EXPERIMENTAL COMPARISON OF THE EFFICACY OF VARIOUS
AGENTS ON THE ENHANCEMENT OF MYOCARDIAL PROTECTION

KEICHIRO KATSUMOTO, M.D. AND TADASHI INOUE, M.D.

Experimental studies were carried out to compare the efficacy of various agents such as calcium antagonists, phosphodiesterase inhibitors, adrenocorticosteroids, coenzyme Q₁₀ (CoQ₁₀), insulin, beta-blocking agent and reduced glutathion (GSH) on the enhancement of myocardial protection. Eighty-five isolated rabbit hearts were subjected to 2 hours of cardioplegic arrest, and maximum developed tension, heart rate, coronary blood flow and coronary arteriovenous oxygen difference following reperfusion were compared between groups pretreated with different agents. The greatest value of maximum developed tension was obtained in the verapamil-treated group (0.2–0.5 mg/kg), followed by dilazep (1 mg/kg), pentoxifylline (30 mg/kg) and CoQ₁₀ (10 mg/kg) treated groups. The time required for the recoveries of spontaneous beating (normal sinus rhythm) on reperfusion was shortest (44 ± 8 sec) in the group treated with a cardioplegic solution containing a low concentration of betamethasone (0.03–0.05 mg/ml), but the so-called stone hearts and cardiac arrhythmias were most frequently seen in this group. On the contrary, in the group pretreated with calcium antagonists, the time required for the restoration of sinus rhythm was much longer (88–180 sec). Hence, the shortest recovery time was not necessarily associated with a better recovery of myocardial function.

It is of utmost importance to obtain a state of cardiac standstill at the time of direct surgical interventions to the heart. Although clamping of the aorta (simple anoxic clamping) is perhaps the easiest method to produce an anoxic arrest, it takes several minutes to achieve a complete standstill, and the period of anoxia must be terminated before an irreversible myocardial damage supervenes. In 1959, Shumway¹ stressed the necessity of lowering myocardial temperature and proposed a topical cooling method to prolong the safety limit of simple anoxic clamping. Subsequently, attention was focussed on pharmacological cardiac arrest, so immediate induction of standstill with chemical agents was considered more beneficial in sparing the energy consumed in electromechanical activities of the heart. Thus, potassium arrest has prevailed since the studies of Gay² in 1973 and Tyres³ in 1975.

It is generally believed that 2 phenomena would take place in these procedures, i.e., 1) an ischemic contracture which occurs during the ischemic period and 2) a reperfusion injury occurring after the restoration of coronary flow to the ischemic myocardium. The purpose of the present study is to find chemical agents that would most satisfactorily prevent these 2

Key Words:
Myocardial protection
Cardioplegia
Verapamil
Calcium antagonist

(Received October 9, 1980; accepted October 15, 1982)
Department of Thoracic and Cardiovascular Surgery, Keio University, School of Medicine, Tokyo, Japan
The outline of this paper was presented at the 43rd Annual Scientific Session of the Japanese Circulation Society in Tokyo, April 5, 1979.
Present mailing address: Keiichiro Katsumoto, M.D., Saitama National Hospital, 2-1 Suwa, Wako City, Saitama 351, Japan

Japanese Circulation Journal Vol. 47, March 1983
undesirable phenomena. Selection of the agents was based on the following theoretical considerations: 1) energy sparing, negative inotropic agents would avoid energy utilization during ischemia; 2) calcium antagonists would prevent myocardial contracture and 3) adrenocorticosteroids could exert membrane stabilizing effects.

