The additive protective effects of cardioplegia with slow-channel blockers during ischemic cardiac arrest in guinea pig heart: a comparative study of Nifedipine, Verapamil and Diltiazem

Rıza DOĞAN, Ali SARIGÜL, Bora FARSÅK, Selim İSBİR, Meltem TUNCER, Ediz DEMİRPENÇE, Kamer KILINÇ, Metin DEMİRCİN

Dept. of Thoracic and Cardiovascular Surgery, Physiology, Biochemistry, Medical School of Hacettepe University, Ankara, TURKEY

This study was designed to compare the effects of calcium channel blocking agents nifedipine (0.075 mmol/L), verapamil (1.1 mmol/L) and diltiazem (0.03 mmol/L) on myocardium after global ischemia and reperfusion in the modified Langendorff model. Thirty-two isolated guinea pig hearts were divided into four groups (n:8) and subjected to 90 min of normothermic global ischemia, followed by 30 min of reperfusion. Cardioplegic arrest was achieved by adding one of the three Ca²⁺ channel blockers to St. Thomas’ Hospital cardioplegic solution (CTHCS). The percent recovery of cardiac function was improved by the addition of Ca²⁺ channel blockers to STHCS. Decreased lipid peroxidation and adenosine triphosphate (ATP) catabolism, protected total glutathione levels and ATP content of myocardium was observed with diltiazem, verapamil and nifedipine when compared STHCS group. These results confirmed that addition of Ca²⁺ channel blockers, in especially diltiazem can enhance cardioplegic protection. [Turk J Med Res 1997; 15(2):49-55]

Key Words: Myocardial protection, Cardioplegia, Nifedipine, Verapamil, Diltiazem, Calcium channel blocker

Nowadays, cardiac surgery is safe and effective with the current myocardial protection techniques. Reduction of myocardial ischemia is the most important factor for the success of the operation. Although, cold cardioplegia yields excellent outcome in myocardial protection, sometimes poor functional recovery is encountered. In order to maintain basic cellular metabolism, ionic equilibrium and membrane integrity, myocardium has been shown to be associated with exacerbation of cellular injury: Reperfusion occasionally potentiates the release of intracellular enzymes, influx of Ca²⁺, breakdown of sarcolemmal phospholipids, and disruption of cell membranes, which either alone or in combination result in ultimate cell death. Events known as reperfusion injury; rather than, a result of biochemical changes during ischemia, specifically occur during reperfusion (1-5).

Current evidence leads to three major hypotheses concerning the mediators of reperfusion injury. These are (1) free radical hypothesis (2), the loss of sarcolemmal phospholipids hypothesis and (3) the calcium overloading hypothesis (1,4,5).

The role of calcium ion in the pathophysiology of myocardial ischemia and reperfusion was first hinted at by Shen and Jennings (6). Myocardial ischemia is characterized by a rise of cystolic hydrogen ion and a depletion of high-energy phosphates. The degree of calcium overload, induced by ischemia has been correlated with mitochondrial dysfunction and impaired ATP (adenosine triphosphate) generating capacity (7,8).

Many previous reports have shown that, calcium antagonists, as an additive to cardioplegic solutions (9-16) or administered intravenously before the onset of ischemia (17,18) can improve cardiac functional recovery after reperfusion.

Since calcium ion accumulation is believed to be one of the primary factors that participate in myocardial injury, we proposed to test the protective effects of calcium channel blockers, such as nifedipine, verapamil and diltiazem, as cardioplegic additivies.

The aim of the present study was to evaluate the effects of calcium channel blockers on (i) heart protection and myocardial recovery after 30 min of global ischemia in Langendorff perfused guinea pig hearts; (ii) lipid peroxidation, lactate, glutathione, hypoxanthine and ATP levels in myocardial tissue; and (iii) creatine kinase release in the coronary effluent.

MATERIALS AND METHODS

Experimental Protocol

Thirty-two male Duncan-Hartley guinea pigs weighing 250-320 gr were used in this study. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National
The animals were anesthetized by ether and after intravenous administration of heparin (200 U) hearts were rapidly removed and quickly mounted on a non-circulating Langendorff perfusion column. Retrograde perfusion was established at a pressure of 100 cm H₂O with an oxygenated normothermic, modified Krebs-Henseleit bicarbonate buffer. The perfusion buffer consisted of: 118 mM/L NaCl, 4.7 mM/L KCl, 25 mM/L NaHCO₃, 1.2 mM/L KH₂PO₄, 1.2 mM/L MgSO₄, 1.2 mM/L CaCl₂, and 11.1 mM/L glucose. The solution was equilibrated with 95% oxygen and 5% carbon dioxide to achieve a pH of 7.4 at 37°C.

