Myocardial protection in chronic volume-overload hypertrophy of immature rat hearts

Abstract

Objective. The benefit of cardioplegic cardiac arrest for protection of the immature myocardium is controversial. We therefore investigated the efficacy of (1) topical hypothermia alone (2) slow cooling by coronary perfusion hypothermia and (3) cardioplegic cardiac arrest plus topical cooling for protection of isolated immature rat hearts (age: 28 days).

Methods. The isolated perfused rat heart model was used. Hearts were subjected to 8 h of global ischemia at 10°C. The study was conducted after clinically relevant conditions of volume-overload myocardial hypertrophy had been established non-invasively by lifelong feeding of a diet low in iron. Parameters of left ventricular function, endothelial function, the metabolic status and myocardial injury were measured.

Results. Topical hypothermia provided superior protection of hypertrophied hearts with recovery of maximum developed left ventricular pressure and rate of pressure rise at 41.2% ± 22.3% and 34.5% ± 20.7% (mean ± standard deviation) of pre-ischemic values (P < 0.05 versus slow cooling and versus cardioplegia plus topical hypothermia). The same pattern of recovery was observed among control hearts. The recovery of endothelial function following protection by topical hypothermia alone measured 55% ± 41% in hypertrophied hearts and 62% ± 37% in control hearts, but was not recordable in all other groups. In hypertrophied hearts post-ischemic myocardial high energy content was significantly improved with topical hypothermia alone for protection when compared to the other methods. Creatine kinase leakage during reperfusion did not differ significantly among the experimental groups.

Conclusion. Rapid cooling by topical hypothermia alone provides superior protection of hypertrophied – and non-hypertrophied – immature rat hearts to additional slow pre-arrest cooling. Use of St. Thomas’ Hospital cardioplegic solution No. 2 (STS 2) does not improve protection, and even hinders functional recovery in hypertrophied immature hearts. Endothelial injury caused by cold asanguinous perfusates, including cardioplegia, interferes with the recovery of vascular function, which in turn, may limit mechanical function.

Key words: Myocardial protection · Immature heart · Cardioplegia · Hypertrophy
Inadequate myocardial protection remains a major cause of death after the repair of congenital heart defects, despite experimental evidence of increased resistance to ischemia in the immature myocardium [9]. Thus, the benefit of currently used methods for protection of the immature myocardium, such as cardioplegic cardiac arrest or extended pre-arrest cooling, is controversial and it has been suggested that topical hypothermia alone may be just as effective [16]. Other reports, however, indicate severe contracture caused by rapid cooling of the immature myocardium [23].

The explanation for inadequate protection in young hearts may relate in part to the differences in metabolic profiles of adult and pediatric hearts. Other common factors of congenital cardiac defects, however, such as chronic cyanosis and myocardial hypertrophy may also contribute to inadequate protection in this age group [26]. With regard to this aspect, previous reports have emphasized the importance of using experimental models that simulate clinically relevant conditions before drawing conclusions about the safety of cardioprotective strategies [15]. One common feature of many congenital cardiac defects with an intracardiac shunt is the rapid development of postnatal myocardial hypertrophy as a sequel of chronic volume-overload. We therefore simulated this pathway to early postnatal myocardial hypertrophy by employing an experimental model of nutritional anemia, which was induced in neonatal rats by feeding them a diet low in iron. This measure has been described as leading to rapid development of heart hypertrophy by compensatory hypercirculation [22]. Unlike other studies, this experimental model allowed for the development of the sequelae of chronic volume-overload non-invasively. Control experiments in non-hypertrophied hearts were performed to assess the impact of hypertrophy on the efficacy of protection.

At the age of 28 days, hearts of rats were subjected to isolated perfusion in order to determine the efficacy of (1) rapid cooling to 10°C by topical hypothermia alone, (2) slow pre-arrest cooling by perfusion hypothermia and (3) cardioplegic cardiac arrest using St. Thomas’ Hospital cardioplegic solution No. 2 (STS 2) plus topical cooling for myocardial protection during global ischemia at 10°C. Four indices of myocardial injury and protection — myocardial function, endothelial function, creatine kinase (CK) leakage and myocardial energy-rich phosphates were assessed.