MATERIALS AND METHODS

Eighty-five albino rabbits were used in the experiment. The animals were anesthetized with an intravenous injection of 50 mg of sodium pentobarbital per kg of body weight, and the right femoral vein and artery were inserted with 21G Teflon cannulae. Sodium heparin (300 units/kg) was used as an anticoagulant. A number of agents were given to the animals as pretreatment, or before the induction of cardioplegic arrest, and their efficacy in myocardial preservation was compared. Three calcium antagonists were treated, i.e., verapamil (0.2–0.5 mg/kg), dilazep (1 mg/kg) and nifedipine (30–50 µg/kg in one group and 10–20 µg/kg in the other). Other agents in this pretreated group included pentoxifylline (30 mg/kg), coenzyme Q_{10} (CoQ_{10}) (10 mg/kg), CoQ_{10} solvent (1 ml/kg), propranolol (0.1 mg/kg), betamethasone (4 mg/kg) and reduced glutathion (GSH, 1.0 g/kg). All these agents were injected intravenously 3–5 min before the induction of cardioplegic arrest. Another group of agents were administered not as a pretreatment, but at the time of induction of standstill, being mixed with the cardioplegic solution. They included insulin (0.016 units/ml of cardioplegic solution), betamethasone either in high (0.11–0.21 mg/ml solution) or low (0.03–0.05 mg/ml) concentration, aminophylline (0.5–1.67 mg/ml) and GSH (4–20 mg/ml). After pretreatment with one of the above-mentioned agents, arterial blood was withdrawn through the cannula inserted into the femoral artery, whereas the same amount of Ringer’s solution was simultaneously infused into the femoral vein to maintain the circulating blood volume constant. When 100-120 ml of blood was withdrawn, a median sternotomy was made and the heart was quickly excised within 30 sec. Twenty ml of a cooled (14°C) cardioplegic solution was then infused via the excised stump of the aortic root. The cardioplegic solution was prepared by adding 1.5 mEq of KCl and 500 units of heparin to 150 ml of Solita T-2®, with a final composition of 30 mEq/L of K, 84 mEq/L of Na, 76 mEq/L of Cl, 20 mEq/L of phosphate and 3.2 g/dl of glucose. Its osmolarity was 396 mOsm/L, and its pH about 6.0.

The infusion was immediately followed by a complete cardiac standstill, and the heart was immersed in cold Ringer’s solution (14°C). During 2 hours of cardioplegic arrest, 20 ml of the same cardioplegic solution was infused into the aortic root every 20 min (rate of infusion: 0.25 ml/sec). With a wide left atriotomy, the mitral leaflets were detached from the annulus. A 6–0 Nylon thread was tied to the tip of the chordae tendineae of the anterior papillary muscle, and a 4–0 Nylon thread was tied to the apex of the heart, so that the anterior papillary muscle was suspended between a fixed point of the reservoir and a force-displacement transducer. After 2 hours of cardioplegic arrest, the heart was reperfused with autologous blood (37°C) at a flow rate of 50 ml/min. The reperfusion was carried out using a roller pump, a polyvinyl tube of 3.5 mm internal diameter, a heat exchanger, and an overflow circuit to prevent an excessive rise of the perfusion pressure (Fig. 1). The perfusion pressure was initially kept relatively low at 20–30 mmHg, by adjusting the height of the overflow outlet, and then was gradually increased over a period of 5 min to attain a final level of 80–90 mmHg.

