Effect of Dilazep on Myocardial Contractility Following Acute Ischemia and Reperfusion in Isolated Blood-Perfused Canine Left Ventricular Muscle

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SUMMARY

Myocardial protection by dilazep HCl, an antianginal drug and a potent calcium antagonist, against myocardial damage following acute ischemia and reperfusion was studied with respect to myocardial contractility in isolated blood-perfused canine left ventricular muscle. Myocardial function was expressed by percent recovery rate of maximal net developed tension. 1) The coronary infusion of dilazep revealed significant myocardial protection during normothermic ischemic arrest of 45 min and reperfusion. 2) The intravenous administration of dilazep to the support dog and Young's infusion also showed significant myocardial protection during normothermic ischemic arrest of 45 min and reperfusion. Dilazep showed no persistent depression of myocardial contractility due to its calcium antagonistic effect during reperfusion. 3) The combination of intravenous administration of dilazep to the support dog, Young's infusion, and hypothermia showed significant myocardial protection during prolonged ischemia and reperfusion even in hypertrophied ventricle. These results demonstrate that dilazep provides effective myocardial protection during ischemic arrest and reperfusion by preventing abnormal calcium accumulation in myocardial cells during reperfusion. No persistent depression of myocardial contractility during reperfusion may support dilazep's clinical application as a myocardial protective agent in open-heart surgery.

Additional Indexing Words:
Isolated blood-perfused canine left ventricular muscle Dilazep HCl Myocardial protection Myocardial contractility Acute ischemia and reperfusion Hypertrophied ventricle Reperfusion injury

A reperfusion injury that has been noticed in the field of myocardial protection in cardiac surgery is that the swollen mitochondria obtained from

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ischemic myocardium contain deposits of calcium phosphate and amorphous matrix densities, and particularly, many of these changes become more intense when blood flow is restored.\textsuperscript{1,2} The mechanism of the uptake of calcium and matrix density has not been established. However, it appears to be related to a defect in cellular permeability or mitochondrial function.\textsuperscript{3,4} Moreover, it is likely that the reperfusion injury may be a contributing factor in transient myocardial dysfunction.\textsuperscript{5}

It is apparent that a series of potent calcium antagonists block only calcium influx without affecting simultaneous Na\textsuperscript{+} movement which is connected with the action potential. This electromechanical dissociation leads to diminished ATP consumption by the cardiac muscle, and to diminished contractile force of the myocardium. As a result, both CP and ATP levels in the heart muscle increase, resulting in a diminution of myocardial O\textsubscript{2} demand by the heart.\textsuperscript{6-8} Therefore, it is assumed that some potent calcium antagonists favorably reduce the myocardial damage of reperfusion injuries by blocking calcium influx and increasing ATP in the myocardium.

The present studies were designed to assess the myocardial protective effect against ischemic arrest of dilazep HCl,\textsuperscript{9} recognized as an antianginal drug and a potent calcium antagonist, by investigating myocardial contractility in isolated blood perfused canine left ventricular preparation.

\textbf{MATERIALS AND METHODS}

The experimental preparation reported previously,\textsuperscript{10} which consisted of the isolated canine left ventricular muscle perfused with blood according to the technique described by Chiba,\textsuperscript{11} was used in the present studies. Mongrel dogs of both sexes were anesthetized with an intravenous administration of 30 mg/Kg of pentobarbital and heparinized with 1 mg/Kg of heparin, and the heart was removed. A part of the left ventricular muscle along the anterior descending branch of the left coronary artery was quickly excised and bathed in cold Tyrode's solution. The wet weight of the excised muscle was approximately 10 to 15 Gm. The left anterior descending artery was cannulated with a small polyethylene catheter and perfused with arterial blood led from a carotid artery of the support dog at a constant rate of 8.4 ml/min. by means of a Harvard pump. A pneumatic resistance was placed in parallel with the perfusion system to obtain a constant pressure of 100 mmHg. The actual coronary flow measured from the blood leaving the isolated ventricular muscle ranged from 7.1 to 8.3 ml/min with an average of 7.6±0.3. The isolated blood-perfused left ventricular muscle was suspended in a cupshaped glass container, which was maintained at a constant temperature of 37°C, with the 2 parts of the muscle fixed to a stainless steel bar. The upper part of the isolated ventricular muscle was attached by a silk thread to a force-displacement transducer (Nihon Kohden SB-1TA). The volume of the container was 65 ml and was initially filled with warmed Tyrode's solution, which was gradually displaced by the
blood leaving the isolated ventricular muscle. Two streams of blood, over-flowing from the container and passing through the pneumatic resistance, were collected in a blood reservoir and then returned to the support dog through a jugular vein. The muscle was electrically paced (3 mA) at a rate of 70/min. The muscle was loaded with a resting tension of 2 Gm. The isometric tension and the rate of rise of tension development (dT/dt) were continuously recorded on an ink-writing rectigraph (Nihon Kohden WI-680G) through a carrier amplifier (Nihon Kohden AP-600G) and an electronic differentiator (Nihon Kohden ED-600G). The diagram of the blood-perfused system of the left ventricular canine myocardium is shown in Fig. 1.