Materials and methods

Male Wistar rats were used for all the studies. The animals received human care in compliance with the “Principles of Laboratory Care” formulated by the National Society for Medical Research and the “Guide for the Care and Use of Laboratory Animals” prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH publication No. 80-23, revised 1978).

Induction of myocardial hypertrophy

Since mating, anemia was induced in mother animals by feeding a diet low in iron (diet C 1038, iron content: 5.1 mg/kg) and distilled water. After birth, litters were reduced to eight male members and the mother animal. After weaning on the 18th day, litter mates received a low iron diet for 10 days before the perfusion experiments were performed on the 28th day.

Perfusion methodology

Rats were injected with sodium heparin (1 mg per rat intraperitoneally). After 30 min, the animals were anesthetized by inhalation of diethyl ether and 1 ml of blood was withdrawn by puncture of the inferior caval vein for the measurement of hemoglobin and hematocrit after laparotomy. Then the chest was opened and the thoracic cavity was filled with cold (4°C) Krebs-Henseleit buffer. With the heart thus immersed in cold buffer, the pulmonary artery was incised near its origin and the aorta was cannulated with a blunt-ended 16 G needle (1 mm OD). The heart was then excised and mounted to the perfusion apparatus via the aortic cannula. Perfusion of the coronary arteries was performed according to the Langendorff technique [18] at a perfusion pressure of 60 mmHg (80 cm H2O) using oxygenated modified Krebs-Henseleit buffer of the following composition (mmol/l): NaCl, 118; KH2PO4, 1.2; KCl, 4.9; CaCl2, 2.5; MgSO4, 1.2; NaHCO3, 25 and glucose, 11.1. The perfusate was filtered before use (5 µm pore size), aerated with a mixture of 95% oxygen and 5% carbon dioxide and kept at 37°C. Each heart was housed in a thermostatically controlled heart chamber and maintained at 37°C during the pre-ischemic and post-ischemic periods. During a 15-min washout period after cannulation, an intraventricular balloon was inserted into the left ventricle (LV) through the mitral valve. The ultrathin balloon was designed so as to match the ventricular dimensions of the heart. The balloon was filled with distilled water and attached to a pressure transducer through a fluid-filled tube. The volume of the balloon was adjusted by means of a water-tight microsyringe attached to the side arm of the transducer. The LV pressure signal was recorded and processed on-line using an Analog-Digital Converter (Plugsys, Type 663)2. Data processing was performed using an IBM-compatible personal computer equipped with standard laboratory software (Hemodyn).2

Measurements

Pre-ischemic baseline measurements of systolic LV function and coronary flow were performed 15 min after the onset of in vitro perfusion during isovolumic contractions by inflating the intraventricular balloon to a LV end-diastolic pressure (LVEDP) of 10 mmHg. The volume necessary to produce this pressure (V10) was registered. Left ventricular maximum systolic pressure (LVP) and its first derivative (dP/dt max.) were recorded. Coronary endothelial function was assessed by change of coronary flow in response to the endothelium-dependent vasodilator acetylcholine (10−6 M), which was administered during 5 min as a supplement to the perfusate via a separate perfusion column. From pilot studies, this acetylcholine concentration was found to accomplish a constant increase in coronary flow in our experimental system.

The recovery of systolic function and coronary flow was measured 60 min after the onset of reperfusion at an LVEDP of 10 mmHg.

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The necessary volume (V10) was recorded and – in relation to the pre-ischemic volume – judged as an estimate for recovery of diastolic function. The recovery of endothelial function was estimated by the coronary flow response to acetylcholine at 40 min of reperfusion in relation to the pre-ischemic response. Blood hemoglobin concentration was measured with an automated analyzer using a standard test kit (Merckotest Haemoglobin 3317). Hematocrit was assessed by centrifugation of blood in the heparinized hematocrit capillaries. Samples for measurement of CK leakage were obtained from the coronary effluent at 2, 10, 20, and 40 min during reperfusion. Each sample was stored at 4°C until it could be assayed using a standard enzymatical test (Granutest 2.5).