The time from the start of reperfusion to the restoration of spontaneous cardiac rhythm was measured. Thirty min after the beginning of reperfusion, one ml of a 2% CaCl₂ solution was
<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Body weight (kg)</th>
<th>Restoration of beating* (sec)</th>
<th>Heart rate (beats/min)</th>
<th>Maximal developed tension (g)</th>
<th>p-value</th>
<th>Coronary blood flow (ml/min)</th>
<th>Coronary A-V O2 difference (% saturation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control</td>
<td>9</td>
<td>3.2 ± 0.1</td>
<td>121 ± 13</td>
<td>149 ± 23</td>
<td>0.8 ± 0.2</td>
<td>4.6 ± 1.4</td>
<td>7.0 ± 1.1</td>
<td></td>
</tr>
<tr>
<td>2. Verapamil (0.2-0.5 mg/kg)</td>
<td>9</td>
<td>3.3 ± 0.2</td>
<td>135 ± 19</td>
<td>193 ± 9</td>
<td>9.4 ± 1.1</td>
<td>p &lt; 0.001</td>
<td>13.7 ± 3.4</td>
<td>6.9 ± 0.8</td>
</tr>
<tr>
<td>3. Dilaze (1.0 mg/kg)</td>
<td>4</td>
<td>3.7 ± 0.2</td>
<td>88 ± 20</td>
<td>150 ± 14</td>
<td>5.9 ± 1.0</td>
<td>p &lt; 0.001</td>
<td>20.5 ± 4.9</td>
<td>3.5 ± 0.9</td>
</tr>
<tr>
<td>4. Nifedipine (30-50 μg/kg)</td>
<td>10</td>
<td>3.3 ± 0.1</td>
<td>155 ± 40</td>
<td>212 ± 21</td>
<td>4.6 ± 0.7</td>
<td>p &lt; 0.001</td>
<td>8.8 ± 2.3</td>
<td>8.9 ± 1.2</td>
</tr>
<tr>
<td>5. Nifedipine (10-20 μg/kg)</td>
<td>5</td>
<td>3.1 ± 0.2</td>
<td>180 ± 40</td>
<td>160 ± 10</td>
<td>1.0 ± 0.4</td>
<td>ns</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>6. Propranolol (0.10 mg/kg)</td>
<td>5</td>
<td>3.9 ± 0.2</td>
<td>65 ± 25</td>
<td>159 ± 24</td>
<td>4.2 ± 0.7</td>
<td>p &lt; 0.001</td>
<td>9.5 ± 2.1</td>
<td>7.4 ± 1.0</td>
</tr>
<tr>
<td>7. Pentoxifylline (30 mg/kg)</td>
<td>6</td>
<td>3.4 ± 0.2</td>
<td>105 ± 7</td>
<td>205 ± 26</td>
<td>6.3 ± 1.4</td>
<td>p &lt; 0.001</td>
<td>11.0 ± 3.6</td>
<td>4.8 ± 1.1</td>
</tr>
<tr>
<td>8. Betamethasone (4.0 mg/kg)</td>
<td>4</td>
<td>3.3 ± 0.4</td>
<td>61 ± 17</td>
<td>167 ± 22</td>
<td>4.5 ± 1.5</td>
<td>p &lt; 0.01</td>
<td>7.0 ± 3.6</td>
<td>9.0 ± 3.0</td>
</tr>
<tr>
<td>9. CoA (1.0 ml/kg)</td>
<td>3</td>
<td>3.1 ± 0.1</td>
<td>113 ± 50</td>
<td>176 ± 26</td>
<td>5.7 ± 2.3</td>
<td>p &lt; 0.01</td>
<td>19.2 ± 4.8</td>
<td>3.3 ± 0.7</td>
</tr>
<tr>
<td>10. CoA placebo (10 ml/kg)</td>
<td>3</td>
<td>3.8 ± 0.1</td>
<td>170 ± 10</td>
<td>175 ± 19</td>
<td>4.7 ± 0.3</td>
<td>p &lt; 0.001</td>
<td>14.6 ± 3.9</td>
<td>4.7 ± 0.9</td>
</tr>
<tr>
<td>11. GSH (1.0 g/kg)</td>
<td>8</td>
<td>3.3 ± 0.2</td>
<td>90 ± 15</td>
<td>164 ± 22</td>
<td>1.5 ± 0.5</td>
<td>ns</td>
<td>11.1 ± 5.7</td>
<td>—</td>
</tr>
<tr>
<td>12. Aminophylline (0.5-1.67 mg/ml)</td>
<td>4</td>
<td>2.9 ± 0.2</td>
<td>113 ± 42</td>
<td>207 ± 26</td>
<td>2.5 ± 0.7</td>
<td>p &lt; 0.05</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>13. Betamethasone (0.11-0.21 mg/ml)</td>
<td>4</td>
<td>3.1 ± 0.2</td>
<td>56 ± 11</td>
<td>208 ± 17</td>
<td>5.2 ± 1.6</td>
<td>p &lt; 0.01</td>
<td>12.5 ± 3.2</td>
<td>6.0 ± 0.9</td>
</tr>
<tr>
<td>14. Betamethasone (0.03-0.05 mg/ml)</td>
<td>4</td>
<td>3.0 ± 0.3</td>
<td>44 ± 8</td>
<td>112 ± 34</td>
<td>2.0 ± 0.7</td>
<td>p &lt; 0.05</td>
<td>2.4 ± 0.9</td>
<td>6.7 ± 1.2</td>
</tr>
<tr>
<td>15. GIK (0.016 units insulin/ml)</td>
<td>3</td>
<td>3.5 ± 0.04</td>
<td>145 ± 45</td>
<td>158 ± 18</td>
<td>4.6 ± 1.2</td>
<td>p &lt; 0.001</td>
<td>8.3 ± 4.3</td>
<td>6.0 ± 2.1</td>
</tr>
<tr>
<td>16. GSH (4-20 mg/ml)</td>
<td>4</td>
<td>3.2 ± 0.2</td>
<td>186 ± 95</td>
<td>182 ± 29</td>
<td>1.4 ± 0.8</td>
<td>ns</td>
<td>5.3 ± 1.9</td>
<td>4.0 ± 1.0</td>
</tr>
</tbody>
</table>

