodynamic parameters

Table 1 shows the haemodynamic parameters. The administration of miglitol had no effect on blood pressure or heart rate.

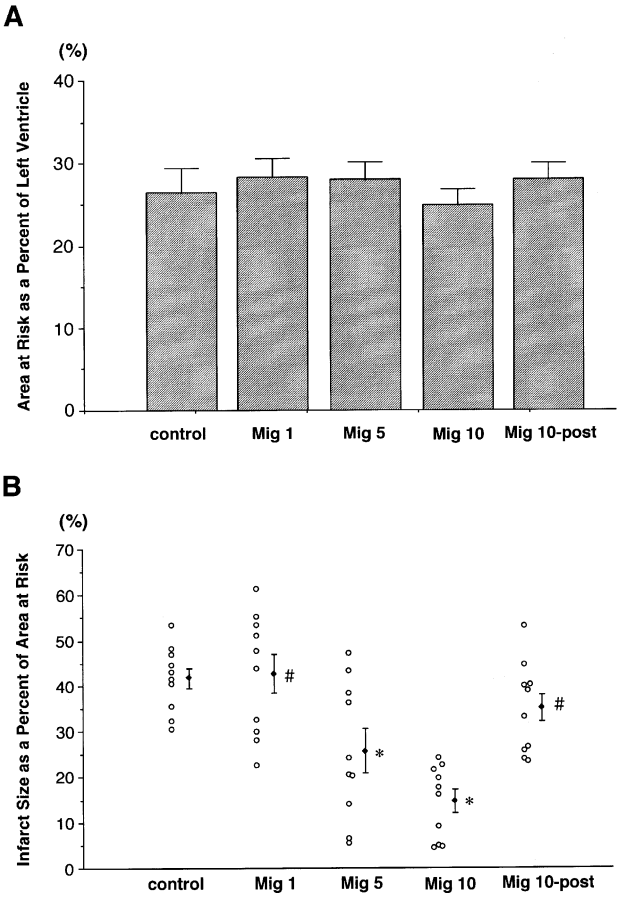

Figure 1 Comparisons of area at risk as a percentage of left ventricle (A), and of infarct size as a percentage of area at risk (B). Mig1, Mig5, and Mig10 represent the doses (mg kg^{-1}) of miglitol injected before ischaemia. Mig10-post represents the dose (mg kg^{-1}) of miglitol injected before reperfusion. One-way analysis of variance combined with Bonferroni's *post hoc* test for multiple comparisons was used. *$P<0.05$ compared with the saline group. #$P<0.05$ compared with the Mig 10 group. Bars represent means±s.e.mean.

Infarct size

There was no significant difference in the mean percentages of area at risk (per cent of left ventricle) among the control, Mig1, Mig5, Mig10 and Mig 10-post groups (Figure 1A). As shown in Figure 1B, the infarct size as a percentage of area at risk was similar between the Mig 1 group and the saline control group, but significantly and dose-dependently reduced in the Mig 5 and Mig 10 groups compared with the saline control group. However, the infarct size was significantly greater in the Mig 10-post group than the Mig 10 group, and showed no significant difference between the Mig-10 post and the saline control group.

Myocardial glycogen and lactate

The myocardial glycogen content (mg g^{-1} wet tissue) was significantly reduced in the ischaemic region compared to the non-ischaemic region both in the saline and the miglitol treatment (1, 5, 10 mg kg^{-1}) groups at 30 min of ischaemia (Figure 2A). Concomitant with the reduction in glycogen, the lactate accumulation (mg g^{-1} wet tissue) was significantly higher in the ischaemic region than in the non-ischaemic region in both saline and miglitol treatment (1, 5, 10 mg kg^{-1}) groups at 30 min of ischaemia (Figure 2B). Compared with saline, pretreatment with miglitol significantly preserved the glycogen

