N-Methyl-1-Deoxynojirimycin (MOR-14), an α-Glucosidase Inhibitor, Markedly Reduced Infarct Size in Rabbit Hearts

Masazumi Arai, MD; Shinya Minatoguchi, MD; Genzou Takemura, MD; Yoshihiro Uno, MD; Tatsuya Kariya, MD; Hisato Takatsu, MD; Takako Fujiwara, MD; Masaya Higashioka, MS; Yoshiaki Yoshikuni, PhD; Hisayoshi Fujiwara, MD

Background—N-methyl-1-deoxynojirimycin (MOR-14), an α-glucosidase inhibitor, reduces the glycogenolytic rate by inhibiting the α-1,6-glucosidase of glycogen-debranching enzyme in the liver, in addition to possessing an antihyperglycemic action by blocking α-1,4-glucosidase in the intestine. Because the reduction of the glycogenolytic rate may be one of the mechanisms of myocardial protection in ischemic preconditioning, the compounds inhibiting myocardial α-1,6-glucosidase may be protective against ischemic damage. Thus, we investigated whether MOR-14 could inhibit α-1,6-glucosidase and reduce the infarct size in rabbit hearts without collateral circulation.

Methods and Results—MOR-14 dose-dependently decreased the α-1,6-glucosidase activity in rabbit heart extract. A tracer study demonstrated the myocardial uptake of a considerable amount of MOR-14 sufficient to fully inhibit α-1,6-glucosidase. To assess the infarct size–reducing effect of MOR-14, 54 rabbits were subjected to 30-minute coronary occlusion followed by 48-hour reperfusion. Preischemic treatment with 25, 50, and 100 mg/kg of MOR-14 dose-dependently reduced the infarct size to 26±4%, 19±3%, and 14±2% of the area at risk, respectively, compared with the saline control (45±5%) without altering the blood pressure or heart rate. Another 40 rabbits given 100 mg of MOR-14 or saline 10 minutes before ischemia were euthanized at 10 or 30 minutes of ischemia for biochemical analysis. MOR-14 decreased the α-1,6-glucosidase activity to ≈20% in vivo, reduced the glycogen breakdown, and attenuated the lactate accumulation at both 10 and 30 minutes of ischemia.

Conclusions—Preischemic treatment with MOR-14 preserved glycogen, attenuated the accumulation of lactate, and reduced the myocardial infarct size by 69%. This cardioprotective effect was independent of changes of blood pressure and heart rate or regional blood flow. It may be associated with α-1,6-glucosidase inhibition, because MOR-14 markedly decreased the α-1,6-glucosidase activity in the heart.

Key Words: glucose ■ ischemia ■ metabolism ■ myocardial infarction ■ pharmacology

Brief episodes of ischemia and reperfusion before prolonged ischemia, called “ischemic preconditioning,” have had a marked cardioprotective effect against ischemia-reperfusion injury in the hearts of rats, rabbits, dogs, and pigs. Because preconditioning the heart with brief ischemia-reperfusion is not practical for patients, it is desirable to precondition the heart with chemicals (“pharmacological preconditioning”) that exert a beneficial effect similar to ischemic preconditioning. Generally, adenosine A1 receptor, protein kinase C, free radicals, and the KATP channel have been considered to be mediator(s) of ischemic preconditioning. In addition, preserved intracellular pH and decreased lactate accumulation by a reduction of anaerobic glycolysis might have a positive beneficial effect. The reduction of glycolysis is related to the reduction of glycogenolysis during sustained ischemia that may have been caused mainly by a decrease in glycogen after ischemic preconditioning. Therefore, we hypothesized that the pharmacological inhibition of glycogenolysis might also protect the myocardium against ischemic injury.

α-Glucosidases are classified into α-1,1-, α-1,2-, α-1,4-, and α-1,6-glucosidases. Because some α-glucosidase inhibitors, such as acarbose and voglibose, have an antihyperglycemic action by inhibiting the α-1,4-glucosidase that induces the breakdown of oligosaccharides into absorbable monosaccharides in the intestine, the inhibitors have been universally used for the treatment of patients with diabetes mellitus. It is reported that 1-deoxynojirimycin, an α-glucosidase inhibitor, inhibits α-1,6-glucosidase of glycogen-debranching enzymes in the liver and reduces the glycogenolytic rate, in addition to inhibiting α-1,4-glucosidase in the intestine. However, it is unknown whether this effect is also present in the heart. An α-glucosidase inhibitor, N-methyl-1-deoxynojirimycin (MOR-14, synthesized by Nippon Shinyaku Co, Ltd) (Fig 1) inhibits α-1,4-glucosidase in the intestine. Thus, in the present study, we examined whether...
MOR-14 could block the α-1,6-glucosidase activity in the rabbit heart without collateral circulation to inhibit glycogenolysis and reduce infarct size.