Apical force displacement was used in order to measure the cardiac contractile force. A 7% silk ligature was attached to the left ventricular apex and connected to the Grass® FT 03C force displacement transducer (Grass Instrument Co., Quincy, Mass., USA). The transducer output was displayed continuously on a Grass® model 5 polygraph (Serial 7D531 V3, Grass Instrument Co., Quincy, Mass., USA). After waiting 15 minutes of stabilization period the preischemic heart rate and ventricular contractile force were recorded.

Ischemic cardiac arrest was induced by clamping the aortic cannula. Then the hearts were arrested by introducing one of the cardioplegic solutions, using reservoir located 60 cm above the heart and attached to a side arm of the aortic cannula for 3 min. Through the ischemic arrest period the hearts were kept at 37°C with isotonic saline-jacketed heart chamber. At the end of 90 min global ischemia the hearts were reperfused with Krebs-Henseleit solution for 30 min at 37°C. The heart rate and ventricular contractile force were recorded every five minutes of reperfusion period. Coronary effluent was collected before cardioplegia and throughout the reperfusion period for cumulative creatine kinase (CK) release as a tissue damage marker. In all instances the left ventricular free wall was resected and stored until the tissue lactate, total glutathione, lipid peroxides (expressed by malondialdehyde-MDA-), hypoxanthine (Hpx), adenosine triphosphate (ATP) measurement were carried out.

Four different cardioplegic solutions were used to arrest the hearts. Hearts of Group I (control group) were arrested with the basic St. Thomas' Hospital cardioplegic solution (STHCS). The composition of the solution is shown in Table 1. In groups II, III and IV, Ca²⁺ channel blockers nifedipine (0.075 mmol/L), verapamil (1.1 mmol/L), and diltiazem (0.03 mmol/L) were added to the ST CHS, respectively. Each group contained eight hearts.

### Biochemical Determination

Frozen tissues were immediately weighed and homogenized in 10 volumes of ice-cold phosphate buffer (50 mM, pH 7.4), using a glass-glass homogenizer. All the biochemical determinations were done on this homogenate.

#### Table 1. St Thomas' Hospital cardioplegic solution (STHCS)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium chloride</td>
<td>110.0</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>16.0</td>
</tr>
<tr>
<td>Magnesium chloride</td>
<td>16.0</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>1.2</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>10.0</td>
</tr>
<tr>
<td>PH adjusted to 7.8</td>
<td></td>
</tr>
<tr>
<td>Osmolarity=324 mOsm/kg H₂O</td>
<td></td>
</tr>
</tbody>
</table>

Tissue lipid peroxide levels, expressed by malondialdehyde (MDA) were determined by the method of Uchiyama and Mihara (19).

The thiobarbituric acid reactive substances (TBARS) were calculated as nanomol per gram wet tissue, and tetramethoxy-propane was used as standard.

One ml homogenate was deproteinized with equal volume of cold 8% (v/v) perchloric acid. After centrifugation the supernatant was saved for the determination of lactate, hypoxanthine and glutathione. Tissue lactate concentrations were determined from this supernatant as described (20). One ml of supernatant was neutralized with 0.65 ml of K₂PO₄, (0.7 M) for hypoxanthine and glutathione determinations. The precipitate was removed by centrifugation. Hypoxanthine concentrations were determined by measuring xanthine oxidase-catalyzed conversion of hypoxanthine into uric acid (21). The hypoxanthine levels were calculated taking the molar absorbitivity of uric acid as 12.200 M⁻¹ cm⁻¹. In these determinations hypoxanthine standard was also used. Standard and samples were studied under the same conditions. Both calculations gave the same results. Tissue hypoxanthine levels were calculated as nanomol per gram of wet tissue.

Total glutathione levels were determined according to the procedure of Tietz (22), using glutathione reductase and NADPH. Total glutathione levels are expressed as millimolar (mM).

