Initial results of a clinical study: adenosine enhanced cardioprotection and its effect on cardiomyocytes apoptosis during coronary artery bypass grafting

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Abstract

Objective: Apoptosis has been considered as one of the mechanisms of cardiomyocyte loss during open heart surgery. Adenosine is cardioprotective against ischemia-reperfusion injury in experimental models. The aim of this study was to find out whether the administration of single-dose adenosine added to blood cardioplegia is effective in decreasing the apoptosis process. Methods: In a double-blinded randomized control intervention study, 40 patients were enrolled for elective coronary artery bypass grafting. In the adenosine group (n = 20) patients received 250 μg/kg adenosine in the aortic root after cross-clamping followed by cold blood cardioplegia. In the control group (n = 20) patients had only antegrade cardioplegia. Left ventricular tissue samples (from apex) were taken before and after the bypass. The apoptotic cells were identified by dUTP nick-end labeling (TUNEL) using an apoptosis detection kit. The number of TUNEL-positive cardiomyocytes was expressed as percentage of the total number of cardiomyocytes in histological tissue sections. Results: The groups were closely identical in demographic data, cross-clamp time, cardiopulmonary bypass time and weaning time. The postoperative cardiac index and other hemodynamic parameters, including the patterns of CK-MB, did not show statistically significant differences. In the tissue samples there were an equal number of patients who developed apoptosis after the cross-clamp. Although the frequency of apoptosis in the control group was two times higher than in the adenosine group, this was statistically not significant. Conclusions: Adenosine enhanced blood cardioplegia could not prevent myocardial apoptosis completely. However, it seems to be that adenosine might influence the frequency of apoptosis and this needs to be considered in future investigations.

Keywords: Adenosine; Apoptosis; Cardioplegic solution; Cardiopulmonary bypass; Myocardial protection

1. Introduction

Apoptosis of cardiomyocytes has been implicated in loss of functional myocardium during many forms of cardiac pathology such as ischemia-reperfusion injury, heart failure, cardiomyopathy and myocarditis [1,2]. Apoptosis during cardiac surgery could be triggered by several myocardial stresses, including an intrinsic pathway caused by hypoxia and oxidative stress/reactive oxygen species or an extrinsic pathway triggered by elevated humoral factors released by inflammatory response mediators due to operative trauma, cardioplegia and cardiopulmonary bypass (CBP) [3—5]. Indeed, animal and human studies have demonstrated that cardioplegic arrest during open heart surgery has been associated with induction of endothelial cell and cardiomyocyte apoptosis [3,6,7,19,20].

Various cardioplegic protocols have been evaluated to decrease the apoptotic process. Cold blood cardioplegia has been reported to be superior to cold crystalloid cardioplegia in inhibiting myocardial apoptosis during ischemic arrest [8,9]. It is currently the most commonly used technique to maintain a safe form of cardiac arrest during cardiac surgery.

Adenosine is an endogenous substance with well-documented cardioprotective properties against ischemia-reperfusion injury [10]. Several studies have shown promising...
results with adenosine as an adjunct to hyperkalemic cardioplegia, both in large animal models, [11] and in clinical trials [12]. However, the effects of adenosine on cardiomyocyte apoptosis remain largely unknown.

Adenosine induces rapid cardioplegic arrest by inhibiting sinus and atrioventricular node function [13]. The cardioprotective effect is shown to be due to both A1 receptor and A2 receptor activation [14,15]. The mitochondrial ATP sensitive K+ channel (mitoKATP) activation downstream of the signal transduction pathway from adenosine receptor activation [16].

The aim of this study was to evaluate the hypothesis that adding 250 μg/kg adenosine directly into the aortic root as an adjuvant to cold blood cardioplegia is successful in modifying the occurrence of cardiomyocyte apoptosis in the left ventricle of patients undergoing elective coronary artery bypass grafting.