Methods

Materials

MOR-14 and [3H]sucrose were purchased from Amersham Ltd. BSA (fraction V) and [14C]glucose were bought from Sigma Chemical Co or Wako Pure Chemical Industries Ltd and were of reagent grade.

In Vitro Study

The inhibitory action of MOR-14 against myocardial α-1,6-glucosidase was first examined in rabbit heart extracts. For the preparation of α-1,6-glucosidase, rabbit heart was homogenized in a fivefold volume (wt/vol) of ice-cold buffer containing 100 mM glycylglycine (pH 7.4), 10 mM EDTA, 10 mM mercapto-ethanol, and 100 mM NaF. α-1,6-Glucosidase activity was determined by measurement of [14C]glucose incorporation into glycogen, according to the procedure of Nelson and Larner. The substrate mixture contained 44 mM glycylglycine (pH 6.5), 12.5% rabbit liver glycogen, 2.5 mM [14C]glucose (20 μCi/μmol), 2.1 mM EDTA, 4.1 mM mercaptoethanol, 0.02% gelatin, and MOR-14 (0, 0.01, 0.03, 0.1, 0.3, or 1.0 μmol/L).

In Vivo Study

Surgical Preparation

Male Japanese White rabbits, each weighing 1.7 to 2.3 kg, were anesthetized with 30 mg/kg sodium pentobarbital and mechanically ventilated with room air. For rabbits receiving the 48-hour reperfusion, all surgical procedures were performed aseptically. A polyethylene catheter (0.9-mm lumen diameter) was inserted into the internal carotid artery and was advanced ~1 cm toward the heart for the drug and saline administration and blood pressure monitoring. Blood pressure was measured with a fluid-filled pressure transducer connected to the end of the cannula. After a left thoracotomy was performed in the third intercostal space, the heart was exposed and connected to the end of the cannula. After a left thoracotomy was performed in the third intercostal space, the heart was exposed and connected to the end of the cannula.

Infarct Size

To investigate the infarct size–reducing effect of MOR-14, 54 rabbits were assigned randomly into drug treatment or saline control groups. There were four drug treatment groups, ie, three preischemic treatment groups given 100 mg/kg (100/0 group, n=10), 50 mg/kg (50/0 group, n=11), or 25 mg/kg (25/0 group, n=11) of MOR-14 10 minutes before ischemia, and one prereperfusion treatment group given 100 mg/kg of the drug 5 minutes before reperfusion (0/100 group, n=11). In all treatments, the injected volume was ~1 ml/kg body wt. In the control group (n=11), an equivalent volume of saline was injected 10 minutes before ischemia. After the treatment, the coronary artery was occluded for 30 minutes and reperfused. The blood pressure and heart rate were monitored throughout the experiment until 20 minutes after reperfusion and were recorded at baseline, at 0, 1, 3, 5, 10, 20, and 30 minutes of ischemia, and at 5, 10, and 20 minutes of reperfusion. The rabbits were then allowed to recover from anesthesia. Forty-eight hours after reperfusion, the rabbits were reanesthetized, and the hearts were excised. After the coronary artery was reoccluded at the identical position, monastral blue dye was retrogradely infused into the ascending aorta at 80 mm Hg to determine the area at risk. Because we left the string beneath the coronary artery at the occluded site after reperfusion and closed the chest, it was easy to identify the location of the previous coronary ligation. The left ventricle was sectioned into five slices parallel to the atrioventricular ring. Each slice was weighed, incubated in a 1% solution of triphenyltetrazolium chloride at 37°C to visualize the infarct area, and photographed. The areas of the ischemic region and the infarcted myocardium were traced on each slice of left ventricle and multiplied by the weight of the slice, then expressed as a fraction of the risk region or left ventricle for each heart.

