Cardioprotective Effect of K-7259, a Novel Dilazep Derivative, against Ischemia-Reperfusion Damage in Isolated, Working Rat Hearts

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ABSTRACT—Global ischemia (15 min) followed by reperfusion (10, 20 or 30 min) was performed in isolated, working rat hearts. Ischemia depressed mechanical function, which was not restored by reperfusion of 20 min. Preischemic administration of K-7259 (N,N'-bis[4-(3,4,5-trimethoxyphenyl)butyl]homopiperazine dihydrochloride) (1, 5 or 10 μM) decreased the function before ischemia, but it attenuated the ischemia-induced dysfunction during reperfusion (20 min). Postischemic administration of K-7259 (10 μM) or dilazep (20 μM) also attenuated the ischemia-induced dysfunction during reperfusion (30 min). Ischemia-reperfusion (10 min) increased the tissue malondialdehyde level, and postischemic administration of K-7259 (10 μM) or dilazep (20 μM) attenuated the malondialdehyde accumulation. K-7259 has a cardioprotective effect when given either before or after ischemia.

Keywords: K-7259, Working rat heart, Ischemia

Dilazep has been demonstrated to attenuate myocardial ischemic damage (1, 2). The cardioprotective action of dilazep has been considered to be due to potentiation of the cardiovascular effects of adenosine, such as vasodilating and negative chronotropic effects, through inhibition of adenosine uptake into cardiac and endothelial cells (3). According to Meghji et al. (4), concentration of dilazep required to inhibit adenosine uptake into the cardiac cell is high (more than 100 μM) in the rat when compared to other species. Nevertheless, dilazep is capable of attenuating ischemic derangements in rat heart at concentrations below 10 μM (2). The cardioprotective effect of dilazep, therefore, may be due to a mechanism other than the potentiation of the cardiovascular effects of adenosine.

K-7259 (N,N'-bis[4-(3,4,5-trimethoxyphenyl)butyl]homopiperazine dihydrochloride) is a novel derivative of dilazep. It was first considered that K-7259 potentiates the action of adenosine as does dilazep because it is a derivative of dilazep. However, the potentiating action of K-7259 on the cardiovascular effects of adenosine has been found to be about 1/1000 times that of dilazep (5). If potentiation of the adenosine-mediated cardiovascular effects is not a major cause of the cardioprotective effect of dilazep, K-7259 could be also effective in attenuating ischemia-reperfusion damage. In the present study, therefore, we examined whether K-7259 has a cardioprotective action against ischemia-reperfusion damage in the isolated, perfused working rat heart.

Male Sprague-Dawley rats (from Animal Laboratory for Medical Research in Asahikawa Medical College, Asahikawa), weighing 280–340 g, were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). After thoracotomy, the hearts were initially perfused by the Langendorff method at a constant pressure of 90 cmH2O for 10 min and then perfused by the working heart method at a left atrial filling pressure of 12.5 cmH2O and an afterload pressure of 90 cmH2O for 15 min (6). The solution for perfusion was a modified Krebs-Henseleit bicarbonate (KHB) buffer containing: 119.4 mM NaCl, 4.7 mM KCl, 2.9 mM CaCl2, 1.2 mM MgSO4, 1.2 mM KH2PO4, 25.0 mM NaHCO3, 11.0 mM glucose and 0.5 mM EDTA-2Na, equilibrated with a gas mixture of 95% O2 + 5% CO2 and maintained at 37 C. Global ischemia (Ischemia) was induced by lowering the afterload pressure from 90 cmH2O to 0 cmH2O (i.e., no-afterload ischemia). Reperfusion was induced by returning the afterload pressure to its
initial level. Aortic pressure was recorded with a pressure transducer placed in the aortic cannula, and heart rate was counted from the recorded aortic pressure tracings. Cardiac mechanical function was defined as rate pressure product (RPP: heart rate multiplied by peak aortic pressure). Coronary flow (defined as the flow coming from the cannula inserted into the pulmonary artery) was measured with a graded glass cylinder.

Two kinds of experiments, preischemic administration and postischemic administration experiments, were carried out. In the former, hearts were subjected to 15-min ischemia and then 20-min reperfusion. K-7259 was administered to the perfusion solution 5 min before starting ischemia until the end of ischemia, and then reperfusion was performed using normal KHB. In this series of experiments, hearts were divided into 4 groups: control (no drug, n=8), K-7259 (1 µM, n=5), K-7259 (5 µM, n=5) and K-7259 (10 µM, n=7) groups. In the latter experiments, hearts were subjected to 15-min ischemia and then 30-min reperfusion, and K-7259 or dilazep was given to the heart only during the first 5 min after reperfusion. There were 3 groups in this series of experiments: control (no drug, n=4), K-7259 (10 µM, n=4), dilazep (10 µM, n=5) and dilazep (20 µM, n=5) groups.