For the determination of myocardial ATP content, specimens obtained from myocardium were immersed in liquid nitrogen and then freeze-dried at 50°C. Specimens were analyzed by high-performance-liquid-chromatography using the techniques described by Hull-Ryde (23). Tissue ATP levels were calculated as pmol/g dry weight. Kreatine kinase (CK) enzyme was measured with an automated analyser using creatine kinase EC 2.7.3.2 (Boehringer, Mannheim) kits, and expressed as IU/min gr heart.

#### Expression of Results

**The following calculations were made**

**Arrest Time:** Time (seconds) from the onset of cardioplegic infusion until the heart arrests.

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Table 2. The effects of the addition of nifedipine, verapamil and diltiazem to the STHCS upon post ischemic recovery of cardiac function

<table>
<thead>
<tr>
<th>Arrest time (Sec)</th>
<th>Total pre Arrest beats</th>
<th>Percent recovery of cardiac function</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Heart rate</td>
</tr>
<tr>
<td>STHCS (control)</td>
<td>63.4±18.0</td>
<td>72.2±8.3</td>
</tr>
<tr>
<td>STHCS+Nifedipine</td>
<td>50.2110.1</td>
<td>49.6±7.1*</td>
</tr>
<tr>
<td>STHCS+Verapamil</td>
<td>50.6±7.9</td>
<td>42.1±9.6*</td>
</tr>
<tr>
<td>STHCS+Diltiazem</td>
<td>49.1±6.5</td>
<td>44.3±7.8*</td>
</tr>
</tbody>
</table>

STHCS: St Thomas' Hospital cardioplegic solution

The results are indicated mean±SEM. Each group consisted of 8 hearts.

*(p<0.05) Indicates significant difference between the value indicated and STHCS group.

Table 3. Pre ischemic and reperfusion period contractile force (gr contractility/gr heart weight) values.

<table>
<thead>
<tr>
<th>Group I (Control)</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preischemic (15' min)</td>
<td>21.4±43.1</td>
<td>22.1±1.8</td>
<td>20.6±2.4</td>
</tr>
<tr>
<td>Reperfusion period (min)</td>
<td>14.2±2.4</td>
<td>18.8±2.7</td>
<td>17.611.1</td>
</tr>
<tr>
<td>5</td>
<td>9.9±1.7</td>
<td>15.3±2.0</td>
<td>15.2±1.6</td>
</tr>
<tr>
<td>10</td>
<td>8.4±2.4</td>
<td>13.211.1</td>
<td>12.1±1.9</td>
</tr>
<tr>
<td>20</td>
<td>7.9±4.5</td>
<td>11.9±2.4</td>
<td>12.0±0.8</td>
</tr>
<tr>
<td>25</td>
<td>7.8±3.9</td>
<td>11.9±1.6</td>
<td>11.6±1.2</td>
</tr>
<tr>
<td>30</td>
<td>7.7±2.8</td>
<td>12.0±2.1</td>
<td>11.6±1.4</td>
</tr>
</tbody>
</table>

Total pre arrest beats: Number of heart beats during the 3 min of cardioplegia infusion.

Percentage recovery of heart rate (HR) = Post-ischemic heart rate / Pre-ischemic heart rate × 100

Percentage recovery of contractile force = Post-ischemic contractile force / Pre-ischemic contractile force × 100

Percentage recovery of heart work = Post-ischemic heart work / Pre-ischemic heart work × 100

Data and Statistics

All values are expressed as the mean±standard error of the mean (SEM). For statistical analysis; analysis of variance, Mann-Whitney U, and Kruskal-Wallis one-way anova test as were used where appropriate. A p value <0.05 was considered to be significant.

RESULTS

Hemodynamic data

Two hearts in group I and one in group III developed irreversible ischemic contracture at the end of 90 min of normothermic global ischemia. As shown in Table 2, there were no significant difference in arrest time among the groups. The number of total pre-arrest beats were 72.2±8.3 in STHCS group. Although there were no significant difference between groups II-IV, the difference was found to be significant between the drug treated groups and control group (p<0.05).

The preischemic (15' min) and post ischemic left ventricular contractile force values obtained from each group was shown in Table 3. Contractile force-time graphy in the reperfusion period was shown in Fig. 1.