At the end of reperfusion, hearts were rapidly frozen with Wollenberger tongs precooled in liquid nitrogen. After perchloric acid extracts had been prepared from tissue samples according to the method of Lowry and Passonneau, the tissue was dried for 48 h and 80°C before weighing the myocardial dry weight [19]. Creatine phosphate and ATP levels (μmol/g dry weight) were assessed using a standard enzymatical assay [6].

Preservation techniques and experimental groups

The perfusion experiments were performed in six experimental groups (n = 13 in each group). Animals of groups 1 to 3 were exposed to chronic nutritional anemia and animals of groups 4 to 6 served as age-matched controls. At the end of the pre-ischemic control perfusion period, three different methods for protection during global myocardial ischemia of 8 h at 10°C were applied.

Protocol 1 (rapid cooling by topical hypothermia alone). In hearts of groups 1A and 1C pre-ischemic perfusion was discontinued and cardiac arrest was induced simultaneously by rapid topical cooling with cold (4–6°C) Krebs-Henseleit perfusate.

Protocol 2 (slow cooling by perfusion hypothermia). In hearts of groups 2A and 2C hypothermia was induced slowly over a time interval of 10 min from normothermia to 10°C by perfusion with oxygenated perfusate at gradually decreasing temperatures. During this period, the temperature inside the water-jacketed perfusion chamber of the heart was adjusted to the temperature of the perfusate thermostatically.

Protocol 3 (single-dose cardioplegia plus topical hypothermia). Hearts of groups 3A and 3C were arrested with STS 2, which was administered at a temperature of 10°C through a side arm of the aortic cannula from a reservoir located 60 cm above the heart for 3 min. The composition of STS 2 was as follows: NaCl 110 mmol/l, KCl 16 mmol/l, MgCl2 16 mmol/l, CaCl2 1.2 mmol/l, NaHCO3 10 mmol/l (pH adjusted to 7.8, osmolarity = 324 mOsm/l). Topical hypothermia was applied additionally as described in protocol 1.

After initial protection, all the hearts were disconnected from the perfusion apparatus, placed in a beaker containing Krebs-Henseleit perfusate and stored in a refrigerator for 8 h at 10°C. In separate experiments hearts from anemic and non-anemic control animals (three per group) were perfused during 10 min at normothermia for washout of the blood components. Subsequently, perfusion was discontinued and the hearts were embroiled in plastic (Technovit 7100). Then, transversal histologic sections were prepared and stained with hematoxylin-eosin for estimation of the degree of myocardial hypertrophy as a result of nutritional iron restriction.

Statistical analysis

All data are expressed as the mean and standard deviation (SD). Non-parametric methods were used for data evaluation. The comparison of group means was carried out by the Kruskal-Wallis Test [17]. When significant differences were found, further analysis by the Mann-Whitney Test was performed. Statistical significance was set at P < 0.05.

Results

Basal characteristics

The basal characteristics are summarized in Table 1. The body weight of anemic animals was significantly lower than that of the control animals. A low-iron diet resulted in marked reduction in serum hemoglobin concentration and hematocrit. Among anemic hearts, the myocardial dry weight/body weight ratio was significantly greater than in controls. The mean myocardial dry weight was higher in anemic hearts than in the corresponding controls. Representative histologic sections of hearts from an anemic animal and a non-anemic control animal are shown in Figures 1A and 1B. Massive right and left ventricular hypertrophy developed under conditions of low-iron nutrition.

Pre-ischemic myocardial function

Pre-ischemic myocardial function is summarized in Table 2. Left ventricular maximum systolic pressure and dP/dt max. were significantly increased in anemic hearts, when compared to corresponding control hearts. Coronary flow was increased in anemic hearts (P < 0.05 versus corresponding controls). The numerical values of V10 were higher in anemic hearts than in the corresponding controls. No differences were observed between anemic hearts and control hearts in pre-ischemic heart rates and increase in coronary flow by acetylcholine infusion. Within the anemia groups and control groups no significant differences were observed during pre-ischemic perfusion.