After the ischemic arrest designed in the experiments the isolated ventricular muscle was reperfused with arterial blood from the support dog until the myocardial contractility fully recovered at the myocardial temperature of 37°C. The maximal net developed tension and dT/dt at this point were referred to as a percent recovery rate in comparison with those before ischemic arrest.

In the present studies, 3 serial experiments were designed. Experiment I, which was performed to investigate the effect of dilazep infused directly into coronary artery on the ischemic ventricular muscle, consisted of 4 groups. In Group I, the control, coronary perfusion was stopped and the isolated ventricular muscle was bathed in Tyrode's solution for a period of 45 min at 37°C in 5 dogs; in Group II the muscle was bathed in Tyrode's solution at 30°C in 5 dogs. The myocardial temperature rapidly equilibrated with the temperature of the Tyrode's solution in which the muscle was bathed. In Group III Young's solution (0.81% potassium citrate, 2.46% magnesium sulfate, and 0.001% prostigmine) adjusted to pH 7.8 with sodium bicarbonate which was usually used to obtain rapid

Fig. 1. Schematic presentation of the isolated canine left ventricular muscle perfused with the blood introduced from a carotid artery of support dog.
arrest after aortic cross-clamp in clinical use was infused for a period of 2 min at a rate of 8.4 ml/min immediately after the acute ischemia which was maintained for 45 min at the myocardial temperature of 37°C in 4 dogs. In Group IV 0.03 mg/Kg of dilazep dissolved in 15 ml saline was infused under the same condition as that of Group III in 5 dogs.

Experiment II was designed to investigate the effect of dilazep administered intravenously to the support dog, comparing the effects of direct infusion into coronary artery and intravenous administration of dilazep in relevance to the clinical use. In the experiment 0.3 mg/Kg of dilazep was administered intravenously to the support dog 15 min prior to the acute ischemia and 5 to 7 ml of Young’s solution was infused into coronary artery to produce acute arrest immediately after the acute ischemia which was maintained for 45 min at the myocardial temperature of 37°C in 4 dogs.

Experiment III, which was designed to investigate the effect of intravenous administration of dilazep and hypothermia on the ischemic ventricular muscle in hypertrophied ventricle, consisted of 2 groups. In Group I coronary perfusion was stopped and the isolated hypertrophied canine left ventricular muscle was bathed in Tyrode’s solution for a period of 150 min at the myocardial temperature of 17°C in 9 left ventricular hypertrophied dogs. In Group II 0.3 mg/Kg of dilazep was administered intravenously to the support dog 15 min prior to the acute ischemia and Young’s solution was infused immediately after the acute ischemia which was maintained for 150 min at the myocardial temperature of 17°C in 4 isolated hypertrophied canine left ventricular muscles. Left ventricular hypertrophy was produced by means of creating supravalvular aortic stenosis (AS) by banding 200±10 days prior to study. The systolic pressure gradient obtained in left ventricular hypertrophied dogs was approximately 50 mmHg. Myocardial calcium content was measured by atomic absorption spectrometry. A statistical analysis was performed by means of Student’s t-test with p<0.05 as the level of significance. The values experimentally obtained were expressed as mean±SD.

Results

Experiment I: The results are summarized in Fig. 2.

Group I (control group): (Normothermic ischemic arrest of 45 min) The percent recovery rates of the maximal net developed tension were 70±5 (mean±SD). A rectigram obtained from a preparation in Group I is shown in Fig. 3.