Biochemical Determinations

Forty additional rabbits were used to assess the in vivo effect of MOR-14 on myocardial glycogen metabolism and lactate accumulation in the ischemic myocardium. The rabbits were randomized to receive either 100 mg/kg of MOR-14 or saline 10 minutes before ischemia and were killed at 10 or 30 minutes of ischemia. Hearts were excised, and transmural samples, each weighing ~200 mg, were taken from the center of the ischemic region and the opposite nonischemic region. The border of the ischemic region was defined by the distribution of cyanosis and marked on the epicardium in ink. The samples were frozen immediately and stored at −80°C until measurement. Samples were weighed, homogenized, and used for the following measurements.

Myocardial glycogen and lactate

For myocardial glycogen measurement, 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. Lactate in the extracts was measured spectrophotometrically by monitoring the hydrogen peroxide formation resulting from the enzymatic reaction with lactate oxidase. For precise analysis, we estimated the glycogen reduction and the lactate accumulation in ischemic myocardium as a difference in the concentration of glycogen and lactate between ischemic and nonischemic areas in each heart.

α-1,6-Glucosidase activity

As already mentioned, the α-1,6-glucosidase activities of the frozen samples were determined by measurement of the [14C]glucose incorporation into glycogen. The homogenate was incubated with the substrate mixture containing 47 mM glycylglycine (pH 6.5), 13.3% rabbit liver glycogen, 2.7 mM [14C]glucose (20 μCi/μmol), 2.2 mM EDTA, 4.4 mM mercaptoethanol, and 0.02% gelatin. The α-1,6-glucosidase activity is expressed as pmol glucose incorporated per minute per mg protein. The amount of protein was determined by a dye-binding assay method with BSA.

Glycogen phosphorylase activity

Like α-1,6-glucosidase, the glycogen phosphorylase activities were measured in the direction of glycogen synthesis according to the procedure of Stalmans and Hers. For the measurement of glycogen phosphorylase a, an active form of glycogen phosphorylase, 133 mM/L maleate (pH 6.5) and 1.3 mM/L caffeine were added to the substrate mixture containing 1.3% rabbit liver glycogen,
13 mmol/L \[^{14}C\]glucose-1-phosphate \((0.2 \mu Ci/\mu mol), 267 \text{ mmol/L NaF}, 6.7 \text{ mmol/L EDTA}, \text{ and } 6.7 \text{ mmol/L mercaptoethanol to specifically inhibit phosphorylase } b, \text{ an inactive form of glycogen phosphorylase, and allow the determination of phosphorylase } a \text{ without the interference of phosphorylase } b. \text{ The total phosphorylase } (a+b) \text{ activity was measured after the addition of } 33 \text{ mmol/L glycylglycine (pH 6.5) and } 6.7 \text{ mmol/L AMP, which increases the activity of phosphorylase } b \text{ with little effect on phosphorylase } a, \text{ instead of maleate and caffeine. For both assays, the homogenate of the myocardial samples was incubated for 20 minutes. The reaction was stopped by the addition of } 0.2N \text{ HCl. The enzyme activity was measured by } ^{14}C \text{ radioactivity counted in the glycogen with a liquid scintillation counter and is expressed as } \text{nmol glucose-1-phosphate incorporated per minute per milligram protein.}

**Cellular Uptake of MOR-14**

To assess the myocardial cellular uptake of MOR-14, we performed a tracer study. Six isolated rabbit hearts were perfused with a buffer containing \[^{14}C\]MOR-14 and \[^{3}H\]sucrose (specific activity, 223 mCi/mol for both). After constant perfusion, the radioactivities of \[^{14}C\] and \[^{3}H\] in the myocardium and the perfusate were measured with a liquid scintillation counter. The cellular uptake of MOR-14 was determined by the method of Angello et al.\(^{22}\) and Rattigan et al.\(^{23}\) \[^{14}C\]MOR-14 was prepared as described by Faber et al.\(^{24}\) The specific activity of \[^{14}C\]MOR-14 was calculated to be 55.8 mCi/mmol.