2. Materials and methods

2.1. Patient selection

After institutional approval by Tampere University Hospital Ethics Committee, the protocol for this prospective randomized, double-blind, placebo-controlled study was reviewed by National Agency for Medicines, Finland. All 40 patients gave their informed consent. The patients were scheduled for elective coronary revascularization using on-pump cardiopulmonary bypass technique.

Patient selection was planned to result in homogenous study and control groups. The exclusion criteria were diabetic patients with sulfonylurea medication, unstable angina, recent myocardial infarction within the last month, redo cardiac operation, preoperative diagnosis of asthma, chronic obstructive pulmonary disease (COPD), kidney function impairment or liver dysfunction. Patients with poor left ventricular function (ejection fraction EF ≤ 30%), valvular disease, and those receiving corticosteroids were considered not eligible.

2.2. Anesthesia

A radial artery line and a pulmonary artery catheter were inserted for hemodynamic monitoring. Anesthesia was induced with propofol (0.5—1.0 mg/kg), sufentanil (0.6—0.8 μg/kg) and cis-atracurium. Sufentanil infusion was continued with a rate of 0.03—0.05 μg/kg min. Sevoflurane was used as the main anesthetic agent throughout the operation, and also provided during the cardiopulmonary perfusion with a vaporizer attached to the fresh gas inlet. It is important to consider that sevoflurane may have an effect on the KATP-channels comparable to that of adenosine.

2.3. Operative and perfusion techniques

The surgical techniques were standardized in all cases. A median sternotomy was performed, and one internal thoracic artery and from one to four peripheral vein grafts from the lower extremities were taken in each case. Radial artery graft was harvested whenever indicated. Cardiopulmonary bypass was established with regular cannulation technique using mild hypothermia (35°C) with nonpulsatile flow with a membrane oxygenator. The circuit was primed with 1500 ml of Ringer’s acetate. The proximal anastomoses were constructed during a single cross-clamping period.

2.4. Cardioplegia and adenosine administration

Patients were allocated into two groups. In the adenosine group, 20 patients received adenosine 250 μg/kg into the aortic root just after cross-clamping. This dose was chosen in view of a pilot study to be the lowest effective dose to stop the myocardium. Twenty patients in the control group received normal saline as placebo. All patients received routine blood cardioplegia delivered through antegrade route. The effect of adenosine is known to be dependent on the temperature [11]. Therefore, the first cardioplegia infusion was given at normothermia. After reaching asystole, cardioplegia temperature was lowered to 10—12°C. Subsequent 1 min antegrade cardioplegia infusions were administered after completion of each distal anastomosis, and final warm antegrade cardioplegia (37°C) was given for 3 min before the removal of the aortic clamp.

2.5. Tissue harvesting

Two samples of left ventricular apex were harvested from each patient in both groups. The first sample was obtained after CBP was established immediately before aortic cross-clamp by oblique introduction of Tru-Cut needle (PRECISA® 14G × 150 mm) into the left ventricle apical wall. The second sample was taken from the same location by the same needle before cardiopulmonary bypass was stopped. The puncture sites were secured with small 4-0 Prolene (Ethicon) stitch even when no bleeding occurred. Myocardial tissue (5—10 × 3 mm) was immediately frozen in liquid nitrogen for histological studies.

2.6. Assessment of apoptosis

Apoptosis was detected using the TUNEL (terminal transferase mediated dUTP nick-end labeling) assay, as previously described. [17,18] In brief, paraffin-embedded myocardial sections were heated in sodium citrate solution and digested with proteinase-K to expose DNA. The DNA strand breaks were then labeled using terminal transferase with digoxigenin-conjugated dUTP and visualized using alkaline phosphatase immunohistochemistry (IHC). To confirm optimal sensitivity of the assay, it was standardized with the use of serial sections treated with DNase I to induce enzymatic DNA fragmentation (positive control of apoptosis). The amount of apoptotic cardiomyocytes was calculated in an average of 34 microscopic fields in each sample (magnification ×250) and expressed as the percentage of the TUNEL positive cardiomyocyte nuclei from the total number of cardiomyocyte nuclei. Only the nuclei surrounded by myofilaments were considered as cardiomyocytes.
2. Sample collection and hemodynamic measurements