Group II: (Ischemic arrest of 45 min at the myocardial temperature of 30°C) The percent recovery rates of the maximal net developed tension were 100±3, indicating a significant increase (p<0.01) compared with those of Group I.

Group III: (Young’s infusion and normothermic ischemic arrest of 45 min) The percent recovery rates of the maximal net developed tension were 70±3, indicating statistically no significance compared with those of the control group (Group I). The muscle became flaccid promptly after the
Fig. 2. Effect of coronary infusion of Young's solution and dilazep on myocardial contractility after normothermic ischemic arrest of 45 min and reperfusion.

Fig. 3. A rectigram of maximal net developed tension and dT/dt before and after normothermic ischemic arrest of 45 min in isolated blood-perfused canine left ventricular muscle. The percent recovery rates of maximal net developed tension and dT/dt are 70 in this case.

infusion of Young's solution and did not contract or fibrillate thereafter.

Group IV: (Dilazep infusion and normothermic ischemic arrest of 45 min) The percent recovery rates of the maximal net developed tension were 100±4, indicating a significant increase (p<0.01) compared with those of the control group. The muscle behaved like those in Young's infusion after the infusion of Dilazep.
Experiment II: Experiment II was designed to investigate the effect of dilazep administered intravenously on the ischemic heart, however, as shown in Fig. 4, when dilazep was administered intravenously to the support dog 15 min prior to acute ischemia myocardial contraction was maintained for approximately 10 min after the acute ischemia. Consequently, the percent recovery rate of maximal net developed tension was approximately 40. Therefore, Young's infusion into the coronary perfusion route, as shown in Group III of Experiment I, to produce acute arrest was added. The percent recovery rates of the maximal net developed tension were 100±3, indicating a significant increase (p<0.01) compared with those of Group I (control group) and Group III (Young's infusion) of Experiment I, and the same percent recovery rate as that of Group IV of Experiment I.

Changes of myocardial calcium content before and after normothermic ischemic arrest of 45 min are shown in Fig. 5. In the control group (Group I) of Experiment I in which acute ischemia was maintained for 45 min at 37°C myocardial calcium content significantly increased after reperfusion, while in Experiment II myocardial calcium content showed no significant change after reperfusion.

During reperfusion after ischemia the calcium antagonistic effect was investigated by means of a frequency-force-relationship. As shown in Fig. 6, a positive staircase phenomenon was found, suggesting washout of dilazep from myocardium during reperfusion.

![Fig. 4. Effect of intravenous administration of dilazep to the support dog without Young cardioplegia on myocardial contractility after acute ischemia. Myocardial contraction maintains for approximately 10 min after normothermic ischemic arrest.](image-url)
Fig. 5. Myocardial calcium contents before ischemia and at the end of reperfusion after normothermic ischemic arrest of 45 min in control group and in the group of intravenous administration of dilazep with Young cardioplegia.

Fig. 6. Effect of intravenous administration of Dilazep (0.3 mg/Kg) and Young cardioplegia on Frequency-Force-Relation during reperfusion.

**Experiment III:** The results of Groups I and II are summarized in Fig. 7.

Group I (control group): (Ischemic arrest of 150 min at the myocardial temperature of 17°C in hypertrophied left ventricular muscle) The percent recovery rates of the net developed tension were 70±5.

Group II: (Intravenous administration of dilazep, Young’s infusion and
ischemic arrest of 150 min at the myocardial temperature of 17°C in hypertrophied left ventricular muscle) The percent recovery rates of the maximal net developed tension were 92±10, indicating a significant increase (p<0.05) compared with those of Group I. Throughout Experiments I, II, and III the percent recovery rates of the dT/dt showed the same tendency as those of the maximal net developed tension.

**DISCUSSION**

The protective effects of topical cardiac cooling against myocardial damage during ischemic cardiac arrest in cardiac surgery advocated by Schumway and his associates are already well recognized. Recently, various potassium-based cardioplegic solutions which were first advocated by Melrose and Young have been assessed experimentally with respect to myocardial protection and several of these cardioplegic solutions have been used clinically with topical cardiac cooling or with perfusion cooling. Moreover, metabolic arrest with procaine, hypocalcemia, magnesium, fluoride, adrenochrome, and tetrodotoxin or metabolic myocardial protection with allopurinol and high-energy solutions for myocardial protection during ischemic myocardium have been investigated. On the other hand, it has been noted that reperfusion injuries may play an important role in the development of myocardial damage after ischemic arrest. Recent studies have shown a rapid increase in intracellular calcium ion concentration associated with a reduction in mitochondrial metabolism. Therefore, besides the protection against myocardial damage during ischemic arrest, the
prevention of reperfusion injuries is considered to be an important problem for myocardial protection in cardiac surgery.