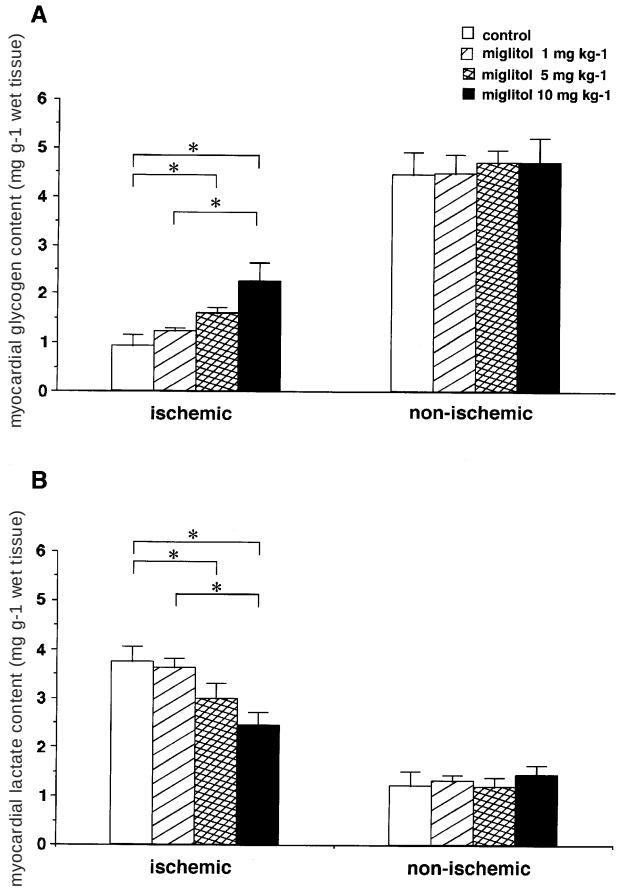

Figure 2 Myocardial glycogen (A) and lactate (B) content (mg g^{-1} wet tissue) at 30 min of ischaemia. Both glycogen and lactate in the transmural myocardial samples in each ten rabbits treated with miglitol (1, 5 and 10 mg kg^{-1}) and saline (control) before ischaemia were measured spectrophotometrically using enzymatic reactions. Open columns indicate control group, and shaded and closed columns indicate miglitol groups. One-way analysis of variance combined with Bonferroni's *post hoc* test for multiple comparisons was used. $*P<0.05$ for each comparison. Bars represent means $\pm$s.e.mean.

content and significantly reduced the lactate accumulation in a dose dependent manner in the ischaemic myocardium at 30 min of ischaemia.

Discussion

The present data demonstrates that (1) pre-ischaemic treatment, but not pre-reperfusion treatment, with miglitol dose-dependently reduced the myocardial infarct size induced by 30 min ischaemia and 48-h reperfusion in rabbits, and (2) miglitol significantly preserved the glycogen content and significantly reduced the lactate accumulation in the ischaemic myocardium at 30 min of ischaemia in a dose dependent manner. This is the first report on the myocardial infarct size-reducing effect of miglitol.

It has already been reported that mean plasma concentration of miglitol 1 h after oral administration of 10 mg kg^{-1} in rabbits reached 21.5 μg ml^{-1} (personal communication, Beckermann B., Brendel E. and Hucke F., Institute of Pharmacokinetics, Institute of Clinical Pharmacology International. Bayer AG, Elberfeld, Germany), and miglitol administered orally was almost completely absorbed. Therefore, the doses of 1, 5 and 10 mg kg^{-1} of miglitol were used in the present i.v. study. The plasma concentrations of miglitol immediately before occlusion were 22.0 ± 0.1, 10.0 ± 0.8 and 3.3 ± 0.2 μg ml^{-1} in the Mig 10, Mig 5 and Mig 1 groups, respectively. The infarct size-reducing effect was not seen in the Mig 1 group, but was seen in the Mig 5 and Mig 10 groups. These data suggest that the minimum plasma concentration of miglitol required to reduce infarct size is greater than or equal to 10 μg ml^{-1} and is within the plasma concentration range which can be reached by oral administration in rabbits.

It has previously been established that the infarct size as a percentage of area at risk is approximately 42% in rabbit hearts with reperfusion after 30 min ischaemia, as was also shown in the present study. This model has been used for many infarct size studies, such as studies on ischaemic preconditioning (Thornton *et al.*, 1990).

Intravenous injection of miglitol did not cause any change in blood pressure or heart rate which might influence the infarct size. Plasma glucose level may affect the infarct size (Kersten *et al.*, 1998). Generally, the hypoglycemic effect of miglitol through inhibition of α-1,4-glucosidase in the intestine is observed only when miglitol is orally administered together with carbohydrates that are digestible by α-glucosidase (Bischoff, 1994). However, the inhibiting effect of miglitol on glycogenolysis which is associated with α-1,6-glucosidase may be also involved in one of the mechanisms for the hypoglycemic effect (Bollen & Stalmans, 1989; Bollen *et al.*, 1988). Therefore, we measured plasma glucose level in the present study. The data showed that intravenous injection of miglitol did not affect the plasma glucose level. This suggests that hypoglycemic effect of miglitol intravenously injected is minimal. In addition, the infarct size-reducing effect is unlikely to be due to effects on the regional blood flow, because collateral circulation in the rabbit heart is minimal (Harken *et al.*, 1981).