The hearts in study groups showed better preservation of left ventricular contractile function. At the 30' min
of reperfusion, contractile force was reduced to 54.5±7.1%, 56.2±4.0%, and 58.1±2.6% of their control values for groups II, III and IV respectively (p<0.05 as compared to STHCS group).

Percentage recovery of postischemic heart work, were better in the groups in which nifedipine, verapamil and diltiazem were added to the STHCS. Although there were no significant difference between these groups, the differences were significant when compared to control.

Metabolic effects of global ischemia

Biochemical determinations of the reperfused myocardium were shown in Table 4. Tissue lactate and Hpx concentrations were unexpectedly low in the STHCS group. This may be the sign of inhibited glycolysis (p<0.05 as compared to the other groups).

Lipid peroxidation was significantly decreased in the fourth group (p<0.05 vs. control). Although MDA levels in group II and III were lower than the control, the difference was not found to be significant. Although, the difference between the study groups and control was found to be significant, according to the myocardial glutathione content, the best results were obtained in the last group (p<0.05 as compared to the other groups).

Tissue MDA and glutathione contents showed that there was a strict correlation between the depletion of glutathione content and increased lipid peroxidation. As ATP concentration was significantly decreased in the control group, Ca2+ channel blockers were found to be effective for the maintenance of tissue ATP levels. According to the myocardial functional and biochemical data, there was a strict correlation between the tissue glutathione, ATP contents and postischemic contractile function.

Initial and reperfusion period CK release and coronary flow data (Table 5) showed that nifedipine cardioplegia has no superiority when compared with the control group.

Although, there was a significant increase in CK leakage as compared to the group III and group IV, best results for coronary flow was achieved in the last two groups.

<table>
<thead>
<tr>
<th>Table 4. The effects of calcium channel blockers on tissue lactate, MDA, Hpx, total glutathione and ATP content.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tissue Lactate</strong></td>
</tr>
<tr>
<td><strong>umol/gr wet weight</strong></td>
</tr>
<tr>
<td>STHCS</td>
</tr>
<tr>
<td>STHCS+Nifedipine</td>
</tr>
<tr>
<td>STHCS+Verapamil</td>
</tr>
<tr>
<td>STHCS+Diltiazem</td>
</tr>
<tr>
<td>Left ventricular tissue before hypoxia as control</td>
</tr>
</tbody>
</table>

All results are the mean and the standard error of the mean. Each group consisted of 8 hearts.

*<p>0.05 indicates significant difference between the value indicated and STHCS group.

MDA: Malondialdehyde, Hpx: Hypoxanthine, ATP: Adenosine triphosphate

<table>
<thead>
<tr>
<th>Table 5. Preischemic and reperfusion period CK leakage and coronary flow values (*p&lt;0.05 vs. control).</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Preischemic</strong></td>
</tr>
<tr>
<td><strong>CK leakage (IU/L min gr. Heart)</strong></td>
</tr>
<tr>
<td><strong>Group I (Control)</strong></td>
</tr>
<tr>
<td><strong>Group II (Nifedipine)</strong></td>
</tr>
<tr>
<td><strong>Group III (Verapamil)</strong></td>
</tr>
<tr>
<td><strong>Group IV (Diltiazem)</strong></td>
</tr>
<tr>
<td><strong>Coronary Flow (ml/min gr heart)</strong></td>
</tr>
<tr>
<td><strong>Group I</strong></td>
</tr>
<tr>
<td><strong>Group II</strong></td>
</tr>
<tr>
<td><strong>Group III</strong></td>
</tr>
<tr>
<td><strong>Group IV</strong></td>
</tr>
</tbody>
</table>

DISCUSSION

The protection of myocardium so as to minimize the postischemic impairment of left ventricular function is a major concern during cardiac surgery. During the ischemic period, oxidative phosphorylation is impaired due to the lack of oxygen, and therefore high energy phosphates (primarily adenosine 5'-triphosphate (ATP) and creatine phosphate) are depleted (24-26). At the early stages of ischemia, glycolysis is stimulated to compensate the energy need. However, in prolonged ischemia, glycolysis is inhibited by the development of tissue acidosis and the accumulation of several metabolites including citrate (3,4,25).