Post-ischemic myocardial function

Hearts of anemic animals. Recovery of myocardial and endothelial function following 1 h of reperfusion is shown in Table 3. In hypertrophied hearts, the recovery of LVP and dP/dt max. was greatest after protection with the use of topical hypothermia alone (41.2% ± 22.3% and 34.5% ± 20.7%, respectively). The numerical differences in the recovery of LVP were statistically significant in comparison to group 2 A (slow cooling) whereas differences in recovery of dP/dt max. were significant to groups 2 A and 3 A (STS 2). The recovery of V10 was highest in group 1 A, however, this numerical difference was significant in comparison to group 2 A only. No differences among anemia groups were observed with regard to recovery of coronary flow and heart rate. The post-ischemic increase

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4 Haereraus Kulzer, Wertheim, Germany
Table 1 Baseline characteristics. (Groups 1, rapid cooling by topical hypothermia alone; groups 2, slow cooling; groups 3, St.Thomas' Hospital cardioplegia No. 2 plus topical hypothermia; A chronic anemia; C control)

<table>
<thead>
<tr>
<th>Group</th>
<th>1 A</th>
<th>2 A</th>
<th>3 A</th>
<th>1 C</th>
<th>2 C</th>
<th>3 C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>78.0 ± 10.4*</td>
<td>71.9 ± 13.1*</td>
<td>68.8 ± 11.2*</td>
<td>100.4 ± 27.3</td>
<td>102.3 ± 23.4</td>
<td>91.9 ± 21.8</td>
</tr>
<tr>
<td>Myocardial dry weight (mg)</td>
<td>95.2 ± 17.3*</td>
<td>107.4 ± 17.8*</td>
<td>117.9 ± 32.2*</td>
<td>78.8 ± 20.7</td>
<td>72.3 ± 15.9</td>
<td>76.0 ± 33.7</td>
</tr>
<tr>
<td>Myocardial dry weight/ body weight (mg/g)</td>
<td>1.26 ± 0.17*</td>
<td>1.53 ± 0.35*</td>
<td>1.75 ± 0.54*</td>
<td>0.80 ± 0.16</td>
<td>0.71 ± 0.07</td>
<td>0.84 ± 0.39</td>
</tr>
<tr>
<td>Hemoglobin (mg/dl)</td>
<td>3.6 ± 0.9*</td>
<td>3.5 ± 1.0*</td>
<td>3.3 ± 0.5*</td>
<td>9.6 ± 1.9</td>
<td>10.3 ± 2.1</td>
<td>10.7 ± 1.6</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>10.7 ± 5.5*</td>
<td>7.7 ± 5.1*</td>
<td>8.0 ± 2.6*</td>
<td>26.7 ± 5.1</td>
<td>31.6 ± 5.2</td>
<td>29.7 ± 4.8</td>
</tr>
</tbody>
</table>

** P < 0.05

Table 2 Baseline measurements. (Groups 1, rapid cooling by topical hypothermia alone; groups 2, slow cooling; groups 3, St. Thomas' Hospital cardioplegia No. 2 plus topical hypothermia; A chronic anemia; C control)

