Nicorandil Improves Post-Ischemic Myocardial Dysfunction in Association With Opening the Mitochondrial KATP Channels and Decreasing Hydroxyl Radicals in Isolated Rat Hearts

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Background Nicorandil has been reported to induce cardioprotection by opening the mitochondrial KATP channels. However, whether nicorandil affects reactive oxygen species is unclear.

Methods and Results The hearts of male Sprague-Dawley rats were excised and perfused on a Langendorff apparatus with Krebs-Henseleit solution with a gas mixture of 95% O2 and 5% CO2. 1 mmol/L of nicorandil was given 10 min before ischemia. Left ventricular developed pressure (LVDP, mmHg), ±dP/dt (mmHg/s) and coronary flow (ml/min) were continuously monitored. All hearts were perfused for a total of 120 min consisting of a 30 min pre-ischemic period, followed by a 30 min global ischemia and 60 min reperfusion with and without 5-hydroxydecanoic acid sodium salt (5-HD), a mitochondrial KATP channel blocker. The concentrations of 2,3-dihydroxybenzoic acid (2,3-DHBA), an indicator of hydroxyl radicals, in the perfusate during reperfusion period were also measured. Nicorandil significantly improved LVDP and ±dP/dt, and increased coronary flow during reperfusion. Pretreatment with 5-HD abolished the improvement of LVDP and ±dP/dt, and the increase in coronary flow induced by nicorandil. Nicorandil significantly attenuated the concentrations of 2,3-DHBA during reperfusion, which were restored by 5-HD.

Conclusion Nicorandil is protective against post-ischemic left ventricular dysfunction in association with opening the mitochondrial KATP channels, decreasing hydroxyl radicals and increasing coronary flow in the isolated rat heart. (Circ J 2006; 70: 1650–1654)

Key Words: Hydroxyl radicals; Mitochondrial KATP channels; Myocardial dysfunction; Nicorandil

It has been reported that the opening of mitochondrial KATP channels induces cardioprotection. Nicorandil, a KATP channel opener, which is currently clinically used for the treatment of coronary artery disease, has been reported to reduce the myocardial infarct size when given before ischemia in animal studies. Recent studies have suggested that the opening of mitochondrial KATP channels induces cardioprotection by generating reactive oxygen species (ROS). However, production of ROS, such as superoxide anion and hydroxyl radicals, during the reperfusion period has been regarded as a predominant mediator of ischemia–reperfusion injury. We hypothesized in the present study that nicorandil reduces the production of ROS during reperfusion because nicorandil has been reported to reduce the myocardial infarct size. Therefore, we aimed to clarify: (i) whether nicorandil is protective against post-ischemic myocardial dysfunction; and (ii) whether cardioprotective effect of nicorandil is related to scavenging hydroxyl radicals and opening the mitochondrial KATP channels in isolated rat hearts.

Methods

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

Animal Selection

Male Sprague-Dawley rats (Chubu-Kagaku-Shizai, Nagoya, Japan) each weighing 200–300 g were used. None of the rats had any clinically evident infections.

Perfusion of Isolated Rat Heart

Male Sprague-Dawley rats were anesthetized with pentobarbital (60 mg/kg, ip) and the hearts were excised in 4°C saline and perfused with oxygenated buffer within 30 s. Hearts were retrogradely perfused on a Langendorff apparatus with Krebs-Henseleit solution (5.5 mmol/L glucose, 1.2 mmol/L Ca2+, 4.7 mmol/L KCl, 25.0 mmol/L NaHCO3) with a gas mixture of 95% O2 and 5% CO2. The perfusion pressure was kept at 80 mmHg. A water-filled polyethylene
Nicorandil Reduces Hydroxyl Radical Production

Circulation Journal Vol. 70, December 2006

Experimental Protocol of Cardiac Function

All hearts were perfused for a total of 120 min consisting of a 30 min pre-ischemic period, followed by a 30 min global ischemia and 60 min reperfusion at a temperature of 37°C. As shown in Fig 1, 4 groups were studied: control group (n=10), hearts treated with saline; the nicorandil group (n=10), hearts treated with 1 mmol/L of nicorandil; the nicorandil +5-hydroxydecanoic acid sodium salt (5-HD) group (n=10), hearts treated with 100 μmol/L of 5-HD during the 10 min of pre-ischemic period in the nicorandil group; and the 5-HD group (n=10), hearts treated with 100 μmol/L of 5-HD during the 10 min of pre-ischemic period.