Hemodynamic monitoring comprised measurement of heart rate (HR), mean arterial pressure (MAP), mean pulmonary artery pressure (MPAP), pulmonary capillary wedge pressure (PCWP), and cardiac output (CO). Derived cardiovascular variables, cardiac index (CI), systemic vascular resistance index (SVRI), and pulmonary vascular resistance index (PVRI) were calculated from standard formulas. All cardiac output measurements were based on the thermodilution technique, and data collection was started as baseline measurements before anesthesia induction.

Preoperative baseline creatine kinase (CK-MB) and hemodynamic measurements were obtained, followed by serial postoperative hemodynamic measurements up to the first postoperative morning, and CK-MB determinations were done after transfer to ICU, then after 12 h and in the first postoperative morning. Reference values for CK-MB were 0—25 U/l.

Statistical analysis was performed using SPSS for Windows software, version 9.0 (SPSS; Chicago, IL, USA). The Mann—Whitney U test was used to distinguish demographic differences between the groups. Continuous variables were analyzed by analysis of variance (ANOVA) for repeated measures. Logarithmic transformation was used, as the variables were not normally distributed. Statistical significance was attributed to p value <0.05.

3. Results

At the time of this study there was no data valid enough to guide the sample sizing when apoptosis was considered as the biological marker to test the efficacy of adenosine during myocardial protection; thus this study represent a pilot model of the current study design.

The study cohort included 40 patients between the ages 46 and 73 years, 90% of them were male patients. Baseline patient data and operative data were homogenous in the two groups, as summarized in Table 1. Perioperative myocardial function, and EF, baseline hemodynamics and the level of creatine kinase MB fraction (Table 2), were not significantly different between the adenosine group and the controls. There were no cross-over cases between the adenosine and control groups, as summarized in Table 1. Perioperative myocardial function, and EF, baseline hemodynamics and the level of creatine kinase MB fraction (Table 2), were not significantly different between adenosine group and the controls.

No significant statistical difference.

2.7. Sample collection and hemodynamic measurements

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n = 20)</th>
<th>Adenosine (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean ± SD)</td>
<td>63.4 ± 8.8</td>
<td>65.5 ± 8.2</td>
</tr>
<tr>
<td>Man/woman</td>
<td>19/1</td>
<td>17/3</td>
</tr>
<tr>
<td>No. of grafted vessels (median)</td>
<td>3 (3)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>CPB time (min)</td>
<td>94 ± 25</td>
<td>92 ± 16</td>
</tr>
<tr>
<td>CX time (min)</td>
<td>79 ± 26</td>
<td>72 ± 18</td>
</tr>
<tr>
<td>Weaning time (min)</td>
<td>15 ± 6.4</td>
<td>19 ± 8</td>
</tr>
</tbody>
</table>

No significant statistical difference.

CPB, cardiopulmonary bypass; CX, cross-clamp.

Table 2

Baseline hemodynamic variables in the control and adenosine groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n = 20)</th>
<th>Adenosine (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (beat/min)</td>
<td>53 ± 24</td>
<td>50 ± 23</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>73 ± 10</td>
<td>70 ± 10</td>
</tr>
<tr>
<td>PCWP (mmHg)</td>
<td>11 ± 8</td>
<td>10 ± 7</td>
</tr>
<tr>
<td>CI (l/min m²)</td>
<td>2.6 ± 1.7</td>
<td>2.4 ± 1.5</td>
</tr>
<tr>
<td>LVSVI (g m²/m²)</td>
<td>48 ± 20</td>
<td>46 ± 19</td>
</tr>
<tr>
<td>EF (%)</td>
<td>63 ± 42</td>
<td>61 ± 39</td>
</tr>
<tr>
<td>CK-MB (U/l)</td>
<td>3 ± 1</td>
<td>3 ± 1</td>
</tr>
</tbody>
</table>

No significant differences between the groups.

