Metabolic changes induced by ischemia and cardioplegia: a study employing cardiac microdialysis in pigs

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Abstract

Objective: The present study investigates dynamic changes of myocardial metabolism in response to ischemia, cardioplegia, and extracorporeal circulation (ECC) in order to differentiate between the contributing effects of each of these interventions. Furthermore, warm blood cardioplegia versus empty beating of the heart were compared as methods to resuscitate the ischemic myocardial metabolism.

Methods: Swedish Landrace pigs on ECC (ECC) were compared with pigs on ECC with warm ischemic cardiac arrest (ischemia) or on ECC with warm ischemic arrest followed by warm blood cardioplegia (ischemia-cardioplegia), using sham-operated pigs as controls (n = 7 in each group). Microdialysis probes were placed on the surface of the left ventricle and in the femoral artery for serial evaluation of metabolites in the intracardiac extracellular fluid and arterial blood. When hearts started in ventricular fibrillation (VF), it was electroconverted after 10 min of normal blood reperfusion. If VF started after 10 min of reperfusion electroconversion was immediately performed.

Results: There were no differences between groups in arterial contents of serine, citrulline, arginine, inosine, hypoxanthine, guanosine, aspartate, glutamate, pyruvate, or asparagine throughout the observation period. Systemic lactate increased in pigs subjected to ischemia (P < 0.001) or ischemia and cardioplegia (P = 0.002), highest in the ischemia only group (P = 0.002). In left ventricular microdialysates, lactate increased in pigs subjected to ischemia alone (P < 0.001 vs. ECC) and ischemia and cardioplegia (P = 0.004 vs. ECC). Guanosine increased in ischemia versus ECC (P = 0.002), while hypoxanthine was increased in microdialysates of both ischemic (P = 0.002) and ischemic-cardioplegic (P = 0.001) pig hearts. Inosine was increased in pigs subjected to ischemia and cardioplegia (P < 0.001 vs. ECC). All ischemic hearts started with VF, but while in the warm ischemia group VF started within 10 min of reperfusion, the ischemia-cardioplegia group had a longer asystolia with VF starting 11–22 min of blood reperfusion.

Conclusion: The heart should be allowed to start empty beating rather than by the use of warm continuous blood cardioplegia. Microdialysis and sampling of interstitial metabolites may be advantageous when an increased sensitivity is needed or when repeated blood sampling is difficult or contraindicated in monitoring of the myocardium.

Keywords: Cardioplegia; Microdialysis; Myocardial metabolism; Myocardial protection

1. Introduction

Extracorporeal circulation (ECC) itself causes a systemic inflammatory response, evident as endotoxin formation, complement activation, activation of circulating leukocytes and their soluble ligands, increase of systemic cytokines, and activation of fibrinolysis and hemostasis among the events occurring [1,2]. Additionally, the procedure on the heart itself potentially includes several detrimental factors; surgery per se, the hyperkalemic cardioplegic solution, ischemia, and reperfusion, causing postcardioplegic cardiac dysfunction [3,4].

When attempting to assess the metabolic response to extracorporeal circulation and myocardial ischemia-reperfusion during open heart surgery, the most commonly employed tool is biochemical evaluations of serum or plasma constituents, possibly in combination with small
tissue biopsies and hemodynamic parameters. However, changes during ischemia, cardioplegia, reperfusion, and extracorporeal circulation may be subtle and factors of importance may remain undetected. The microdialysis technique is based on a small dimensional, permeable probe in a fixed position allowing diffusion of extracellular molecules to be collected at timed intervals through dialysis [5,6]. The microdialysis technique has been widely employed for sampling of extracellular fluid in the brain for a long time, but has only recently been adapted to the heart [6]. With this technique, dynamic changes in the extracellular space, which comprises about 20% of the tissues, may be detected [5]. Cardiac microdialysis has been employed for studies of purine and glucose metabolism in regionally ischemic pig and dog hearts, and/or to investigate ischemic preconditioning in this context [7–9]. In patients during open heart surgery intracardial increases of interstitial amino acids and lactate have been detected [10,11]. Furthermore, troponin T levels in the microdialysate were far higher than in serum [12].