<table>
<thead>
<tr>
<th>Group</th>
<th>1 A</th>
<th>2 A</th>
<th>3 A</th>
<th>1 C</th>
<th>2 C</th>
<th>3 C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum peak developed pressure (mmHg)</td>
<td>149 ± 23*</td>
<td>164 ± 40*</td>
<td>171 ± 24*</td>
<td>128 ± 19</td>
<td>134 ± 22</td>
<td>122 ± 28</td>
</tr>
<tr>
<td>Maximum peak dP/dt (mmHg/s)</td>
<td>2625 ± 290*</td>
<td>2468 ± 436*</td>
<td>2827 ± 633*</td>
<td>2113 ± 270</td>
<td>2150 ± 270</td>
<td>1904 ± 465</td>
</tr>
<tr>
<td>V10 (μl)</td>
<td>188 ± 45*</td>
<td>196 ± 39*</td>
<td>203 ± 72</td>
<td>143 ± 44</td>
<td>132 ± 22</td>
<td>173 ± 23</td>
</tr>
<tr>
<td>Coronary flow (ml/min)</td>
<td>10.6 ± 2.8*</td>
<td>10.4 ± 2.7*</td>
<td>9.3 ± 1.9*</td>
<td>6.7 ± 1.9</td>
<td>6.3 ± 1.8</td>
<td>6.8 ± 1.2</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>344 ± 38</td>
<td>351 ± 53</td>
<td>336 ± 74</td>
<td>330 ± 70</td>
<td>323 ± 76</td>
<td>354 ± 31</td>
</tr>
<tr>
<td>Increase in coronary flow by acetylcholine infusion (%)</td>
<td>28.8 ± 19.0</td>
<td>38.4 ± 27.9</td>
<td>30.9 ± 20.9</td>
<td>31.0 ± 35.0</td>
<td>17.6 ± 21.4</td>
<td>21.7 ± 19.3</td>
</tr>
</tbody>
</table>

* V10 balloon volume necessary to achieve an LV end-diastolic pressure of 10 mmHg
* P < 0.05 versus corresponding control group

Table 3 Recovery of myocardial function at 60 min of reperfusion and increase in coronary flow following acetylcholine infusion at 40 min of reperfusion (in % of pre-ischemic value). (Groups 1, rapid cooling by topical hypothermia alone; groups 2, slow cooling; groups 3, St. Thomas' Hospital cardioplegia No. 2 plus topical hypothermia; A chronic anemia; C control; NR not recordable)

<table>
<thead>
<tr>
<th>Group</th>
<th>1 A</th>
<th>2 A</th>
<th>3 A</th>
<th>1 C</th>
<th>2 C</th>
<th>3 C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum peak developed pressure</td>
<td>41.2 ± 22.3*</td>
<td>14.1 ± 10.6</td>
<td>27.6 ± 20.9</td>
<td>44.4 ± 19.4</td>
<td>33.9 ± 27.5</td>
<td>35.5 ± 27.1</td>
</tr>
<tr>
<td>Maximum peak dP/dt (mmHg/s)</td>
<td>34.5 ± 20.7**</td>
<td>13.0 ± 8.2</td>
<td>19.1 ± 18.3</td>
<td>41.4 ± 20.1**</td>
<td>27.2 ± 20.7</td>
<td>33.9 ± 26.7</td>
</tr>
<tr>
<td>V10</td>
<td>29.9 ± 22.5*</td>
<td>12.5 ± 6.2</td>
<td>17.3 ± 14.7</td>
<td>48.1 ± 35.4</td>
<td>28.7 ± 16.8</td>
<td>36.9 ± 25.7</td>
</tr>
<tr>
<td>Coronary flow</td>
<td>32.4 ± 12.4</td>
<td>39.3 ± 22.4</td>
<td>39.8 ± 9.2</td>
<td>42.1 ± 13.2</td>
<td>35.6 ± 23.5</td>
<td>31.7 ± 15.8</td>
</tr>
<tr>
<td>Heart rate</td>
<td>73.8 ± 25.9</td>
<td>55.6 ± 36.5</td>
<td>70.6 ± 27.7</td>
<td>77.9 ± 13.8</td>
<td>79.8 ± 29.7</td>
<td>65.0 ± 25.1</td>
</tr>
<tr>
<td>Increase in coronary flow by acetylcholine infusion (%)</td>
<td>55 ± 41</td>
<td>NR</td>
<td>NR</td>
<td>62 ± 37</td>
<td>NR</td>
<td>NR</td>
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</tbody>
</table>

* P < 0.05 versus group 2 A; ** P < 0.05 versus group 2 A and 3 A; *** P < 0.05 versus group 2 C
Fig. 1 Representative transversal myocardial sections as seen on light microscopy. (A) Heart from control animal at 28 days of age with normal septal, right and left ventricular wall thickness. (B) Heart from animal exposed to lifelong nutritional iron restriction at 28 days of age: massive biventricular hypertrophy.

in coronary flow as a response to acetylcholine infusion measured 55% ± 41% of the pre-ischemic baseline in group 1A (anemia, rapid cooling), whereas it was not recordable in groups 2A (anemia, slow cooling) and 3A (anemia, STS 2).