CI: cardiac index; CK-MB: creatine kinase MB-fraction; EF: ejection fraction; HR: heart rate; LVSVI: left ventricular stroke work index; MAP: mean arterial pressure; PCWP: pulmonary capillary wedge pressure.

3.1. Apoptosis

We were able to analyze apoptosis only in 29 patients from the total number 40 patients, 13 patients in the adenosine group and 16 patients in the control group.

The other 11 patients were excluded from the analysis because only very few cardiomyocytes could be found in their tissue sections (<10 microscopic fields).

In the preischemic samples, apoptotic cardiomyocytes were not detected in any patient. In contrast, scattered myocytes with intensely TUNEL-positive nuclei were detected in eight patients in both groups and the baseline data did not show significant difference between these subgroups (Table 4). The mean value of the apoptotic cells in the control was 0.03 ± 0.07% (SD) with the 95% confidence interval (0.03; 0.7). This is almost twice the mean in the adenosine group 0.017 ± 0.0% (SD) with confidence interval (0.00; 0.03). However, this difference was not statistically significant (p = 0.73). When patients were allocated into quartiles depending on the percentage of apoptosis (low, medium and high percentage), there was a non-significant tendency towards more apoptosis in the control than adenosine group in all quartiles (p = 0.268) (Table 5).

A statistical model built to study correlations between the cross-clamp time, weaning time, CK-MB values and degree of apoptosis with respect to site, number, and severity of the obstruction within the coronary arteries did not show significant correlations between these parameters.

4. Discussion

Despite the recent advances in myocardial protection and cardioplegia formulations, recent clinical and experimental studies have shown that apoptosis frequently occurs after...
cardiopulmonary bypass [19,20,22]. It is a point of controversy whether ischemia alone or the combination of ischemia and reperfusion activates the apoptotic machinery in cardiomyocytes [21—23].

The main aim of our study was to investigate the effect of adding adenosine to the blood cardioplegia in order to decrease the development of cardiomyocyte apoptosis during cardiopulmonary bypass.

The rationale for studying adenosine in the setting of open heart surgery is based on the considerable experimental evidence that adenosine reduces both myocardial stunning and infarct size [10—14]. Although the exact mechanism underlying the cardioprotective effect of adenosine is unknown, the beneficial effects of this agent appear to be related to activation of specific adenosine receptor subtypes, at least three of which (A1, A2a and A3) may be involved [14,15]. Experimental findings indicate that adenosine is most effective in protecting a reversibly injured heart when administered before ischemia, most likely by activation of cardiac myocytes A1 and A3 receptors. Preischemic adenosine administered before ischemia, most likely by activation of cardiac myocytes A1 and A3 receptors. Preischemic adenosine administration could further significantly reduce the cardiomyocyte apoptosis rate [24]. It is a point of controversy whether ischemia alone or the combination of ischemia and reperfusion activates the apoptotic machinery in cardiomyocytes [21—23].

There was no significant difference between the adenosine and control group in regard to the demographic preoperative clinical data, the cross-clamp or weaning time. However, we found a wide range of apoptotic response between the patients. Although the difference in the mean apoptotic indexes between the two groups was statistically not significant, the index in the control group was twice as high as in the adenosine group. This indicator is giving some evidence that adenosine could be capable of producing a remarkable effect. As we used the minimal effective dose to induce rapid myocardial arrest, it might be possible that modification of the dose and timing of adenosine administration [24] could further significantly reduce the cardiomyocyte apoptosis. On the other hand, we concentrated in this initial study only in low-risk coronary artery disease patients with well-preserved myocardial function, and,