The present study aimed to investigate dynamic changes in myocardial metabolism in response to ischemia, cardioplegia, and ECC in order to differentiate between the contributing effects of each of these interventions. Furthermore, we wanted to compare warm blood cardioplegia versus empty beating of the heart as methods to resuscitate the metabolism of the ischemic myocardium. Pigs on cardiopulmonary bypass were employed, and the microdialysate was collected serially for evaluation of metabolites of the intracardiac extracellular fluid.

2. Methods

2.1. Animal preparation

The study was approved by the Ethics Committee for Animal Research at the Karolinska Institute, and the animals were cared for according to ‘Principles of Laboratory Animal Care’ by the National Society for Medical Research. Twenty-eight Swedish domestic pigs of either sex weighing 40–50 kg were used. After an overnight fast and premedication with ketalar (20 mg kg$^{-1}$) and 0.5 mg atropine i.m., anesthesia was induced with 300–600 mg pentobarbital i.v. The animals were ventilated (Harvard Apparatus) with a mixture of oxygen and air. The ventilator was adjusted according to blood gases. Anesthesia was maintained with continuous infusion of fentanyl 14 μg kg$^{-1}$ h$^{-1}$, midazolam 0.18 mg ml$^{-1}$ h$^{-1}$, and pancuronium 1.7 mg ml$^{-1}$ h$^{-1}$. Via the right femoral artery a catheter was placed in the abdominal aorta for monitoring of arterial pressure and blood sampling. A triple lumen venous catheter was placed in the inferior vena cava through the right femoral vein for infusions and transfusion. Five hundred milliliters of Ringer acetate was infused before start of extracorporeal circulation, and if necessary additional Ringer acetate was used. A Foley catheter and temperature probe were introduced surgically into the bladder. The temperature was kept between 37.5 and 38.5 °C with heated mattresses and ECC throughout the experiment.

2.2. Extracorporeal circulation

After sternotomy, cannulation sutures were put on the ascending aorta and the right atrium for ECC. The ascending aorta was cannulated with a Sarns 22F cannula for inflow, and the right atrium with a Sarns single two-stage 32F cannula for outflow. The left ventricle was vented by a 16F catheter inserted through the left atrium, and connected to the venous line for passive drainage. ECC was conducted with non-pulsatile flow of 75–80 ml kg$^{-1}$ min$^{-1}$ using a roller pump (7400 Sarns, Ann Arbour, MI, USA) and a membrane oxygenator (Maxima, Medtronic Blood System, Anaheim, CA, USA). The machine was primed with 1200 ml Ringer acetate and 7500 U heparin. Heparin was infused to keep the activated clotting time over 480 s. PaO$_2$ was kept at 15–20 kPa, and PaCO$_2$ at 4.2–5.8 kPa. During ECC mean arterial blood pressure was maintained at 60–90 mmHg without any difference between groups.

2.3. Microdialysis

Microdialysis probes with a membrane length of 30 mm (Human Probe, CMA Microdialysis AB, Stockholm, Sweden) and a cut-off at 20 kDa were placed 3–4 mm below the surface of the left ventricular mid-region as well as in the femoral artery and fixed in position. The probes were perfused with Ringer’s solution at a flow rate of 1 μl min$^{-1}$ employing a high precision microinfusion pump (CMA Microdialysis AB) to maintain a constant flow rate. Every 15 min the dialysates were collected from both sites, and frozen at −20 °C until analyzed.

Samples were analyzed for the purine metabolites inosine, hypoxanthine, and guanosine. To evaluate possible anaerobic metabolism, lactate and pyruvate were measured, and the ratio calculated. For parameters of nitric oxide synthesis and metabolism arginine and citrulline were measured, and the arginine/citrulline ratio calculated. The amino acids serine, aspartate, glutamate, and asparagine were used as indicators of possible protein degradation.