Measurement of Hydroxyl Radicals

The 2,3-dihydroxybenzoic acid (2,3-DHBA), an indicator of hydroxyl radicals, was measured using a high performance liquid chromatography coupled with electrochemical detection in the control group (n=10), nicorandil group (n=10), nicorandil+5-HD group (n=10), and 5-HD group (n=10). This method is based on the chemical reaction of salicylic acid with hydroxyl radicals, yielding 2,3-DHBA, 2,5-DHBA and catechol as its derivatives in an approximate proportion of 49%, 40% and 11%, respectively. It is reported that salicylic acid with a concentration of 1 mmol/L traps approximately 10% of the theoretically possible hydroxyl radicals formed in vivo. We used 2,3-DHBA as an indicator of hydroxyl radicals in the present study. The experimental procedure for the measurement of 2,3-DHBA was the same as described above, except that the hearts were perfused with the buffer containing 1 mmol/L salicylic acid. The pH of the buffer was re-adjusted after the addition of salicylic acid. Then, the hearts were subjected to global ischemia for 30 min and were reperfused for 30 min. The effluent was collected at the end of the perfusion and at 1, 2, 3, 5, 10, 15 and 30 min in the reperfusion period and stored at −80°C until assay with high performance liquid chromatography coupled with electrochemical detection (Coulochem II, MC Medical, Tokyo, Japan).

Statistical Analysis

All values are presented as the means ± SEM. The difference in hemodynamic variables over the time course between the control and the drug-treated groups was assessed by 2-way repeated measures analysis of variance. Student’s t-test was used to assess the differences between the 2 groups. Differences with p<0.05 were considered statistically significant.
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Effect of Nicorandil on Post-Ischemic Recovery of LV Function

During the pre-ischemic period, all measured parameters such as LVP, +dP/dt and coronary flow were comparable between the groups. Time course changes in LVP, ±dP/dt and coronary flow in the control, nicorandil, nicorandil + 5-HD and 5-HD groups are shown in Figs 2–4. Treatment with nicorandil improved the post-ischemic LVDP and ±dP/dt significantly better than the untreated control hearts at 15, 30, 45 and 60 min of reperfusion. Treatment with nicorandil increased the coronary flow at 15, 30, 45 and 60 min of reperfusion. Pretreatment with 5-HD abolished the improvement in LVDP and ±dP/dt, and the increase in coronary flow induced by nicorandil.

Effect of Nicorandil on 2,3-DHBA Concentration, an Indicator of Hydroxyl Radicals, in the Effluent

As shown in Fig 5, the 2,3-DHBA concentration significantly increased during reperfusion in the control group. Treatment with nicorandil significantly decreased the baseline concentration of 2,3-DHBA and significantly attenuated the rise of 2,3-DHBA concentration during reperfusion.

Chemicals Used

Five-HD was purchased from Sigma Chemical (St Louis, MO, USA). Nicorandil was generously donated by Chugai Pharmaceutical Company (Tokyo, Japan).
Pretreatment with 5-HD abolished the attenuation of the increase in 2,3-DHBA concentration during reperfusion induced by nicorandil. The 5-HD by itself did not affect the 2,3-DHBA concentrations.

Discussion

The present findings show that: (i) treatment with nicorandil significantly improved the recovery of LVDP and ±dP/dt during reperfusion after 30 min of global ischemia; (ii) the effect of nicorandil was blocked by pretreatment with a K_ATP channel blocker, 5-HD; and (iii) nicorandil significantly prevented the release of 2,3-DHBA in the effluent during reperfusion. Pretreatment with 5-HD restored the attenuation of 2,3-DHBA concentration by nicorandil.

Treatment with nicorandil significantly improved the post-ischemic myocardial dysfunction in the present study, which is consistent with results from other studies showing that nicorandil improves the post-ischemic myocardial dysfunction.12-13