Table 3
Postoperative hemodynamic variables in the control and adenosine groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n = 20)</th>
<th>Adenosine (n = 20)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 h</td>
<td>6 h</td>
</tr>
<tr>
<td>HR (beat/min)</td>
<td>77</td>
<td>81</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>68</td>
<td>74</td>
</tr>
<tr>
<td>PCWP (mmHg)</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>CI (l/min·m²)</td>
<td>3</td>
<td>3.1</td>
</tr>
<tr>
<td>LVSWI (g m/m²)</td>
<td>33</td>
<td>36</td>
</tr>
</tbody>
</table>

Data represented as mean value. No significant differences between the groups.

CI: cardiac index; CK-MB: creatine kinase MB-fraction; EF: ejection fraction; HR: heart rate; LVSWI: left ventricular stroke work index; MAP: mean arterial pressure; PCWP: pulmonary capillary wedge pressure.

Fig. 1. CK-MB activity in the control and adenosine groups. No significant differences were detected between the groups. (CK-MB1 indicates preoperative value, CK-MB2 immediately after the operation, CK-MB3 after 12 h and CK-MB4 1st postoperative morning.) Reference values were 0—25 U/l.

Table 4
Baseline characteristics for patient who developed apoptosis in both groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control (n = 8)</th>
<th>Adenosine (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>63 ± 7.3</td>
<td>63 ± 6.8</td>
</tr>
<tr>
<td>Man/woman</td>
<td>8/0</td>
<td>8/0</td>
</tr>
<tr>
<td>No. of grafted vessels</td>
<td>3 (3.4)</td>
<td>3 (3.4)</td>
</tr>
<tr>
<td>EF</td>
<td>58 ± 9.7</td>
<td>64 ± 5.8</td>
</tr>
<tr>
<td>CPB time</td>
<td>100 ± 11.5</td>
<td>94 ± 20</td>
</tr>
<tr>
<td>CX time</td>
<td>73 ± 5</td>
<td>79 ± 5</td>
</tr>
<tr>
<td>Weaning time</td>
<td>17 ± 8.5</td>
<td>15 ± 9.3</td>
</tr>
</tbody>
</table>

No significant statistical difference.

CPB, cardiopulmonary bypass; CX, cross-clamp; EF, ejection fraction.

Data presented as mean ± SD.
therefore, adenosine effect may have been more limited than in patients with more severe myocardial functional impairment. We can only speculate that, in more advanced cases the protective effect of adenosine might have been more pronounced. Similarly, with a larger study population and longer reperfusion, the difference might be more pronounced. Further studies with longer follow-up time would be needed to study the effects of adenosine on apoptosis during open heart operation as negative results might be due to an insufficient number of patients. In conclusion adenosine enhanced blood cardioplegia could not prevent myocardial apoptosis completely. Therefore, it seems to be that adenosine might influence the frequency of apoptosis, and this needs to be considered in the future investigations.

Acknowledgements

We would like to present our appreciation and thanks to Heini Huhtala, statistic lecturer, School of Public Health University of Tampere, Finland.

References


Appendix A. Conference discussion

Dr G. Szabo (Heidelberg, Germany): In the present study the authors investigated the effects of adenosine in a clinical trial, and I find it very good that they also presented the negative results here. We saw that in most of the parameters there was no effect of adenosine. I find personally that not only positive but also negative effects should be published.

Adenosine is well known for its potent vasodilatation. However, it promotes also glycolysis, activates potassium-sensitive ATP channels, and strongly inhibits neutrophil function, such as the production of free radicals. In positive but also negative effects should be published.
showed a protective effect using the same dose of adenosine as this study and higher expressions, followed by a study 2 years ago in a very similar setting as yours. However, it should also be noted that there are some negative trials, such as a large trial published in Circulation in 1998 with 250 patients and a study in 2000 from the Tampere group. This is the first question. If I look at the paper which was published in 2000 in the Journal of Cardiothoracic and Vascular Anesthesia and the paper that you sent to the European Journal of Cardiothoracic Surgery, the question that is raised is, is the study which was published 7 years ago and the study presented here the same? The only new aspect is the investigation of apoptosis. If in fact it is, it should be stated here and also in the paper that this is a post hoc analysis regarding apoptosis markers. If this was a new study, I would have serious ethical concerns in setting up a clinical study which had been shown ineffective 7 years ago.