Hearts of control animals. The same rating in recovery as in anemic hearts was observed in control hearts. The highest numerical recovery values in LVP and dP/dt max. were measured after topical cooling alone (44.4% ± 19.4% and 41.4% ± 20.1%). However, except for recovery in dP/dt max. in comparison to group 2C (slow cooling) differences failed to achieve statistical significance. The response to acetylcholine measured at 62% ± 37% of the pre-ischemic value after topical cooling, but was not recordable with the other protection methods tested.

Myocardial high energy phosphates

As shown in Fig. 2, the post-ischemic myocardial concentrations in ATP and creatine phosphate ranged from 2.8 to 6.3 μmol/g dry weight and 6.4 to 13.5 μmol/g dry weight, respectively. In comparison to pre-ischemic controls, postischemic ATP values were reduced between 70 and 85% whereas values for creatine phosphate were decreased between 50 and 80%. Among the anemia groups, ATP values were significantly increased in group 1A (topical cooling alone) when compared to groups 2A (slow cooling) and 3A (STS 2). The highest myocardial concentration of creatine phosphate among anemia groups was also measured in group 1A (P < 0.05 versus group 2A). Among the control groups no statistically significant differences were observed in the post-ischemic myocardial concentration of high energy phosphates.

Creatine kinase leakage

The mean values for CK leakage during reperfusion varied widely within a range of 1400 and 5300 mU/min per g dry weight among the experimental groups. No time- or group-dependent statistically significant differences were observed among the groups.

Discussion

The objective of this study was to assess the efficacy of measures for myocardial protection in immature hearts employing clinically relevant experimental conditions of chronic volume-overload with subsequent development of compensatory myocardial hypertrophy. The results of this study indicate that neither administration of STS 2 nor slow pre-arrest cooling by means of coronary perfusion with Krebs-Henseleit solution improve myocardial protection when compared to the protection afforded by topical hypothermia alone. This observation included measures for recovery of systolic ventricular function (LVP, dP/dt max.), diastolic ventricular function (V10) and recovery of endothelial function.

Several experimental studies have indicated a greater inherent tolerance to ischemia of immature hearts compared with the adult heart [7, 14]. In contrast to this observation, a strong correlation of early postoperative death after pediatric cardiac operations with the duration of myocardial ischemia has been described [9]. A reason for this discrepancy may be that most of the studies evaluating cardioprotective strategies in immature hearts were conducted in healthy animals. Little is known about the impact of cyanosis and/or hypertrophy, as observed with many congen-
However, several factors must be considered, when comparing our results with those of other studies.