**Myocardial Ultrastructure**

Eight rabbits were given 100 mg/kg of MOR-14 or an equivalent volume of saline 10 minutes before ischemia. Thirty minutes after coronary occlusion, the hearts were excised, and myocardial samples were obtained from the ischemic region and the nonischemic region. Each sample was fixed with 2.5% glutaraldehyde in 0.1 mol/L phosphate buffer and postfixed with 1% osmium tetroxide. For each heart, four representative blocks from the ischemic and nonischemic region were thin-sectioned, mounted on plain copper grids, stained with uranyl acetate and lead citrate, and examined under a transmission electron microscope.\(^{25}\)

**Statistical Analysis**

All data are presented as mean±SEM. Risk and infarct sizes were compared among the groups by one-way ANOVA combined with Bonferroni’s post hoc test for multiple comparisons. The difference in blood pressure and heart rate over the time course between the control and the drug-treated groups was assessed by two-way repeated-measures ANOVA. Student’s \(t\) test was used to assess the differences in glycogen, lactate, and enzyme activities between pairs of groups. Differences with \(P<.05\) were considered significant.

**Results**

**In Vitro Study**

The inhibitory action of MOR-14 against \(\alpha\)-1,6-glucosidase was examined in vitro. In rabbit heart extracts, MOR-14 dose-dependently inhibited \(\alpha\)-1,6-glucosidase (Fig 2). The \(IC_{50}\) value of MOR-14 for rabbit \(\alpha\)-1,6-glucosidase activity was 0.03 \(\mu\)mol/L.

**In Vivo Study**

**Infarct Size**

Of the 54 rabbits used for the infarct-size study, 1 rabbit in the 25/0 group died of ventricular fibrillation. One rabbit in the 0/100 group died before completion of the experiment of an undefined cause. An additional 2 rabbits with areas at risk <15% of the left ventricle were excluded to avoid an inaccurate infarct size definition as the percent of area at risk. The areas at risk were not significantly different among the groups (Fig 3A). The mean infarct size, assessed by tetrazolium staining and expressed as a percentage of the area at risk, was significantly and dose-dependently reduced in the preischemic treatment groups of 100 mg/kg \((14.4\pm2.2\%, n=10), 50 \text{ mg/kg } (18.7\pm2.6\%, n=10), \text{ and } 25 \text{ mg/kg } (25.7\pm4.4\%, \text{ respectively.})

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Figure 2. Effect of MOR-14 on activity of \(\alpha\)-1,6-glucosidase in rabbit heart extracts. Results are mean±SEM of six observations.

Figure 3. Comparison of area at risk as percentage of left ventricle (A) and of infarct area as percentage of area at risk (B). 100/0, 50/0, 25/0, and 0/100 represent doses (mg/kg) of MOR-14 injected before ischemia/before reperfusion. One-way ANOVA combined with Bonferroni’s post hoc test for multiple comparisons was used. *\(P<.05\) vs saline group, #\(P<.05\) vs 0/100 treated group. Bars represent mean±SEM.
n = 10) compared with the saline control group (44.6 ± 5.2%, n = 10) or the prereperfusion treatment group of 100 mg/kg (39.5 ± 4.2%, n = 10) (Fig 3B). There were no significant differences in infarct size between the saline control group and the prereperfusion treatment group, indicating that only preischemic treatment with MOR-14 reduced infarct size. The administration of MOR-14 had no effect on blood pressure or heart rate (Table). The five groups showed similar blood pressures and heart rates during the experiments.

**Myocardial Glycogen and Lactate**

The myocardial glycogen content (mg/g wet tissue) was significantly reduced in the ischemic region compared with the nonischemic region both in the saline and the pretreatment (100 mg/kg of MOR-14) groups at 10 minutes of ischemia (saline, n = 10, 1.1 ± 0.1 versus 2.5 ± 0.2; and MOR-14, n = 10, 1.5 ± 0.2 versus 2.5 ± 0.2) and at 30 minutes of ischemia (saline, n = 10, 0.4 ± 0.03 versus 2.7 ± 0.3; and MOR-14, n = 10, 1.3 ± 0.1 versus 2.6 ± 0.2) (Fig 4A). The lactate level (mg/g wet tissue) was significantly higher in the ischemic than in the nonischemic myocardium in both the groups at 10 minutes of ischemia (saline, 3.6 ± 0.2 versus 1.7 ± 0.1; and MOR-14, 2.6 ± 0.1 versus 1.5 ± 0.2) and at 30 minutes of ischemia (saline, 3.9 ± 0.2 versus 1.3 ± 0.1; and MOR-14, 2.6 ± 0.2 versus 1.4 ± 0.1) (Fig 4B). In the saline group, myocardial glycogen consumption and lactate accumulation were measured spectrophotometrically by use of enzyme reactions. Glycogen reduction and lactate accumulation in ischemic myocardium were assessed by difference in content of glycogen and lactate between ischemic and nonischemic areas. *P < 0.05 for each comparison. Bars represent mean ± SEM.
mulation in the ischemic area, estimated as a difference in the concentration of glycogen and lactate between nonischemic and ischemic areas, progressively increased between 10 and 30 minutes of ischemia. Glycogen reduction was 1.5±0.1 and 2.2±0.3 mg/g wet wt ($P<.05$) and lactate accumulation, 1.9±0.2 and 2.6±0.2 mg/g wet wt ($P<.05$) at 10 and 30 minutes of ischemia, respectively (Fig 4C and 4D). However, in the drug-treated group, there was no further significant consumption of glycogen or accumulation of lactate in the ischemic area during this period. In this group, glycogen reduction was 1.0±0.2 and 1.3±0.2 mg/g wet wt ($P=NS$) and lactate accumulation, 1.1±0.1 and 1.2±0.2 mg/g wet wt ($P=NS$) at 10 and 30 minutes of ischemia, respectively (Fig 4C and 4D). The glycogen reduction and the lactate accumulation were significantly lower in the pretreatment group than the saline group at both 10 and 30 minutes of ischemia.

