BENEFICIAL EFFECT OF THE ADDITION OF NITROGLYCERIN TO THE CARDIOPLEGIC SOLUTION ON THE COLD-STORRED REPERFUSED ISOLATED RAT HEART

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We discuss here the effect of the addition of trinitroglycerin to the cardioplegic solution on the isolated rat heart after eight hours of storage. This effect was evaluated by measuring cardiac function as well as myocardial calcium and enzymes. Male Wistar rats were divided into three groups based on the concentration of trinitroglycerine in the cardioplegic solution. In the control group, the fluid used was a crystalloid cardioplegic solution (K+ 25 mEq/L) without trinitroglycerin and for groups I and II, trinitroglycerin was added at concentrations of 2 µg/ml and 5 µg/ml, respectively. All hearts were arrested with the cardioplegic solution at 4°C and then immersed for eight hours in Euro-Collins' solution at 4°C. The postperfusion coronary flow showed higher rates for groups I and II than for the control group (p<0.05), while the difference between groups I and II was not significant. In group I, the total concentrations of creatine kinase-MB, lactate and malondialdehyde after reperfusion showed the lowest levels; this group also had the lowest content of myocardial calcium. These results indicate that the addition of nitroglycerin, especially at a concentration of 2 µg/ml, to the cardioplegic solution elicits better cardiac function for immersed rat heart.

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THE Pharmacologic effect of trinitroglycerin (TNG) on the coronary artery is due not only to the dilatation of the artery but also to the decrease in cardiac afterload1-4 Moreover, TNG has been reported to be an effective coronary vasodilator when used as an adjunct in hypothermic hyperkalemic cardioplegia5-7 In this study, we evaluate the effect of the addition of TNG to a crystalloid cardioplegic solution on the isolated donor heart by focusing on cardiac function and myocardial enzymes and calcium.

MATERIALS AND METHODS
Male Wistar rats weighing 250 to 350 gms were employed. Heparin at a concentration of 1000 units/kg was injected intravenously under ether inhalation anesthesia. The heart was then rapidly isolated and immersed into saline at 4°C To establish the prearrest cardiac function, the aortic and coronary flows for one min were measured against those obtained for an afterload of 80 cmH2O after a working mode of 10 min8 Next, 20 ml/kg body weight of a crystalloid cardioplegic solution (Table I) at 4°C was infused via

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TABLE I COMPOSITION OF THE SOLUTIONS

<table>
<thead>
<tr>
<th>Component (mmol/L)</th>
<th>Cardioplegic solution</th>
<th>Krebs-Henseleit bicarbonate buffer solution</th>
<th>Euro-Collins solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>100</td>
<td>118</td>
<td>—</td>
</tr>
<tr>
<td>KCl</td>
<td>25</td>
<td>4.7</td>
<td>13.3</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>1.0</td>
<td>2.0</td>
<td>—</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>1.0</td>
<td>1.2</td>
<td>—</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>16.7</td>
<td>25</td>
<td>10.0</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>—</td>
<td>1.2</td>
<td>15.1</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>—</td>
<td>—</td>
<td>42.5</td>
</tr>
<tr>
<td>Ca-EDTA</td>
<td>—</td>
<td>0.5</td>
<td>—</td>
</tr>
<tr>
<td>Glucose</td>
<td>—</td>
<td>11</td>
<td>—</td>
</tr>
<tr>
<td>Mannitol</td>
<td>87.9</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

| Osmolarity        | 350 mOsm/L            | 320 mOsm/L                                | 355 mOsm/L            |
| PH                | 7.8 (37°C)            | 7.4 (37°C)                                | 7.2 ~ 7.5             |

TABLE II PERCENTAGE RECOVERY OF CARDIAC FUNCTION AFTER 8 H STORAGE

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Heart rate</th>
<th>Aortic Flow</th>
<th>Coronary Flow</th>
<th>Cardiac output</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>10</td>
<td>96.2 ± 4.1</td>
<td>[−38.2 ± 7.1</td>
<td>a -55.6 ± 6.7</td>
<td>a -43.3 ± 11.0</td>
</tr>
<tr>
<td>I</td>
<td>10</td>
<td>96.8 ± 3.4</td>
<td>[−50.3 ± 6.4</td>
<td>a -78.1 ± 7.5</td>
<td>a -55.9 ± 12.8</td>
</tr>
<tr>
<td>II</td>
<td>10</td>
<td>97.1 ± 3.7</td>
<td>43.1 ± 10.6</td>
<td>76.4 ± 8.7</td>
<td>50.8 ± 15.5</td>
</tr>
</tbody>
</table>

Control rat hearts were given a crystalloid cardioplegic solution free of trinitroglycerin, while group I and II hearts received the fluid added with trinitroglycerin at 2 and 5 μg/ml, respectively. Cardiac function was measured against the afterload of 80 cm H₂O after 45 min in the working mode (60 min after reperfusion).

a: p<0.05.

aortic cannulation. All hearts were immersed for eight hours in the preservation solution (Euro-Collins, Green Cross Corp., Osaka, Japan) (Table I) at 4°C and washed out after preservation with 20 ml/kg of cardioplegic solution at 4°C via the aortic root after preservation.