Level of hypothermia

Improved protection with STS 2 has been reported in immature rats and rabbits [2, 8]. In these studies, however, the myocardial temperature was maintained at normothermia or mild hypothermia during ischemia: Avkiran and Hearse found, in isolated immature rat hearts subjected to normothermic ischemia times ranging from 30 to 150 min, that cardioplegic protection with the use of STS 2 resulted in greater recovery of left ventricular developed pressure when compared to unprotected controls [2]. In another study, employing moderately hypothermic ischemia of 120 min at 28 °C in neonatal isolated rabbit hearts, Bove and colleagues observed the least post-ischemic functional deterioration when STS 2 was administered either as a single dose or as multidose [8]. Our experiments were conducted at 10 °C during myocardial ischemia, a temperature more closely related to clinical conditions. The reason for the even superior results we observed with topical hypothermia alone may be threefold: (1) the efficacy of protection by topical hypothermia at this temperature will be even more pronounced in rat hearts when compared to rabbit or piglet hearts, because homogenous hypothermia may be induced more rapidly in small than in bigger hearts, (2) the unequivocal benefit of cardioplegia may be offset by the detrimental effects of coronary perfusion with crystalloid solutions, which have been observed at lower temperatures [16] and (3) it is possible that the combination of topical cooling at 4–6 °C and hypothermic cardioplegic arrest induces extremely rapid arrest in the immature heart with subsequent myocardial injury caused by rapid cooling contracture [23]. Thus, it remains controversial whether or not the application of cardioplegia additional to hypothermia at 10 °C is beneficial or detrimental. Magovern et al. supposed damaging effects of STS 2 in immature rabbit hearts at 10 °C that are not seen at 28 °C [20]. The results of Kempsford and colleagues differed from those of Magovern, since a trend towards improved protection with the use of STS 2 at this temperature was observed in neonatal rabbit hearts subjected to a global ischemia time of 18 h [16]. Interestingly, multidose cardioplegia afforded improved protection at 20 °C in this study but was deleterious at 10 °C. The authors concluded that multiple infusions might reveal detrimental effects of the provision of fluid for the development of cellular edema at 10 °C rather than at 20 °C, which may be of particular relevance in view of the greater capillary permeability of the immature heart [16]. Thus, our own results confirm, on the one hand, that cardioplegia at 10 °C may be detrimental in the immature heart, even when applied as a single dose and, on the other, slow cooling by means of coronary perfusion with Krebs-Henseleit...
buffer also failed to improve protection. The results were consistent in normal hearts and hearts that were hypertrophied following chronic volume-overload. As discussed below, there is evidence that coronary perfusion with asanguinous solutions at lower temperatures per se, irrespective of perfusate or cardioplegia, is detrimental for the immature heart.

Role of calcium

It has been reported that slow cooling by coronary perfusion with asanguinous perfusates before arrest may lead to a rise in the ATP depletion rate [13]. Since the maintenance of intracellular homeostasis and hence of low cytosolic calcium requires ATP, the depletion of myocardial energy-rich phosphates may finally result in partial cell membrane damage and the sudden influx of calcium into the cell with further deleterious effects. Thus, it is conceivable that detrimental sequelae of cytosolic calcium loading as a result of pre-ischemic hypothermic perfusion with Krebs-Henseleit buffer contributed to the reduced post-ischemic recovery in our experiments after slow cooling prior to ischemia.

The same final pathway may account for the relatively poor protection which we observed with STS 2. This solution was used in our studies as an example of crystalloid cardioplegia that has been well characterized and is in current clinical use [12]. It was developed specifically to protect the ischemic adult heart. The efficacy of this solution in protecting the ischemic child’s heart, however, has been questioned mainly for its calcium concentration of 1.2 mmol/l [3]. This concentration was reported optimal only at normothermic ischemia of immature rabbit hearts [27]. Further studies under hypothermic conditions indicated that the calcium dose-response curve was shifted downward with cold, such that 0.3 mmol/l was the optimal calcium concentration in STS 2 [5]. However, the role of the calcium concentration in cardioplegic solutions for protection of immature hearts is still controversial, since other studies have shown that the calcium concentration has no relation to the post-ischemic recovery of myocardial function [24].

Myocardial protection in hypertrophied immature hearts

One aspect of this study was to assess whether disease states such as hypertrophy following chronic volume-overload alter the response of the immature heart to measures for myocardial protection. Our results indicate that this was not the case, i.e. neither in normal nor in hypertrophied hearts did cardioplegia or slow cooling afford greater protection than topical hypothermia alone. However, the numerical differences between the “topical hypothermia group” and both other groups with reference to post-ischemic recovery of LVP and dP/dt max. tended to be more pronounced in hypertrophied hearts (Table 3). In this context, it warrants consideration that hypertrophy renders the myocardium more susceptible to the development of edema when perfused with asanguinous solutions, irrespective of high (35 mmol/l) or low (5 mmol/l) potassium concentration [21].