Values are given as mean ± SE. * The time required for the appearance of continuous heart beating after reperfusion.
Abbreviations: GSH = reduced glutathion; GIK = glucose insulin potassium; p-value = p-value when compared to the control group;
ns = not significant; — = not determined
dripped into the blood reservoir. The heart rate and the maximal developed tension of the anterior papillary muscle of the left ventricle were then measured using the force-displacement transducer and by applying a gradual traction of the Nylon thread tied to the ventricular apex. Blood collected from the heart was measured using a graduated cylinder and total coronary blood flow was determined. Arteriovenous oxygen difference (%) was measured with oxymetry. An electromogram was obtained by directly recording the ventricular potential through a temporary pacemaker electrode. All the data was expressed as average ± standard error of the mean (SEM), and statistical analyses were made with Student's t-test.

RESULTS

Restoration of Spontaneous Cardiac Rhythmicity after Reperfusion

In non-treated 9 hearts (control group), the time interval from the beginning of reperfusion to the reestablishment of continuous beating of the heart measured 121 ± 13 sec (Table I). Of the various agents tested, betamethasone caused the most marked shortening of this interval to 44 ± 8 sec (p < 0.01) and to 56 ± 11 sec (p < 0.05), when this agent was added to the cardioplegic solution at the concentration of 0.03—0.05 mg/ml and at 0.11—0.21 mg/ml, respectively. Pretreatment with betamethasone also shortened the time required for the restoration of spontaneous rhythmicity to 61 ± 17 sec (p < 0.05).

In the groups pretreated with various calcium antagonists, this time interval measured 155 ± 40 sec with nifedipine (30—50 µg/kg), 88 ± 20 sec with dilazep, and 135 ± 19 sec with verapamil. All these values were not significantly different from the value in the control group. The remaining agents including propanolol, pentoxifylline, CoQ10, CoQ10 solvent, GSH, aminophylline and GIK likewise did not produce significant shortening of this interval, although it is possible that the value obtained after a pretreatment with propanolol (65 ± 25 sec) was not significantly different from the control value of 121 ± 13 sec only because of the small number of hearts tested with this agents (n = 5).

The heart rate measured after 30 min of reperfusion was 149 ± 23 beats/min in the nontreated group. This value in all the groups receiving various test agents ranged from 112 ± 34 (when betamethasone 0.03—0.05 mg/ml was added to the cardioplegic solution) to 212 ± 21 beats/min (in the group pretreated with nifedipine), but did not show any significant difference as compared to the control group.