To assess further effects of MOR-14 on carbohydrate metabolism, the amounts of lactate accumulation and glycogen breakdown were converted from the value of mg/g wet wt shown above to μmol/g wet wt. Then we calculated the ratio of lactate accumulation (μmol/g wet wt) to the breakdown of glycogen measured as glycosyl units (μmol/g wet wt). Their ratios (lactate accumulation to glycogen breakdown) were 2.1±0.2 (17.3±1.6 to 8.2±0.7) at 10 minutes of ischemia and 2.0±0.2 (23.5±2.1 to 12.4±1.4) at 30 minutes of ischemia in the saline group, and 1.8±0.3 (9.8±1.2 to 5.4±0.8) at 10 minutes of ischemia and 1.5±0.2 (10.8±2.1 to 7.3±1.0) at 30 minutes of ischemia in the MOR-14–treated group. The ratio of lactate accumulation to glycogen breakdown at 30 minutes of ischemia was significantly lower in the MOR-14–treated group than in the saline group.

**Glycogenolytic Enzymes**

The α-1,6-glucosidase activities (pmol · min⁻¹ · mg protein⁻¹) in the saline and the drug-treated groups were, respectively, 9.4±0.9 and 2.0±0.2 at 10 minutes of ischemia and 7.8±1.4 and 1.7±0.2 at 30 minutes of ischemia in the ischemic area, and 9.7±0.8 and 1.9±0.1 at 10 minutes of ischemia and 8.9±1.5 and 1.9±0.2 at 30 minutes of ischemia in the nonischemic area (Fig 5). Thus, consistent with glycogen preservation, myocardial α-1,6-glucosidase activity in the drug-treated hearts was strongly inhibited in vivo in both the ischemic and nonischemic myocardium over 30 minutes of ischemia.

In both the saline and the drug-treated groups, the myocardial glycogen phosphorylase $a$ activity (nmol · min⁻¹ · mg protein⁻¹) in the ischemic area was significantly suppressed compared with that in the nonischemic area (saline, 13.3±1.4 versus 20.5±2.0; and MOR-14, 13.8±2.4 versus 21.2±2.4) at 30 minutes of ischemia, presumably by higher tissue levels of anaerobic glycolytic products such as lactate$^{26}$ (Fig 6A). The total phosphorylase activities were not different between the ischemic and the nonischemic areas (Fig 6B). There was no difference in glycogen phosphorylase $a$ and $a+b$ activities between the saline and the drug-treated groups in both the ischemic and the nonischemic regions at given time points, indicating that the treatment with MOR-14 did not alter the glycogen phosphorylase activities.
Discussion
The present data demonstrated that (1) MOR-14 dose-dependently inhibited myocardial $\alpha$-1,6-glucosidase activity in vitro and (2) preischemic treatment with MOR-14 markedly inhibited $\alpha$-1,6-glucosidase activity during 30-minute ischemia in vivo and reduced the infarct size after 48-hour reperfusion. We believe that this is the first report of the inhibition of $\alpha$-1,6-glucosidase in the heart and the reduction of myocardial infarct size by an $\alpha$-glucosidase inhibitor.