The recovery of endothelial function was assessed by the coronary flow response to the endothelium-dependent vasodilator acetylcholine [25]. Unlike with topical hypothermia, there was no measurable recovery of endothelial function with cardioplegia or perfusion hypothermia. Aoki et al. suggested that only excessively cold cardioplegia (<4 °C) might cause endothelial dysfunction in neonatal lamb hearts whereas cardioplagia at 10 °C was not harmful [1]. The discrepancy between this and our results is probably due to differences in the experimental protocols of the two studies. Thus, it is very conceivable that the detrimental effects of a long ischemic interval such as 8 h together with those of cardioplegia lead to suppression of post-ischemic endothelial function that might not be seen when coronary perfusion with crystalloid solution is completely omitted or when the ischemia is of shorter duration, as in the study of Aoki et al. In addition, we cannot preclude that a relatively low infusion pressure of 60 cm H2O caused endothelial dysfunction by shear stress and barotrauma, since even control of the infusion pressure does not guarantee normal shear stress in epicardial coronary arteries [11]. Although the design of this study did not allow for isolation of the effects of shear stress and pressure during infusion of cold asanguinous perfusates, it is evident that impairment of post-ischemic vascular function may limit the recovery of mechanical function [25].

Measurements of post-ischemic myocardial concentrations of ATP and creatine phosphate support the functional results in as much as the highest numerical concentrations of both phosphates were observed after topical hypothermia alone. However, the numerical differences were statistically significant only among hypertrophied hearts following exposure to low-iron diet (Fig. 2).

The wide variability of CK leakage during post-ischemic reperfusion leads us to question the value of this parameter as an indicator of ischemic injury in this experimental system. Since CK leakage is a flow-dependent phenomenon, it is conceivable that small changes in coronary vascular resistance would alter the enzyme washout considerably [16]. This point may be even more relevant in relatively small rat hearts with low post-ischemic coronary flow rates.

Limitations of the study

The interpretation of our results in relation to the clinical situation in pediatric cardiac surgery must be made with caution. The volume of cardioplegic solution delivered per
gram of heart weight was probably much greater than used clinically in the human heart. Thus, a volume-related detrimental mechanism of cardioplegia cannot be discounted. In addition, there are many obvious differences between the isolated rat heart and the human heart in situ. Noncoronary collateral flow could alter the balance between beneficial and detrimental effects of cardioplegia. Sequelae of noncoronary collateral flow, such as rewarming and the return of electrical activity, would require reinfection of hypothermic cardioplegia. This measure would be expected to be more beneficial under such conditions. The ischemia time of 8 h was much longer than employed clinically during surgery of congenital cardiac defects, however preliminary experiments performed with shorter ischemia times were followed by almost complete recovery of myocardial function. In order to allow for the differentiation of the true capabilities of measures for myocardial protection, it was necessary to subject the hearts to prolonged global ischemia at 10°C, such that the recovery of contractile function was markedly reduced. In addition, the relatively poor protection we observed with STS 2 infused at 10°C and the combination with topical hypothermia at 4-6 °C may have caused deleterious differential cooling of the myocardium. Further analysis of this phenomenon would require experiments without topical cooling in addition to cardioplegic protection.

Although low-iron nutrition allows for the development of sequelae of chronic volume-overload non-invasively, this model does not mimic clinical conditions precisely, in that abnormal loading of the right ventricle by an anatomical shunt does not exist. This would lead primarily to right ventricular hypertrophy. Our own observations (Figures 1 A and 1 B) and other reports indicate, however, that low-iron nutrition of young rats results in biventricular hypertrophy [22].

In conclusion, the major point of emerge from this study is that the use of a crystalloid perfusate in the immature heart, particularly at deep hypothermia, is not well tolerated. The adverse consequences of crystalloid perfusion at low temperatures appear to be more pronounced in hypertrophied hearts of chronically anemic young rats than in control hearts. The data advocate the use of topical hypothermia alone for myocardial protection during surgery of congenital cardiac defects when performed in the neonatal period at deep hypothermia and circulatory arrest. In how far other methods, such as blood cardioplegia, improve protection in the immature heart has to be further investigated.

Acknowledgements Supported by a grant from the Deutsche Forschungsgemeinschaft (Bo 172/15-1).

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