In physiologic conditions, cytoplasmic calcium concentration is maintained under 1fJ-M. When calcium concentration is elevated to micromolar levels, calcium-ATPase is activated to pump calcium in to the sarcoplasmic reticulum vesicles. In addition, excess cytosolic calcium is pumped out of the cell or into the mitochondria by other calcium-activated ATPases. Calcium transport against a concentration gradient is strictly dependent on ATP energy. During prolonged ischemia, calcium transport is blocked because of insufficient ATP production and a sharp increase in calcium concentration occurs (27-29).

On reperfusion, more calcium can accumulate in the cytoplasm (24,26). It is well known that the production of free oxygen radicals is increased with the resupply of...
oxygen after ischemia (2,4,30,31). These radicals react with membrane phospholipids to initiate lipid peroxidation which in turn irreversibly inactivates calcium-ATPases (8). In addition to the inhibition of calcium-ATPases, the inhibition of glycolysis is also held responsible for calcium overload (32).

An increased level of calcium activates several metalloproteinases including calpains involved in proteolytic conversion of xanthine dehydrogenase to xanthine oxidase. Calcium can also activate phospholipase A₂, the enzyme that degrades membrane phospholipids (33). Without any doubt, reperfusion is the most effective way to treat the ischemic myocardium. Some authors believe that much of the injury is the consequence of events occurring at the moment of reperfusion, rather than as a result of changes occurring during the ischemic period (2,4).

Despite numerous experimental and clinical studies, ideal myocardial protection has not yet been found. Recent reports on the experimental (9-14) and clinical (15,16) use of calcium channel blockers to limit reperfusion injury have been encouraging. The protective properties of calcium channel blockers include reduction of the rate of extent of injury during ischemia together with combating coronary spasm, reduction of arrhythmia and hypertension, influence automaticity and slow conduction (9,11,14). The purpose of these experiments were to determine, if the addition of nifedipine, verapamil and diltiazem to potassium cryoplegic solution was synergistic in aiding restoration of cardiac function and myocardial protection.

Glutathione, as a cellular antioxidant, protects proteins and other biomolecules from oxidation. The levels of total glutathione were also very low in the control group, showing that this molecule was lost from the tissue. Since no calcium channel blocker was used in the STHCS group and glutathione was lost from the tissue, it was reasonable to suggest that xanthine dehydrogenase was converted into oxidised form by Ca²⁺ and/or by -SH modification. To clarify these statements, the inhibition of glycolysis and conversion of xanthine dehydrogenase to xanthine oxidase must be demonstrated in the STHCS group.

Our study showed that, nifedipine, verapamil and especially diltiazem used as cardioplegic additives can enhance cardioplegic protection against ischemia reperfusion injury.
Kobay kalbinde iskemik kardiyak arrest sırasında yavaş kanaal bloklerine ile kardiyoplejinin ilave koruyucu etkileri; Nifedipine, Verapamil ve Diltiazem'in karşılaştırmalı incelemesi

Bu çalışma; modifıye Langendorff modelinde, kalsiyum kanaal blokleri olan nifedipin (0,075 mmol/L), verapamil (1 mmol/L) ve diltiazem (0,03 mmol/L)’ın global iskemi ve reperfüzyon sonrası myokard üzerindeki etkilerini karşılaştırmak amacıyla planlanmıştır. İzole edilmiş 32 adet kobay kabı, 4 gruba ayrıldı (n=8) ve 90 dk normotermik global iskemiya tabi tutuldu. Kardiyoplejik arrest St. Thomas’ Hastanesi Kardiyoplejik Solüsyonu’na (STHCS) ile Ca++ kanaal bloklerinden alınan eklenmesiyle elde edildi. STHCS’e Ca++ kanaal bloklerini eklenmesiyle kardiyak fonksiyonun iyileşme yüzdesi arttıldı. STHCS grubuna karşılaştırmalı olarak diltiazem, verapamil ve nifedipin eklendiginde myokardı azalmış lipid peroksidasyonu ve adenozin trifosfat (ATP) katabolizması, korunmuş glutatyon seviyesi ve ATP içiğinden zehirlendi. Bu sonuçlar; Ca++ kanaal bloklerinin özelile göre diltiazem’in eklenmesinin kardiyoplejik koruyucu etkisine arttılabileceğini doğruladı. [T Klin Araştırmaları 1997; 15(2):49-55]

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