Maximal Developed Tension following Reperfusion

The maximal developed tension determined after reperfusion in the control group was 0.8 ± 0.2 g. This measurement was significantly increased in most groups treated with various agents (Table I), with the verapamil-pretreated group showing the highest value (9.4 ± 1.1 g, p < 0.001). This was followed by the pentoxifylline group (6.5 ± 1.4 g, p < 0.001), the dilazep group (5.9 ± 1.0 g, p < 0.001) and the CoQ10 group (5.7 ± 2.3 g, p < 0.01). It is interesting to note that the group pretreated with CoQ10 solvent (a surfactant) also showed a significantly higher value of 4.7 ± 0.3 g (p < 0.001). Betamethasone (either with pretreatment or with simultaneous administration with cardioplegic solution), nifedipine (30—50 µg/ml), propanolol, aminophylline and GIK also significantly increased the maximal developed tension, although in lesser degrees than the above-mentioned several agents (Table I).

In contrast, GSH (either with pretreatment or addition to the cardioplegic solution) and a smaller amount of nifedipine (10—20 µg/kg) failed to significantly increase the maximal developed tension. The significant increase in the developed tension produced by a larger amount of nifedipine (30—50 µg/kg), as mentioned above, would thus indicate the need for a larger dose of this agent to achieve a satisfactory myocardial protection.

The hearts in the group receiving a small amount of betamethasone (0.03—0.05 mg/ml) simultaneously with the cardioplegic solution developed the state of the so-called stone hearts after 30 min of reperfusion. This condition was characterized by a low coronary blood flow (2.4 ± 0.9 ml/min), a smaller heart size with hard and cold myocardium and a dark discoloration. These hearts were associated with the lowest mean heart rate (112 ± 34 beats/min) of all groups.

Coronary Blood Flow after Reperfusion

The coronary blood flow attained after 30 min of reperfusion in the control, nontreated group measured 4.6 ± 1.4 ml/min. The blood

Japanese Circulation Journal Vol. 47, March 1983
flow was markedly increased in the groups pretreated with dilazep (20.5 ± 4.9 ml/min), CoQ_{10} (19.2 ± 4.8 ml/min) and CoQ_{10} solvent (14.6 ± 3.9 ml/min), all showing a significant difference (p < 0.05) from the control group (Table I). Although most of the remaining groups were likewise associated with greater values of the coronary blood flow as compared with the control group, the difference were not statistically significant. In general, those groups with lesser coronary blood flows showed greater values of arteriovenous oxygen difference, and vice versa. Thus, the dilazep- and CoQ_{10}-pretreated groups showing the most marked increases in the coronary blood flow were accompanied by the smallest arteriovenous differences of oxygen saturation, measuring 3.5 ± 0.9 and 3.3 ± 0.7%, respectively. However, these values were not significantly different from the value in the control group (7.0 ± 1.1%).

Arrhythmia after Reperfusion

Arrhythmias, including slow ventricular rhythm with atrioventricular block and premature ventricular contraction, were more frequently seen in the groups treated with betamethasone (0.03—0.05 mg/ml added to the cardioplegic solution), GSH, low dosage of nifedipine (10—20 μg/kg) and in the control group than the groups treated with verapamil, dilazep, high dosage of nifedipine, propranolol, pentoxifylline and CoQ_{10}.

DISCUSSION

In order to achieve an immediate and complete cessation of electrical and mechanical activities of the heart required for cardiac surgery, reasonably high concentrations of potassium should be used in the cardioplegic solution. Since potassium per se does not seem to be effective in protecting the myocardium from prolonged periods of ischemic arrest, in the presence of normothermia, hypothermia was introduced as a useful adjunct to cardioplegic arrest, and their combination has become a standard procedure for myocardial preservation as reviewed by Engelman et al.\(^5\) The question as to what degrees of hypothermia would be most desirable still remains to be answered, although Ino\(^6\) suggested the vicinity of 18°C as the temperature of choice. On the other hand, it has been demonstrated that the cell damage caused by ischemia is further aggravated by reperfusion (the so-called reperfusion injury)\(^7,8\) even though the fine structure of the myocardium appears to undergo little change from that prior to the restoration of coronary flow. The mechanism of reperfusion injury has been ascribed to an explosive myocardial edema and accumulation of calcium in the damaged cells.\(^9–12\) Reperfusion injury is known to occur also in the kidney and brain and, in these organs “no reflow” phenomenon\(^13\) resulting from alterations in their high vascular resistance has been held responsible for such a injury.