Generally, it is recognized that an abnormality of the process of excitation-contraction coupling has important effects on tension produced by the heart muscle even in the failing heart. Calcium ions are the link between excitation and contraction. There are many possibilities in which myocardial failure could interfere with the excitation-contraction coupling. It is clear that the complexities of the calcium binding and release mechanism of the sarcoplasmic reticulum may play an important role in myocardial failure. It has been shown that some calcium antagonists (diltiazem and verapamil) favorably reduce myocardial damage after regional myocardial ischemia resulting from ligation of the left descending coronary artery of dogs and global ischemia. Recently, it was demonstrated that nifedipine in a cardioplegic dose (100 \mu g) results in preservation of myocardial structure and function, that is similar to that obtained with potassium cardioplegia. In the present studies, a potent calcium antagonistic drug (dilazep HCl) which was recognized as a new type of antianginal drug was evaluated in its effect on preventing reperfusion injuries after global ischemia by blocking calcium influx into myocardial cells.

Dilazep (1,4-bis-[3-(3,4,5-trimethoxy benzoyl-oxy)-prophyl]-perhydro-1,4-diazepin), which is a derivative of trimethoxy benzoic acid, is a new antianginal drug investigated by Lenke. It has been found to increase coronary flow due to the potentiating effect of adenosine in doses which had almost no effect on systemic arterial pressure or heart rate. In further investigations, it was demonstrated that dilazep had a potent calcium antagonistic effect and a preserving effect on ATP and CP in the heart muscle during hypoxic condition. Moreover, it has been reported that hexobendine, which is a derivative of trimethoxy benzoic acid, protects isolated rabbit atria against the decrease of contractility following anoxia and increases the tolerance to anoxia by about 50% over controls in the doses which do not influence the mechanical properties of the heart. In the present studies, dilazep infusion into the coronary route (Experiment I) has showed a myocardial protecting effect against myocardial damage following normothermic ischemic arrest of 45 min with a rapid arrest by the investigation of myocardial contractility in which it is recognized that the contractility of the ischemic heart declines before biochemical and morphological changes in mitochondria can be detected by presently available techniques. This effect was similar to that of myocardial cooling at 30°C with ischemic period of 45 min. While, Young's infusion which was usually used to obtain acute arrest after aortic cross-clamp in cardiac surgery showed no myocardial protection in the
condition of normothermic ischemic arrest of 45 min. It has been reported
that approximately 10 µg of dilazep caused a 50% increase of coronary blood
flow in normal isolated guinea pig heart. Moreover, Kukovetz reported
that hexobendine, in a dose of 130 µg, increased the tolerance to anoxia in
heart-lung preparation of the rat. Therefore, in the Experiment I 0.03
mg/Kg of dilazep as a test dose which corresponded to one tenth of intra-
venous dose (0.3 mg/Kg) was infused into coronary artery after occlusion of
coronary route. The direct infusion of dilazep in a dose of 0.03 mg/Kg was
followed by a rapid arrest of myocardial contraction. In the Experiment I,
it is assumed that calcium antagonistic effect of dilazep producing a rapid
arrest of myocardial contraction may play an important role for myocardial
protection.