Infarct Size-Reducing Effect of MOR-14
The infarct size--reducing effect was observed in the preischemic treatment groups but not in the prereperfusion treatment group. In addition, the electron microscopic analysis clearly revealed the protective effect of MOR-14 against myocardial cellular damage during ischemia. Therefore, this compound protects the myocardium against ischemic injury but not reperfusion injury. MOR-14 did not have any effect on blood pressure and heart rate, which might alter the infarct size. In addition, the infarct size--reducing effect was also independent of the regional blood flow, because the rabbit heart has no collateral circulation.

The MOR-14 treatment preserved glycogen content and attenuated lactate accumulation in the ischemic myocardium. Although glycogenolysis followed by anaerobic glycolysis is the major metabolic pathway for the energy production during ischemia, accumulated anaerobic glycolytic products, such as lactate, NADH, and H$^+$, may contribute to intracellular acidosis and osmotic load and cause irreversible cell damage. This anaerobic glycolysis-mediated mechanism of ischemic injury has been supported by several observations. Ischemic preconditioning decreases myocardial glycogen content after preconditioning, attenuates lactate accumulation and intracellular acidosis during the sustained ischemia, and reduces the infarct size. In addition, the time course of glycogen repletion after a brief ischemic episode paralleled the loss of the protection from ischemic injury. These findings suggest that the attenuation of glycolytic flux and lactate accumulation is an important mechanism of protection in the ischemic preconditioned heart. In addition, preischemic glycogen reduction and glycolytic inhibition have each been reported to improve the functional recovery of isolated ischemic-reperfused rat hearts.

More recently, Hadour et al demonstrated that the myocardial infarct size was limited in diabetic rabbits, in which the glycogenolytic activity might be suppressed by the low insulin level. Thus, the cardioprotective effects of MOR-14 appear to be associated with the inhibition of glycogenolysis during ischemia.

We found that MOR-14 markedly inhibited the $\alpha$-1,6-glucosidase activity in the rabbit hearts. This suggests that the preservation of glycogen and the attenuation of lactate accumulation by this compound are related to the inhibition of $\alpha$-1,6-glucosidase. In the present study, however, the inhibitory effect of MOR-14 on $\alpha$-1,6-glucosidase was observed in an extract of myocardium in which the cell membranes were disrupted. Therefore, we performed the experiment to assess the myocardial cellular uptake of MOR-14. The amount of MOR-14 trapped by the cells after 10 minutes of perfusion was $10.3 \pm 4.4 \mu$mol/kg tissue, which is much higher than the IC$\text{50}$ value (0.03 $\mu$mol/L) of MOR-14 for rabbit $\alpha$-1,6-glucosidase. Conversely, the concentration of MOR-14 in the perfusate was 200 $\mu$g/mL, which is lower than the plasma concentration of MOR-14 at 5 (470±32) and 10 (392±29) minutes after injection in our infarct study. Thus, the amount of MOR-14 taken up by the cells of the perfused rabbit heart was sufficient to completely block myocardial $\alpha$-1,6-glucosidase activity.
glucosidase activity. Because it is reasonable to assume that membrane function in isolated heart preparations is not fundamentally different from membrane function in vivo, a considerable amount of MOR-14 may also be incorporated and inhibit α-1,6-glucosidase in vivo.

One mole of glycogen measured as glycosyl units gives rise to 2 mol lactate. In the present study, the accumulation of lactate (μmol/g wet wt) during ischemia was actually nearly twice as high as the amount of glucose (μmol/g wet wt) originated from glycogen in the saline group. However, the ratio of lactate accumulation to the amount of mobilized glycogen in the MOR-14–treated group was significantly lower than that in the saline group at 30 minutes of ischemia (1.5±0.2 versus 2.0±0.2, P<.05). This suggests other beneficial effects of MOR-14 on glucose metabolism during ischemia in addition to the glycogenolytic inhibition through the inhibitory effect of α-1,6-glucosidase. That is, MOR-14 might decrease the accumulation of products of anaerobic glycolysis through inhibition of glycolysis itself and/or might stimulate glucose oxidation and divert lactate to be used as a substrate. The stimulation of glucose oxidation also may be cardioprotective, because it allows the production of more ATP per quantity of O2 and reduction in the buildup of H⁺.

Further studies are warranted to clarify whether these metabolic alterations are also involved in the pharmacological effects of MOR-14 and contribute to cardioprotection.