In additional experiments, other hearts were subjected to an experimental protocol that is exactly the same as in the postischemic administration experiments except that reperfusion was performed for 10 min instead of 30 min. Rats were divided into the control (no drug, n=4), K-7259 (10 µM, n=5) and dilazep (20 µM, n=4) groups. Hearts were freeze-clamped (−173°C) 10 min after the start of reperfusion. There was another group, the nonischemia group (n=4), in which the hearts were subjected to neither ischemia nor drug administration but freeze-clamped. The freeze-clamped cardiac tissue samples were analyzed for malondialdehyde (MDA) by HPLC (7, 8). Briefly, MDA was extracted from the pulverized tissue sample with 10 mM phosphate buffer (pH 8.0), and the tissue extract was centrifuged at 10,000 \( \times g \) for 10 min at 4°C, and the supernatant was filtered through a millipore filter (UFC3LCC; Nihon Millipore Kogyo KK, Yonezawa) at 2,000 \( \times g \). The filtered solution (20 µl) was injected into the HPLC system (LC-9A; Shimadzu Corporation, Kyoto), equipped with an ODS guard column (0.4 x 1 cm, Shimadzu Corporation) and ODS column (0.46 x 25 cm, Shimadzu Corporation). MDA was separated with 15% acetonitrile containing 50 mM myristyltrimethylammonium bromide and 1.0 mM Na\( _3 \)HPO\( _4 \), adjusted to pH 6.8, at a flow rate of 1.0 ml/min. The effluent was monitored at 267 nm using a spectrophotometric detector (SPD-2AS, Shimadzu Corporation). The quantitative analysis was performed by comparison with standard curves. MDA standards were prepared by acid hydrolysis of malonaldehyde bis(dimethyl acetal). Before measurement of MDA, we confirmed that neither dilazep nor K-7259 interferes with measurement of MDA.

K-7259 and dilazep were dissolved in the normal KHB buffer. Biochemicals and reagents were purchased from Sigma Chemical Company (St. Louis, MO, USA) or Aldrich Chemical Company (Milwaukee, WI, USA). All data are expressed as means±S.E.M. The significance of difference between means was analyzed by the analysis of variance, followed by Duncan’s multiple-range test.
Differences were considered significant at the level of $P<0.05$.

Figure 1 shows the results of the preischemic administration experiments. A change from the Langendorff perfusion to the working heart perfusion increased RPP markedly. In the control (no drug) group, ischemia decreased both heart rate and aortic pressure to 0 beats/min and 0 mmHg, respectively, and therefore RPP became 0 mmHg/min after ischemia. Reperfusion following ischemia did not increase RPP at all in the control group. Preischemic administration of K-7259 decreased RPP depending on the concentration in the heart before ischemia, and the values of RPP induced by K-7259 (5 or 10 $\mu$M) immediately before ischemia were significantly lower than that in the control group. Even in the presence of K-7259, ischemia decreased RPP to 0 mmHg/min. The drug-treated heart was then reperfused with normal KHB buffer. Reperfusion restored RPP markedly in the heart in which there was preischemic administration of K-7259 (1, 5 or 10 $\mu$M). The degree of recovery of RPP after reperfusion did not always depend on the concentration of K-7259. This is probably because there was a concentration-dependent decrease in RPP with K-7259 before ischemia, which opposed the recovery of RPP after reperfusion. Before ischemia, K-7259 did not increase but decreased coronary flow. Even in the presence of K-7259, ischemia decreased coronary flow to 0 ml/min. In the control group, coronary flow recovered incompletely during reperfusion. In the presence of K-7259, the recovery of coronary flow was accelerated insignificantly or significantly but concentration-dependently.

Figure 2 shows the results of the postischemic administration experiments. In the control group, ischemia decreased RPP to 0 mmHg/min, and reperfusion did not restore RPP. When K-7259 (10 $\mu$M) or dilazep (20 $\mu$M) was given during the first 5 min of reperfusion, there was a significant recovery of RPP during reperfusion. There was no recovery of RPP when dilazep (10 $\mu$M) was given, however.

In the present study, postischemic administration of dilazep (10 $\mu$M) failed to attenuate mechanical dysfunction during reperfusion. In the previous study, however, we have demonstrated that a preischemic administration of dilazep (10 $\mu$M) increases the recovery of mechanical function after ischemia (2). Therefore, the preischemic administration of dilazep is considered more effective in attenuating the mechanical dysfunction induced by ischemia-reperfusion. K-7259 (10 $\mu$M) was administered either before or after ischemia in the present study, and therefore it is possible to compare the degree of recovery of mechanical function between the preischemic administration and the postischemic administration groups. However, there was no significant difference between the

![Fig. 2. The results of the postischemic administration experiments. Effects of K-7259 (10 $\mu$M) and dilazep (10 and 20 $\mu$M) on the rate-pressure product in the hearts subjected to ischemia (for 15 min) followed by reperfusion (for 30 min) are shown. Drugs were administered during the first 5 min after reperfusion. ○, Control; ●, K-7259 (10 $\mu$M); △, Dilazep (10 $\mu$M); ▲, Dilazep (20 $\mu$M). *$P<0.05$ compared with the corresponding value in the control group.](image1)