The samples were diluted 10-fold with distilled water, and automatically injected with refrigerated microsamplers (CMA 200, CMA Microdialysis AB, Stockholm, Sweden) into high precision liquid chromatography systems with UV detection at wavelengths of 214 nm for lactate and pyruvate and 260 nm for hypoxanthine, inosine and guanosine or with fluorescence detection around 495 nm after precolumn derivatization with o-phthalaldehyde for aspartate, glutamate, asparagine, serine, citrulline and arginine. The concentrations were calculated with SP 4290 integrators.
2.4. Experimental protocol

The pigs were randomly assigned by ballot to four different groups prior to the experiments \((n = 7–8\) in each). A schematic presentation of the study protocol is shown in Fig. 1. At the end of surgical preparation, microdialysis probes were placed and fixed, and sampling was started allowing the pigs a 45-min stabilization period before cannulation for ECC. After start of ECC, one group of pigs were subjected to aortic occlusion consisting of 30 min warm \((37^\circ C)\) ischemia followed by a 45-min infusion of antegrade warm blood cardioplegia, and thereafter the aortic cross-clamp was released and the pigs observed for 30 min of reperfusion (group 1, ischemia-cardioplegia, in Fig. 1). Cardioplegia was started with an initial dose of 500 ml of high-potassium, followed by low-potassium warm blood cardioplegia without any substrate-enrichment (for composition of cardioplegia solution, see Table 1). Cardioplegia flow was adjusted to maintain an aortic root pressure of 70–80 mmHg. Other pigs were subjected to the same treatment excluding cardioplegia after global ischemia (group 2, only ischemia). In another group (3, only ECC), ECC was started after cannulation, but no interventions on the heart were performed. The last group of animals were sham operated without any ECC or cardiac interventions (group 4, Sham operated, in Fig. 1). No attempt was done to wean the animals from ECC. In the groups subjected to ischemia only or ischemia reperfusion, if the heart started in ventricular fibrillation (VF), the VF was electroconverted after 10 min of normal blood reperfusion. If VF started after 10 min of reperfusion electroconversion was immediately performed. If three attempts of electroconversion were unsuccessful, another attempt was done 5 min later.

2.5. Statistics

Between group differences were tested by using ANOVA for repeated measures. The sham operated group was not included in the statistical analysis because these animals were stable throughout the experiments and to include them would greatly increase the chances of a type II statistical error. First an overall test of the interaction term for the three intervention groups was performed. Second, if the results showed to be significant, post-hoc tests of the interaction term was performed. All tests were univariate tests for the within-subjects interaction term, using a 5\% significance level \((P < 0.05)\). With a Bonferroni correction in the post-hoc tests the \(P\)-value must be lower than 0.017 to be significant \((0.05/3 = 0.0017\) based on three post-hoc tests).

The arterial measurements were compared from the start of ECC and until the end of the experiment. The myocardial samples were compared as two blocks, one comparison were from the time point when the aorta was cross-clamped against freshly prepared standards.

Table 1: Content of the cardioplegia solution which is mixed 1:4 with blood

<table>
<thead>
<tr>
<th>Component</th>
<th>High K⁺ solution</th>
<th>Low K⁺ solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>KCl (mmol)</td>
<td>101</td>
<td>30</td>
</tr>
<tr>
<td>MgSO₄ (mmol)</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>Glucose 5 g (mmol)</td>
<td>28</td>
<td>28</td>
</tr>
<tr>
<td>THAM (mmol)</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Normal (0.9%) saline to a volume of (ml)</td>
<td>1000</td>
<td>1000</td>
</tr>
</tbody>
</table>

THAM, trishydroxymethyl-amino-metan \((=\) Trometamol).
until the end of infusion of cardioplegia in group I versus the corresponding time block in groups II and III. The second block was from the first time point during reperfusion after cardioplegia in group I versus the corresponding time points in groups II and III.

Mauchly’s test of Sphericity was always tested before each ANOVA, to evaluate if the assumption for variance–covariance matrix was satisfied. If Mauchly’s test showed a non-significant result, the result from the row labeled ‘sphericity assumed’ in the test of within-subjects effects was described. If Mauchly’s test showed a significant result, the result from the row labeled ‘Greenhouse-Geisser’ in the test of within-subjects effects was described. Exact P-values are given except when P < 0.001 or P > 0.2. All values are presented as mean ± S.E.M. Although we regard S.E.M. as suboptimal for showing variations, that error bar was used in order to make the figures readable.

3. Results

All ischemic hearts started with VF, but an important difference was that in the warm ischemia group VF started within 10 min of reperfusion, whereas in the ischemia-cardioplegia group a much longer reperfusion in asystole was observed, and VF started between 11 and 22 min of blood reperfusion.