The hearts under study were divided into three groups according to the concentration of TNG (Nippon Kayaku Corp., Tokyo, Japan) in the crystalloid cardioplegic solution. For the control group, the fluid consisted of the cardioplegic solution containing 25 mEq/L of potassium, while for groups I and II, TNG at respective concentrations of 2 μg/ml and 5 μg/ml was added to the cardioplegic solution.

The heart was then transferred to the perfusion system to measure cardiac function. The Langendorff system was employed to perfuse the heart with a Krebs-Henseleit bicarbonate buffer solution (Table I) at 37°C through the ascending aorta for 15 min under a pressure of 80 cm H₂O. The aortic or coronary flow per minute was measured against the afterload of 80 cm H₂O after 45 min in the working mode (60 min after reperfusion).

Fifteen minutes after termination of the cardiac working mode (30 min after reperfusion), the levels of creatine kinase (CK)-MB isoenzyme, lactate and malondialdehyde (MDA) in the perfusates from the right atrium and the pulmonary artery were measured. Adenosine triphosphate (ATP) was measured immediately after storage and

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TABLE III THE TOTAL CONCENTRATION OF CREATINE KINASE-MB ISOENZYME, LACTATE AND MALONDIALDEHYDE IN CORONARY EFFLUENT

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>CK-MB (U/L/g dry weight)</th>
<th>Lactate (mg/dl/g dry weight)</th>
<th>MDA (nmol/ml/g dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>15</td>
<td>18.3±6.1</td>
<td>-22.8±7.3</td>
<td>-1.68±0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>a</td>
<td>a</td>
<td>a</td>
</tr>
<tr>
<td>I</td>
<td>15</td>
<td>11.3±4.2</td>
<td>-13.6±4.5</td>
<td>-0.72±0.15</td>
</tr>
<tr>
<td>II</td>
<td>15</td>
<td>15.0±6.3</td>
<td>18.1±5.7</td>
<td>1.24±0.20</td>
</tr>
</tbody>
</table>

Fifteen minutes after termination of the cardiac working mode, the levels of creatine kinase-MB, lactate and malondialdehyde were measured in the perfusates from the right atrium and the pulmonary artery.

TABLE IV MYOCARDIAL ADENOSINE TRIPHOSPHATE, Ca²⁺ AND WATER CONTENT

<table>
<thead>
<tr>
<th>Group</th>
<th>ATP (μmol/g dry weight)*</th>
<th>Ca²⁺ (μmol/g dry weight)**</th>
<th>Water content (%)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>17.3±2.1</td>
<td>9.6±3.4</td>
<td>78.1±2.3</td>
</tr>
<tr>
<td></td>
<td>a</td>
<td>a</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>17.2±4.3</td>
<td>-3.8±2.8</td>
<td>78.0±3.1</td>
</tr>
<tr>
<td>II</td>
<td>16.3±4.9</td>
<td>4.0±2.3</td>
<td>78.3±2.4</td>
</tr>
</tbody>
</table>

Adenosine triphosphate (ATP) was measured immediately after storage and the sample for measurement of myocardial calcium and water content was taken at the end of this experiments.

*a: p<0.05.
**n=8 in all of the groups.

The aortic pressure and the heart rate obtained through the aortic cannula were recorded via a pressure transducer (Life Scope 12, Thermal Array Recorder, Nippon Koden Corp.). The aortic and coronary flow per min was measured and the sum was calculated to represent the cardiac output; the indices of cardiac function were expressed as percentages of the individual prearrest control values.