First, it was clearly shown that treatment with nicorandil significantly attenuated the rise in 2,3-DHBA concentration, an indicator of hydroxyl radicals, in the effluent during reperfusion compared with the control, suggesting that nicorandil inhibited the production of hydroxyl radicals in the heart during reperfusion. It has been reported that post-ischemic myocardial dysfunction is mediated at least in part through the generation of ROS, such as superoxide anion, hydrogen peroxide and hydroxyl radical. Among these, hydroxyl radical is reported to play the most important role in post-ischemic myocardial dysfunction.4-5,13 Therefore, it might be possible that the improvement of cardiac function by nicorandil is because of the blockade of hydroxyl radical during reperfusion. Furthermore, attenuation of the concentration of 2,3-DHBA during reperfusion by nicorandil was restored by the treatment with 5-HD, a mitochondrial K_ATP channel blocker, suggesting that the opening of mitochondrial K_ATP channels reduces the production of hydroxyl radicals during reperfusion. This result is consistent with the findings by Ozcan et al16 and Ferranti et al,17 who suggested that the opening of mitochondrial K_ATP channels decreases the generation of ROS. However, Pain et al and Forbes et al7 reported that the opening of mitochondrial K_ATP channels produces the generation of ROS.6,7 Furthermore, Obata and Yamanaka reported that nicorandil increases the concentration of 2,3-DHBA in the rat myocardium and that the increased myocardial interstitial 2,3-DHBA concentration induced by 15 min ischemia was blocked by 5-HD or glibenclamide, suggesting that the blockade of cardiac mitochondrial K_ATP channels reduces hydroxyl radicals in the myocardium.18 The discrepancies between these reports and ours might be explained by the difference in the amount of ROS generated during ischemia and reperfusion. It has been reported that ischemic preconditioning, a brief episode of ischemia and reperfusion, opens the mitochondrial K_ATP channels and produces free radicals and triggers the ischemic preconditioned state.6,7,19 The amount of ROS produced by ischemic preconditioning by the short period of ischemia and reperfusion is considered to be small but that produced by prolonged ischemia and reperfusion is a larger burst of ROS.20 Therefore, it is conceivable that the opening of the mitochondrial K_ATP channels by nicorandil produces a small amount of ROS but suppresses a large amount of ROS production during reperfusion.

The baseline 2,3-DHBA concentration without ischemia was decreased in the nicorandil group. This suggests that nicorandil by itself can scavenge hydroxyl radicals. This is consistent with the previous report showing that nicorandil by itself scavenged hydroxyl radicals produced by hypoxanthine plus xanthine oxidase in the presence of iron.21 However, the decrease in 2,3-DHBA concentration in the effluent after treatment with nicorandil might be at least in part a result of the dilution of the solution induced by an increase of coronary flow. The increase of coronary flow might have increased the washout rate of hydroxyl radicals produced in the myocardium then decreased the damage by hydroxyl radicals at a local site of the myocardium.

There is still the question of whether the decrease in 2,3-DHBA by nicorandil is the cause or the result of attenuating the ischemia–reperfusion injury. To solve this, it is reasonable to examine the effect of antioxidants on post-ischemic cardiac dysfunction. However, previous studies have shown that antioxidant drugs improve post-ischemic cardiac dysfunction in isolated hearts.22,23 Therefore, because nicorandil has been reported to have many beneficial effects, such as opening the mitochondrial K_ATP channels;4-5,13 inhibiting apoptosis;4,15 inhibiting platelet aggregation;15 direct preservation of mitochondrial function;6 and coronary capillary architecture and volume;27 besides free radical scavenging effects, it is still difficult to say whether the decrease in 2,3-DHBA concentration is the main cause of the attenuation of ischemia–reperfusion injury.

Second, it is suggested that the cardioprotective effect of nicorandil is based on the opening of the mitochondrial K_ATP channels in the cardiac myocytes, because the improvement in myocardial dysfunction after global ischemia by nicorandil was completely abolished by pretreatment with a mitochondrial K_ATP channel blocker, 5-HD, in the present study. At present, it is widely accepted that opening of the mitochondrial K_ATP channels is critically important in protecting the myocardium against ischemic injury.28-30

Third, the present study shows that nicorandil significantly increased the coronary flow during reperfusion as compared with the control. The increase in coronary flow during reperfusion by nicorandil was restored by the treatment with 5-HD, a mitochondrial K_ATP channel blocker, suggesting that opening of the mitochondrial K_ATP channels is critically important in protecting the myocardium against ischemic injury.28-30

It has been reported that there is heterogeneity in the vascular response to nicorandil on coronary arteries; nicorandil behaves predominantly as a nitrate in large coronary arteries and behaves as a K_ATP channel opener in small arteries.31 Furthermore, it has been reported that a nitric oxide donor, such as isosorbide dinitrate, increases the diameter of large coronary arteries without affecting coronary blood flow at low doses and increases both coronary diameter and blood flow at higher doses.32

The increase in coronary flow by nicorandil was inhibited by the 5-HD but the curve tended to be situated at higher position in the nicorandil+5-HD group than in the control group, although not significant statistically as shown in
Fig 5. This suggests that this difference might be a result of the nitrate action of nicorandil.

Among many K<sub>ATP</sub> channel openers, nicorandil is the only K<sub>ATP</sub> channel opener that is currently used clinically, and its use has been reported to be associated with better clinical and functional outcomes in patients with acute myocardial infarction. The beneficial effect of nicorandil might be at least in part a result of its effect to decrease the production of hydroxyl radicals.

In conclusion, nicorandil is protective against post-ischemic LV dysfunction in association with opening the mitochondrial K<sub>ATP</sub> channels, decreasing hydroxyl radicals and increasing coronary flow in the isolated rat heart.

References


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