3.1. Arterial samples

There was no difference between groups in systemic serine, citrulline, arginine, citrulline/arginine ratio, inosine, hypoxanthine, guanosine, aspartate, glutamate, pyruvate, or asparagine during the time course (data not shown). Lactate was increased in pigs subjected to only warm ischemia (P < 0.001) and ischemia-cardioplegia (P = 0.002 vs. ECC). The lactate was also higher in the ischemia only group versus ischemia-cardioplegia (P = 0.002) (Fig. 2).

3.2. Left ventricular dialysates

In none of the parameters were any difference found in the time block corresponding to reperfusion after cardioplegia. All the following results are therefore only including the time block corresponding to ischemia and cardioplegia.

The amino acids glutamate, asparagine, pyruvate, ratio lactate/pyruvate, and serine were not significantly different between groups (results not shown).

Lactate concentrations (Fig. 3) were increased in pigs subjected to warm ischemia (group II, P < 0.001) and in the ischemia cardioplegia group (P = 0.004) as compared to only ECC. Lactate tended to be higher in the ischemia-cardioplegia group compared to the ischemia only group (P = 0.062). There was also a tendency that the recovery of lactate was delayed in the ischemia-cardioplegia group.

Guanosine (Fig. 3) was increased in the ischemia only group versus the ECC group (P = 0.002). However, there was neither a difference between ischemia only and the ischemia cardioplegia group (P > 0.2) nor an important difference between the ischemia-cardioplegia group and pigs on ECC (P = 0.085). The recovery was delayed in the ischemia-cardioplegia group compared to the ischemia-only group.

Hypoxanthine (Fig. 3) increased in left ventricular dialysates of pigs subjected to ischemia-cardioplegia (P = 0.001) and ischemia only (P = 0.002) versus the ECC group. There was no difference between the ischemia only and the ischemia-cardioplegia group (P = 0.13). However, the recovery tended to be delayed also for this parameter.

Citrulline (Fig. 3) decreased during ischemia and ischemia-cardioplegia and the decrease was most apparent in hearts of pigs given cardioplegia after warm ischemia. However, there were no important differences between groups (P = 0.15 between groups I and III, and P > 0.2 between groups I and II and groups II and III).

Inosine (Fig. 3) increased in hearts of ischemia-cardioplegic pigs (P < 0.001) compared to the ECC.
However, there was no important difference between the ischemia-cardioplegia group ($P = 0.2$) or between the ischemia-cardioplegia group and the ECC group ($P = 0.10$).

Arginine (Fig. 3) was not different between any of the groups ($P > 0.2$ for all comparisons). The arginine/citrulline ratio was unchanged between groups.

**4. Discussion**

One main finding of the present study was that hearts of pigs subjected to global, warm ischemia had increased purine metabolism, evident as increased extracellular inosine, guanosine and hypoxanthine. At the same time lactate increased significantly in microdialysates, indicating anaerobic metabolism. When microdialysis was performed in femoral artery blood, higher systemic levels of lactate were found in pigs subjected to warm ischemia alone as well as ischemia-cardioplegia. Possibly this was a spill-over effect from the ischemic heart, as the systemic increase came secondary to the cardiac increase. Pyruvate, which is a product of gluolysis and substrate for lactate, did not change in any group either in the arterial blood or in the myocardium.
That ischemia and reperfusion causes anaerobic metabolism and lactate production and increase of metabolites in purine metabolism as seen in the present study is well known. Increased myocardial contents of purine metabolites has been observed in cardiac models of regional ischemia [13,14]. In dogs with occluded left anterior descending coronary artery, adenosine, inosine, hypoxanthine, and xanthine were increased in ischemic myocardium [15], similar to findings in analogous porcine models [7,8]. In an investigation on dogs on extracorporeal circulation and 30-min induced global ischemia, transmural left ventricular biopsies revealed a 50% depletion of ATP during ischemia, accompanied by an increase of nucleosides [16].