A myocardial biopsy was performed after cold storage. The specimens were rapidly frozen in liquid nitrogen and treated with perchloric acid. High performance liquid chromatography was then used to determine the level of ATP in the preparation. The CK-MB activity was measured with the chemiluminescent immunoassay, lactate with the ultra-violet method, and MDA, which was reacted with thiobarbituric acid (TBA), was measured as a lipperoxide value. The measurement of MDA was performed as follows. First, 0.1 ml of sample was put into 1.0 ml of saline. After centrifugation at 4000 rpm for 10 min, the supernatant was transferred and mixed with 4.0 ml of N/12 H₂SO₄. Next, 0.5 ml of 10% phosphotungstic acid was added, followed by centrifugation. The free supernatant was mixed with N/12 N₂H₂SO₄ and the mixture centrifuged again. Thiobarbituric acid was then added to this sediment and heated. After cooling with water, 5.0 ml of n-butanol was added. After centrifugation, the n-butanol layer was used for fluorometric measurement, which was performed at 515 nm excitation and 553 nm emission. Taking the fluorescence intensity of the standard solution (F), which was obtained by reacting 0.5 nmol of tetraethoxypropane with TBA, and that of the sample as f, the lipperoxide concentration (Lp) could be expressed in terms of malondialdehyde. Lp was calculated as following;
$L_p = 0.5 \times t/F \times 1.0/0.02$.

The level of myocardial calcium was measured according to the methods described by Alto and others. Briefly, the coronary vasculature was flushed through with 10 ml of sucrose solution. After flushing, the heart was removed from the perfusion apparatus. The specimens were dried with HNO$_3$ for 12 h at 105°C and extracted with HNO$_3$ for 12 h at 60°C. The supernatant was analyzed with a polarized Zeeman atomic Absorption Spectrophotometer (Hitachi 180-60, Japan). The water content in the myocardium was measured and calculated with the following equation for the heart dried at 80°C for 48 h:

$$\text{Water content (\%) = (Wet weight - Dry weight) / Wet weight} \times 100$$

All results were expressed as the mean ± standard deviation. The data were evaluated by analysis of variance and the unpaired Student $t$ test. Statistical significance was assumed when the $p$ value was less than 0.05.

RESULTS

(1) Cardiac function (Table II)

The heart rate was similar for all three groups. The aortic flow rate for group I was superior to that for the control group and group II. The coronary flow for groups I and II increased more than for the control group ($p < 0.05$), although the difference between groups I and II was not significant. Finally, cardiac output for group I was greater than for the other groups.

(2) CK-MB, lactate and MDA in the coronary effluent (Table III)

The levels of CK-MB and lactate were the lowest for group I. The level of MDA was also the lowest for group I, indicating a significant difference between the control group and the group I ($p < 0.05$).

(3) Myocardial ATP content, calcium and water contents (Table IV)

The myocardial ATP content examined immediately after storage showed no significant differences in all groups. The level of myocardial calcium was the lowest for group I, and showed a significant difference from the control group ($p < 0.05$). Water content showed no significant differences in all groups.

DISCUSSION

TNG has been used for the treatment of ischemic heart disease and systemic hypertension through the mechanism of coronary vasodilation and reduction in systemic venous pressure!–3 However, its precise mechanism has not yet been defined. Itoh et al!4,15 reported that TNG increased the production of cyclic GNP in the porcine coronary artery without producing any change in the amount of cyclic AMP. The main effects of cyclic GNP involve an activation of calcium extrusion and a reduction in the amount of calcium stored in the cells!6–20 Consequently, TNG was found to reduce the free calcium in the myocardium and to promote muscular relaxation.

In this report, the effectiveness of the use of TNG in the cardioplegic solution before and after immersed preservation of isolated rat heart is discussed. The addition of 2 µg/ml of TNG produced a better cardiac function than did the TNG-free solution, while 5 µg/ml of TNG was found less effective. The solution with TNG added resulted in an increase in coronary flow and a marked decrease in the MDA as a lipoperoxidation product and myocardial contents of calcium.

These results suggest that TNG may offer isolated hearts protection against injury during poststorage reperfusion and that its action mechanism may involve the regulation of calcium ions in the myocardium. Our study did not clarify the relationship between the concentration of myocardial calcium and that of thiobarbituric acid (TBA) reactive substance (MDA) generated from lipoperoxide. Moreover, the discrepancy in the preventive efficacy of TNG concentrations of 2 µg/ml and of 5 µg/ml remains to be resolved. Five rats had been used and the cardiac function did not recover 60 min after reperfusion using the solution (Krebs-Henseleit bicarbonate buffer solution) with 2 µg/ml TNG, and the hearts seemed to be edematous. In this study, we used TNG in the cardioplegic solution but not in the reperfusion solution; there was no significant difference in the result of water content between each group. However, further study, such as the change of coronary vascular permeability by the cardioplegic solution with different concentrations of TNG or morpho-
logical change, may be required.

In conclusion, the effect of TNG in terms of a reduction in intramyocardial calcium might thus protect the myocardial membrane from damage and result in minimization of damage otherwise brought about by lipoperoxide, provided TNG is added to the cardioplegic solution. The effects of TNG on calcium movement and production of lipoperoxide during reperfusion following heart storage deserve more detailed studies.

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REFERENCES