From the theoretical standpoint, pretreatment of the myocardium with calcium antagonists before the induction of cardioplegia would prevent reperfusion injury, if intracellular accumulation of calcium does play a role in the development of the latter. In this regard, Clark\(^14\) has reported the effectiveness of nifedipine in an experimental study. As for verapamil, Robb-Nicholson\(^15\) compared verapamil-induced cardiac arrest and potassium arrest using isolated perfused rat hearts, and suggested superiority of verapamil in preserving the myocardium after a prolonged anoxic arrest of over one hour. Slow calcium channels\(^16\) which play a role in myocardial contraction are opened by catecholamines, whereas they are blocked by several compounds known as calcium antagonists including D-600, verapamil and nifedipine. Catecholamines are considered to open energy-dependent slow channels through an increase in cyclic AMP\(^17\). Hence, pentoxifylline, which is a competitive inhibitor of cyclic nucleotide phosphodiesterase, could possibly block the calcium channels and prevent an excessive calcium inflow after reperfusion. Regarding the protective action of verapamil against hypoxia, Nayler\(^18\) demonstrated that this agent successfully antagonized the accumulation of calcium in mitochondria. Based on her studies on CPK and myoglobin release as well as mitochondrial accumulation of calcium, Nayler\(^19\) further indicated that propranolol could be considered even more effective than verapamil in preserving myocardial tissues during periods of both ischemic arrest and reperfusion. One possible advantage of verapamil over propranolol is that the velocity of fiber shortening appeared to be less markedly restricted after a pretreatment with the former than with the latter.\(^20\)

As compared with the above-mentioned 2 agents, adrenocorticosteroids (e.g., methylprednisolone) are said to be less effective in protecting the ischemic myocardium,\(^18\) although an earlier...
study by Toyama and Reis on the recovery of ventricular compliance after reperfusion suggested some myocardial protection afforded by hydrocortisone pretreatment. When a steroid, betamethasone, was added to the cardioplegic solution in a lower dose (0.03–0.05 mg/L) in the present experiment, it only minimally increased the maximal developed tension and even decreased the coronary blood flow, although the time interval for the restoration of spontaneous rhythmicity was the shortest in this group. Betamethasone in a high dosage (0.11–0.21 mg/ml) showed a much greater protective action, still being associated with early recovery of spontaneous beating. Such an early recovery of cardiac rhythmicity may be attributed to the steroid’s stabilizing action on lysosome membrane. The lack of cardioprotective action with a lower dosage of steroid may be ascribed to its insulin antagonistic action. Slower recovery of cardiac rhythmicity was found in the calcium antagonist group probably because of its suppressive action on the sinus node, atrioventricular conduction and on the speed of recovery of myocardial metabolism from cardioplegic arrest.

A clinical study of Codd et al showed that methylprednisolone did not reduce the incidence of perioperative myocardial injury in coronary bypass grafting. Finally, coenzyme Q (ubiquinon) plays an important role in terminal electron transport and in oxidative phosphorylation in the mitochondria. This agent was thus theoretically expected to be effective in protecting the myocardium, and it definitely increased the maximal developed tension as well as the coronary flow on reperfusion. It must also be stressed that, in the present study, the CoQ10 solvent exerted similar protective effects. Although Okamoto has previously reported an improvement of myocardial energy metabolism following anoxic clamping by CoQ10, he did not observe such beneficial effects of the solvent (pulronic F68). Further studies are needed to elucidate the mechanism for this interesting finding.

REFERENCES

3. TYERS GFO, TODD GJ, NIEBAUER IM, MANLEY NY, WALDHAUS FN: JA: The mecha-

Japanese Circulation Journal Vol. 47, March 1983