L-Arginine is a substrate for nitric oxide synthase, with the vasorelaxant nitric oxide and L-citrulline as end-points of the reaction [13]. The ratio between arginine and citrulline is often employed as an indicator of nitric oxide activity. Neither citrulline, arginine, nor the ratio between arginine/citrulline differed between groups, which may indicate no influence on nitric oxide synthase activity.

Free amino acids have previously been found to accumulate in microdialysates of cardioplegic human hearts during open heart surgery, and have been interpreted as markers of myocardial damage [10]. We did not find any changes in the interstitial amino acids serine, aspartate, glutamate, and asparagine, indicating that proteins were not degraded in the present animals.

The second main finding in the present work was that when comparing resuscitation of the ischemic myocardium there was no important metabolic difference whether the hearts were allowed to start empty beating or whether the hearts were perfused with warm cardioplegia. For all the important parameters (lactate, guanosine, inosine, and hypoxanthine) there was a tendency (Fig. 3) that the recovery was delayed in the group that was given cardioplegia after ischemia as compared to the hearts beating during resuscitation. The consequence for cardiac surgery is that in a situation with acute coronary occlusion, cardiogenic shock, and revascularization, once extracorporeal circulation has been established, or at least when the crucial vessels have been revascularized, the heart should be allowed to start beating. There may not be an advantage of longer episodes with continuous cardioplegia ‘to let the heart rest’ while for instance the remaining vessels are bypassed. The empty beating state may offer better resuscitation for the acutely ischemic heart than a longer episode (45 min) of continuous warm cardioplegia. However, theoretically some reperfusion asystole may be the optimal reperfusion [17]. The present work is not contrary to the concept of controlled reperfusion as recommended by Buchberg and Beyersdorf [18,19], as this is a completely different situation.

When the concept of warm continuous cardioplegia was introduced in the early 1990s, there were enthusiastic followers who claimed that now the heart can be cardioplegic almost indefinitely because it is perfused and oxygenated. However, the present investigation is in agreement with subsequent investigations, which have shown that the hyperkalemic, cardioplegic perfusion is indeed injurious or at least suboptimal as compared to normal blood perfusion [20]. A limitation of the present study is that it only shows metabolic changes, and no functional data were included. Function is the only parameter with important clinical relevance, and may not always be significantly correlated to metabolic changes. Furthermore, these animal experiments in non-diseased hearts may also be different from patient hearts.

Microdialysis has only recently been established for studies of the beating heart, although it has been employed in numerous studies of the central nervous system [6]. A major theoretical advantage of the microdialysis technique is the possibility to access the extracellular space in a tissue without taking biopsies. Furthermore, frequent repetitive sampling allows estimation of temporal changes in the tissue, and from a clinical perspective microdialysis may be a good alternative to blood sampling in situations where blood loss is to be avoided. A possible source of error in sampling of dialysates is that mechanical insertion of the probe may cause bleeding, leakage from the intracellular space, induction of inflammation and reactive changes in vascular tone and local blood flow, which potentially may influence the local tissue concentration of metabolites. For this reason a 45-min equilibrium period was employed after probe insertion, and in the time interval all end-points became stable. In patients an early increase in troponin T release was observed shortly after probe insertion, and this peak was gone after 70 min [12]. Our metabolites measured were stable throughout the whole stabilization period. Another advantage of microdialysis as compared to blood sampling is increased sensitivity. Kennergren et al. [10] found that the peak of troponin T release was 300 times higher in microdialysates than in serum from the same patients after cardiac surgery with cardioplegic arrest. It is interesting that now there is microdialysis equipment that allows for continuous measurements of lactate, and the probes can be implanted during surgery and indwelling for up to 100 h [10]. However, for the present work, it is not certain that microdialysis added information about metabolism, which would not be apparent, by sampling blood from the coronary sinus. However, such a comparison was beyond the scope of this work.

5. Conclusions

Metabolically the heart should be allowed to start empty beating as soon as possible after an important ischemic insult. Interestingly the present metabolic data suggest that revascularization should rather be on the on-pump beating heart than by the use of warm continuous blood...
cardioplegia, however, studies including functional parameters are needed. Microdialysis and sampling of interstitial metabolites in the heart may in some situations, when an increased sensitivity is needed or when repeated blood sampling is difficult or contraindicated, have advantages in monitoring of the myocardium.

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