In the intravenous administration of dilazep to the support dog 15 min
prior to acute ischemia produced by Young's infusion (Experiment II), which
was designed to investigate the effect of intravenous administration of dilazep
in relevance to the clinical use, it is thought that dilazep circulates into the
blood stream of support dog and is metabolized in the support dog, and
reaches to the cannulated blood-perfused ventricular muscle preparation of
of recipient dog by 2 min after the intravenous administration of dilazep to
the support dog. Therefore, the ventricular muscle preparation of recipient
dog is perfused by the blood of support dog with dilazep for about 12 min
until acute ischemia without the weakness of myocardial contraction or the
arrest. Dilazep showed as much myocardial protecting effect against myo-
cardial damage following normothermic ischemic arrest of 45 min and re-
perfusion as did the direct infusion into coronary route of dilazep. Young's
infusion was introduced to obtain a rapid arrest because in the intravenous
administration of dilazep to the support dog myocardial contractility was
maintained for about 10 min after occlusion of coronary route. In the
Experiment I Young's infusion showed no myocardial protection in normo-
thermic ischemic arrest of 45 min. Therefore, it is assumed that the myocar-
dial protecting effect in the Experiment II is attributable to the intravenous
administration of dilazep itself. Moreover, in relevance to the clinical ap-
lication the intravenous administration of dilazep is more reasonable, safe
method than the coronary infusion for myocardial protection. It has been
reported that in anesthetized closed-chest dogs the intravenous dose for opti-
mal coronary vasodilation was from 0.1 to 0.15 mg/Kg. Moreover, in the
patients optimal coronary vasodilation was induced by dose of 0.2 mg/Kg. Therefore, it is assumed that 0.3 mg/Kg of dilazep is optimal in the intra-
venous administration to the support dog on the present experimental pre-
paration. Consequently, it is assumed that approximately 0.04 mg of dilazep
is administered to the blood-perfused ventricular muscle preparation of recipient dog during 15 min prior to acute ischemia. It has been recognized that dilazep shows a potent calcium antagonistic effect. However, it is likely that the effect is weakened enough not to influence myocardial contractility by washing out during reperfusion. It has been shown that the administration of calcium antagonist (diltiazem) to dogs with regional myocardial ischemia results in a reduction of the decline of ATP in the ischemic region, lessens the inhibition of anaerobic glycolysis, lowers the tissue level of lactic acid, and improves contractility of heart muscle. In the present studies (Experiment II), the administration of dilazep decreased myocardial calcium ion content by the end of reperfusion, suggesting the prevention of abnormal calcium accumulation in the myocardial cells after reperfusion. Moreover, it has been demonstrated that the administration of dilazep maintains myocardial contraction for approximately 10 min after acute ischemia, suggesting a reduction of the decline of ATP in ischemic myocardium. Therefore, it is assumed that the myocardial protecting effect of dilazep against myocardial damage following acute ischemia may be not only due to a potent calcium antagonistic effect, but also due to a preserving effect of ATP in the ischemic myocardium.

Recent studies have shown that the hypertrophied ventricle is more vulnerable to ischemic damage than is the normal ventricle. Previously, our experiment demonstrated that hypothermia alone is not sufficient to protect against myocardial damage following acute ischemia in hypertrophied ventricle compared with that in non-hypertrophied ventricle because the percent recovery rate of net developed tension following an ischemic period of 150 min at the myocardial temperature of 17°C was 90±9% in non-hypertrophied ventricle and it was 70±19% in hypertrophied ventricle. While, it is indicated that hypothermic potassium cardioplegia effectively attenuates metabolic injury resulting from 1 hour of global ischemia in hypertrophied myocardium. It is found that the intravenous administration of dilazep shows a myocardial protecting effect, however such experimental condition as normothermic ischemic period of 45 min is a severe experimental condition for screening drugs. Practically, the effect of myocardial protection is usually evaluated under the condition of hypothermic ischemic period of 180 min, because the clinical target for myocardial protection is aimed to prevent myocardial damage produced by ischemic period of 180 min. However, in the present experimental preparation such condition as hypothermic ischemic period of 180 min is so much severe as percent recovery rate of developed tension shows only 60% in non-hypertrophied ventricle. Therefore, in the present studies the hypothermic ischemic period of 150 min
is chosen for the experimental condition to evaluate myocardial protection. In the problems of optimal myocardial temperature, it is already accepted that myocardial temperature of 17°C is clinically optimal for myocardial protection. In the present studies (Experiment III), it has been demonstrated that the combination of the intravenous administration of dilazep (0.3 mg/Kg) to the support dog, Young’s solution, and hypothermia provide useful myocardial protection during prolonged acute ischemia up to 150 min and reperfusion even in hypertrophied ventricle. It is likely that the intravenous administration of dilazep reveals an additional effect on myocardial protection produced by hypothermia and Young’s solution, suggesting a clinical application as myocardial protection in open-heart surgery.

REFERENCES