![Fig. 3. The tissue levels of malondialdehyde (MDA) in the nonischemia, control (no drug), K-7259 (10 $\mu$M), and dilazep (20 $\mu$M) groups. The experimental protocol is the same as the postischemic administration experiments except that reperfusion was performed for 10 min instead of 30 min. In the nonischemia group, neither ischemia nor drug administration was performed. Drugs were administered during the first 5 min after reperfusion. Hearts were freeze-clamped immediately after the end of reperfusion. In the nonischemic group, hearts were freeze-clamped without ischemia and drugs. *$P<0.05$, compared with the control group.](image2)
two groups.

Figure 3 shows the results of the additional experiments. The tissue MDA level in the control group, in which ischemia and reperfusion were performed without drugs, was more than double the level in the nonischemic group. The MDA levels in the K-7259 and dilazep groups were significantly lower than that in the control group, suggesting that K-7259 and dilazep attenuated lipid peroxidation of the myocardium induced by ischemia-reperfusion.

The primary mechanism of action of anti-ischemic drugs has been considered to be improvement of the myocardial energy balance between supply and demand by either an increase in coronary blood flow or a decrease in mechanical function of the heart, or both (i.e., preservation of energy). In fact, we have shown that dilazep (5 and 10 μM) decreased mechanical function before ischemia and enhanced the recovery of mechanical function after ischemia (2). The results of the present study also demonstrated that K-7259 (5 and 10 μM) decreased mechanical function before ischemia and enhanced the recovery of mechanical function after ischemia (preischemic administration experiments). Therefore, one of the mechanisms of the beneficial action of K-7259 on the ischemia-reperfusion damage is preservation of energy in the heart. According to Nakagawa et al. (9), dilazep can block the L-type Ca\(^{2+}\) channel in the myocardium. By similar inhibitory action on the Ca\(^{2+}\) channel, K-7259 may decrease mechanical function before ischemia, although there is no information about the effect of K-7259 on the Ca\(^{2+}\) channel.

In contrast, the lowest concentration of K-7259 (1 μM) did not have any significant action on mechanical function before ischemia. In addition, K-7259 (1, 5 and 10 μM) did not increase but decreased coronary flow in the heart before ischemia, although the reason why it decreased coronary flow remains obscure. It should be noted, however, that K-7259 (1 μM) was effective in attenuating the mechanical dysfunction during reperfusion even when it did not have a favorable hemodynamic action before ischemia. These facts suggest that a mechanism other than the preservation of energy is also responsible for the cardioprotective action of K-7259 against ischemia-reperfusion damage. Recently, we have demonstrated that dilazep and K-7259 attenuate mechanical dysfunction induced by lysophosphatidylcholine (10) and long-chain acylcarnitine (11), both of which are amphiphilic metabolites, in the isolated perfused rat heart. These amphiphilic metabolites accumulate in the heart during ischemia-reperfusion producing damage to the heart, and they are regarded as substances involved in production of ischemia-reperfusion damage (12, 13). The beneficial effect of K-7259 against amphiphilic metabolites may contribute to its protective action on the myocardium from ischemia-reperfusion damage.

We have also found that dilazep and K-7259 attenuate hydrogen peroxide (H\(_2\)O\(_2\))-induced mechanical dysfunction in the isolated perfused rat heart (8). Similarly, K-7259 attenuates the H\(_2\)O\(_2\)-induced cell damage in the rat cardiomyocytes (14). Reactive oxygen species such as H\(_2\)O\(_2\) can be generated during reperfusion after ischemia in the myocardium and endothelium, causing lipid peroxidation of the cell membrane, and hence myocardial derangements including mechanical dysfunction (15). There is a possibility, therefore, that dilazep and/or K-7259 is effective in attenuating the mechanical dysfunction during reperfusion even when given only during reperfusion. In fact, reperfusion with dilazep (20 μM) and K-7259 (10 μM) enhanced the recovery of mechanical function after ischemia (posts ischemic administration experiments). In addition, we measured the tissue concentration of MDA, an indicator of lipid peroxidation, in the posts ischemic administration experiments. The tissue MDA concentration was measured shortly (10 min) after the onset of reperfusion, because MDA can be washed out into myocardial interstitial effluent and the tissue concentration decreases by prolongation of the period of reperfusion (7). Posts ischemic reperfusion produced an increase in the tissue concentration of MDA, which was attenuated by reperfusion with dilazep and K-7259. These results suggest that the protective action of posts ischemic administration of dilazep or K-7259 is probably due to its effect to reduce the lipid peroxidation, at least in part.

In conclusion, both preischemic and posts ischemic administration of K-7259 protect the myocardium against ischemia-reperfusion damage. The cardioprotective action of K-7259 may be due to mechanisms including preservation of energy and reduction of lipid peroxidation.

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