Effect of MOR-14 on Glycogen Metabolism

In the saline group, the glycogen reduction and lactate accumulation in the ischemic area increased progressively between 10 and 30 minutes of ischemia. In the pretreatment group, they occurred during the first 10 minutes of ischemia but disappeared between 10 and 30 minutes of ischemia. The difference in glycogen reduction and lactate accumulation between the saline group and the pretreatment group became larger at 30 minutes than at 10 minutes of ischemia. This special feature of the time course of glycogen depletion in drug-treated hearts could be explained by the present observation that pretreatment with the drug markedly decreased the myocardial α-1,6-glucosidase activity but did not affect the glycogen phosphorylase activity during ischemia. The breakdown of glycogen proceeds through the interaction of glycogen phosphorylase and the α-1,6-glucosidase and glucanotransferase activities of the glycogen-debranching enzyme. The external side chains of a glycogen molecule attach through an α-1,6 bond to an adjacent α-1,4–linked chain. Glycogen phosphorylase first releases glucose-1-phosphate until the external chains have been shortened to form maltotriosyl units. Subsequently, glucanotransferase removes the maltotriosyl unit from the α-1,6–linked stub and attaches it to the free C₄ of the main chain. The single remaining α-1,6–linked glucosyl unit is then removed as free glucose by α-1,6-glucosidase, while additional α-1,4–linked glucosyl residues become available for phosphorylase. Therefore, even when α-1,6-glucosidase activity is blocked, some glucose-1-phosphates are released by the action of unaffected phosphorylase, and the glycogen concentration decreases. However, glycogen may be decreased slowly thereafter, because glycogen phosphorylase alone cannot trim the four glucosyl residues located distal to the glucose, which has an α-1,6–linked glucosyl unit.

In the present study, the total glycogen content (mg/g wet wt) in the nonischemic area in the MOR-14–treated group (2.6±0.2) was similar to that in the saline-treated group (2.7±0.3) at 30 minutes of ischemia (ie, 40 minutes after injection of MOR-14). Myocardial glycogen content is regulated by both synthesis and degradation (ie, glycogen turnover). Therefore, when glycogenolysis is inhibited by MOR-14, one would expect an increase in net glycogen. However, Henning et al recently reported that glycogen synthesis ranged from 0.33 to 0.51 μmol glucose·min⁻¹·g dry wt⁻¹ in the aerobic, isolated working rat heart, corresponding to 0.3% to 0.5% of initial glycogen content per minute. These rates of synthesis agree with observations in a rat model reported by Goodwin et al (0.17 to 0.62 μmol glucose·min⁻¹·g dry wt⁻¹). This indicates that, even when the baseline rate of synthesis is completely maintained and degradation is totally stopped, glycogen content increases by only 12% to 20% over 40 minutes, although data from a rabbit in vivo model are unknown. MOR-14 specifically inhibited α-1,6-glucosidase but did not affect phosphorylase activity. In fact, glycogen content in the ischemic region decreased by ≈50% after 30-minute ischemia despite the blockade of α-1,6-glucosidase by MOR-14 in the treated group. This suggests that the glycogenolytic rate mediated by phosphorylase activity is quite high, although the precise rate is unknown in nonischemic rabbit myocardium. In addition, glycogen itself may inhibit glycogen synthesis when degradation of glycogen is blocked. That is, MOR-14 may shut off the glycogen turnover altogether. These suppositions may explain the absence of increase in glycogen levels in the nonischemic area of the MOR-14–treated group. At present, the baseline rate of glycogen turnover is unknown, and how it is affected by MOR-14 in rabbit hearts remains to be clarified.

The reported glycogen concentrations in nonischemic myocardium were quite variable in both rabbits and rats, although the glycogen levels of 2.7 and 2.5 mg/g wet wt in the present study were within the range of reported values. The reason for the variability remains obscure, because the measurement was done by the same standard method. However, the myocardial glycogen levels in rats have recently been reported to be approximately two times higher in the fasted than in the fed state. Therefore, diet milieu before experiment may be one factor causing this variability.

Clinical Benefits of MOR-14

In the present study, MOR-14 clearly protected myocardium against the ischemic induction of infarcts when given just before ischemia but not when given before reperfusion. MOR-14, like other N-substituted derivatives of deoxyynojirimycin, is completely absorbed from the intestines. In addition, when taken orally, MOR-14 develops an antihyperglycemic action through its α-1,4-glucosidase inhibitory action in the intestine. Further studies should be conducted to assess the suitability of this drug for clinical use.
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