The second point, you showed no or marginal differences regarding apoptosis markers. I find it not surprising, because adenosine has a major effect via influencing myocardial metabolism or due to inflammatory effects. So if there is any effect on apoptosis, this is just secondary.

Third, I would like to ask you to speculate why the results of clinical studies are diverging. What role does the time of application of adenosine play and what role does the type of cardioplegia play?

Dr Shalaby: Regarding to the first point this study is a new study. I would like to say that, this is the first work to use blood cardioplegia plus adenosine as a single dose injected directly into the aortic root after cross-clamp and use left ventricular tissue sample to evaluate the apoptotic myocardial changes. From the clinical point of view, there was no significant difference in this regard. It could be the effect of using blood cardioplegia can mask this effect clinically, but at cellular basis we detected some apoptotic difference. As regards the induction of myocardial arrest, it was very fast, and this show the direct effect of adenosine on the potassium channels as a primary effect not as a secondary effect on cardiomyocyte metabolism and inflammation. We were evaluating does this first induction influence the course of cell apoptosis during cardiopulmonary bypass or not.

The second question?

Dr Szabo: What is the role in terms of time of application; before cardioplegia, within the cardioplegic solution, or even during reperfusion, such as the Chinese group published a couple of months ago, and what is the role of the type of the cardioplegia? This is just speculation, but this may explain the diverging results of the numerous clinical studies performed before.

Dr Shalaby: Yes, the timing, of course, is very important because it affects the mechanism of action of adenosine. In the reperfusion, it is related more to the antioxidant and the anti-inflammatory effects. But here we are investigating the induction and the effect on ion transportation.

Dr T. Steensrud (Tromso, Norway): I have one question. Adenosine given in blood has a half-life of 0.6 s, and you gave that in blood. Have you considered that?

Dr Shalaby: Yes, we considered that, and this is why we didn’t mix the adenosine with the blood cardioplegia but we injected adenosine directly through three-way line, intracoronary, then it was followed immediately by the blood cardioplegia. It doesn’t mix.

Dr H. Vanermen (Aalst, Belgium): I want to say something in favor of the presenter here. Everybody knows that I have used endoclamp occlusion for 10 years in all of my endoscopic mitral valve surgeries, and there is just one way to make sure that your endoclamp is well positioned, and that is to induce the fastest cardiac arrest possible. Adenosine does that. There is not a single doubt about that. You give adenosine and you can position the balloon. And usually, and I’ll say 99% of the cases, I don’t see a single heartbeat anymore, which is never the case when you put an external cross-clamp and you give cardioplegia, whether it is blood cardioplegia or St. Thomas, and just wait for the arrest by potassium. That’s one thing.

The other thing is that I think that the effect of cardioplegia can be better, because adenosine prior to the administration of cardioplegia has a vasodilative effect, so you probably reach more areas with your cardioplegia. And if you just inject your cardioplegia immediately, which is cold, it will induce a vasocostrictive effect. Those are my thoughts.

Dr Shalaby: Yes, this is true, and in this group of adenosine, we saw this immediate arrest result, and after a short while we saw fibrillation indicating the end-point of the action of adenosine overlapped by the starting of the arrhestatic action of potassium.

Dr Vanermen: I agree with you. It happens very often. You put an external clamp and you just give cardioplegia without adenosine and you see the heart beat for another 3 min against an obstacle, which I don’t think is favorable for the protection.