It has been reported that 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). Ischaemic preconditioning decreases the myocardial glycogen content after preconditioning, attenuates lactate accumulation and intracellular acidosis during the subsequent sustained ischaemia, and reduces the infarct size (Kida *et al.*, 1991; Murry *et al.*, 1990). The time course of glycogen repletion after a brief ischaemic episode paralleled the loss of the protection from ischaemic injury (Wolfe *et al.*, 1993). Furthermore, McNulty *et al.* (1996) found that ischaemic preconditioning reduced the infarct size under conditions where ischaemic glycogenolysis and lactic acidosis was reduced. Our recent study showed that N-methyl-1-deoxynojirimycin, an α-1,6-glucosidase inhibitor, attenuated glycogen reduction and lactate accumulation during ischaemia, and markedly reduced infarct size in rabbit hearts (Arai *et al.*, 1998). In the present study, we investigated the effect of 1, 5 and 10 mg kg^{-1} of miglitol on glycogen reduction, lactate accumulation and infarct size following 30 min of ischaemia. The data showed that 1 mg kg^{-1} of miglitol which failed to reduce the infarct size also failed to preserve the glycogen content and to attenuate the lactate accumulation. In addition, the effect of miglitol on ischaemic glycogen and lactate content was dose-dependent at the 5 and 10 mg kg^{-1} doses and paralleled its infarct-size reducing effect. Furthermore, the infarct size-reducing effect was observed in the pre-ischaemic treatment with miglitol, but not in the pre-reperfusion treatment, supporting that the mechanism of ischaemic protection is higher tissue pH at the end of ischaemia. These findings suggest that the attenuation of glycolytic flux and

lactate accumulation in ischaemic preconditioning is protective against ischaemic cellular damage, and miglitol or N-methyl-1-deoxynojirimycin reduces the infarct size by reducing the glycogenolytic rate through the inhibition of α-1,6-glucosidase during ischaemia.

Miglitol reduced infarct size more dramatically than it affected glycogenolysis or acidosis. For example, the 5 mg kg^{-1} dose reduced glycogen consumption by only about 11% (from 3.5–3.1 mg g^{-1}), but lactate production by about 31% (from 2.6–1.8 mg g^{-1}), and infarct size by about 38% (from 41.7–25.7%). Therefore, inhibiting glycogenolysis may be a very efficient way of reducing ischaemic acidosis, because the effect is amplified through the process of anaerobic glycolysis.

It is well known that myocardial glycogen depletion and lactate accumulation reach their maximum levels at the endpoint of ischaemia, which correlates with infarct size (Wolfe *et al*., 1993; Barbosa *et al*., 1996). Therefore, we chose 30 min of ischaemia as the time point to assess the effects of miglitol on myocardial glycogen depletion and lactate accumulation in the present study of 30 min occlusion and reperfusion. Meanwhile, it is established that the infarct size itself is determined by the cellular damage during ischaemia and/or within several hours after reperfusion (Fujiwara *et al*., 1989), and the longer the reperfusion time becomes, the more precise the measurement of infarct size using TTC and histological methods become. Therefore, we measured the infarct size at 48 h after reperfusion in the present study. However, it would be useful to look at the time course of glycogen preservation and the reduction of lactate accumulation.

It is well known that 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, as an inhibitor of α-1,4-glucosidase, is a new agent for treating patients with type II diabetes mellitus. Long-term miglitol treatment of 50–100 mg day^{-1} in patients with diabetes mellitus induced a long-lasting reduction of postprandial plasma glucose level without any abnormalities in routine clinical and laboratory examination (Pagano *et al*., 1995). Whilst the treatment of 300 mg day^{-1} did not induce any side effect in routine laboratory examination, clinically patients suffered flatulences, meteorism and diarrhoea (Schnack *et al*., 1989). The dose of miglitol at 5 mg kg^{-1} or 10 mg kg^{-1} used in rabbits of the present study is somewhat high, compared to the clinical dose. However, the relationship between the dose of drugs and the effect generally varies from species to species. Therefore, whether miglitol is also beneficial for patients with coronary heart disease should be examined.

In conclusion, pre-ischaemic treatment with miglitol, a new human anti-diabetic agent, shows a strong protective effect against ischaemic cellular damage in rabbit hearts.

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