Pre-conditioning with adenosine leads to concentration-dependent infarct size reduction in the isolated rabbit heart

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Abstract

Adenosine (ADO) has a cardioprotective effect in ischemia–reperfusion injury when administered both prior to ischemia and during reperfusion. ADO has also been implicated in the mechanism of ischemic pre-conditioning. The aim of this study was to investigate whether there was a concentration-response between the administration of ADO prior to ischemia–reperfusion and reduction in subsequent infarct size. Rabbit isolated perfused hearts were subjected to 45 min ischemia and 180 min reperfusion following pre-treatment with either Krebs Henseleit buffer alone or buffer containing ADO at a range of concentrations (3 μM–100 μM) for 5 min followed by 5 min perfusion with buffer. Infarct/risk ratios were significantly reduced in hearts pre-perfused with higher (> 3 μM) concentrations of ADO (Control, 58.5 ± 1.5%; 3 μM ADO, 51.6 ± 3.0%; 6 μM ADO, 44.1 ± 2.0%; 10 μM ADO, 33.3 ± 1.9%; 100 μM ADO, 26.6 ± 0.9%; 50 μM ADO, 21.6 ± 3.5%; 100 μM ADO, 23.0 ± 0.6%). We conclude that pre-treatment with ADO leads to a concentration-dependent reduction in infarct size.

Keywords: Adenosine; Myocardial infarct size; Rabbit, heart

1. Introduction

Coronary artery occlusion followed by reperfusion leads to infarction of a proportion of the ischemic area. The extent of injury depends on the availability of collateral blood flow, the duration of ischemia, the adhesion, activation and extravasation of neutrophils together with the generation of free radicals. There is continuing interest in the development of strategies to mitigate against ischemia–reperfusion injury.

During ischemia, the release of adenine nucleotides from endothelial cells, cardiac myocytes and purinergic nerves increases substantially [1]. Nucleotides are rapidly degraded by extracellular ectophosphatases to adenosine which accumulates in ischemic tissue and is progressively washed out during reperfusion. Interest in the role of adenosine during ischemia and reperfusion has increased following the observation that a brief period of ischemia–reperfusion can increase tolerance against subsequent sustained ischemia–reperfusion [2]. This phenomenon, termed "ischemic pre-conditioning", is associated with both preservation of function and an anti-arrhythmic action, using both in vivo and in vitro models of myocardial ischemia (see reference [3] for review). The release of adenosine from ischemic cells and its subsequent washout during the pre-conditioning cycle has been implicated in the mechanism of ischemic pre-conditioning [4]. The effector pathway of adenosine-induced protection in pre-conditioning remains unclear but appears to involve A1/A3 (and not A2) receptors on the cardiac myocytes.

In addition to the cardioprotective effect resulting from exposure to adenosine prior to sustained ischemia–reperfusion as observed for pre-conditioning, the presence of increased concentrations of adenosine during reperfusion is also beneficial and further augmentation of adenosine levels during reperfusion offers protection against injury [5,6]. The mechanism of the cardioprotection offered by adenosine in this respect may involve A2-mediated coronary arteriolar vasodilatation [1], inhibition of local release of vasoconstrictors such as noradrenaline [7] and endothelin...
and the inhibition of activation and aggregation of circulating platelets and granulocytes [5,9].

We have recently reported a relationship between elevated adenosine concentration in the coronary perfusate of the rabbit isolated heart and infarct size reduction by comparing two different experimental techniques which have adenosine-dependent mechanisms [10]. In the light of those findings, we have gone on to investigate whether there is a concentration response between the administration of adenosine prior to ischemia–reperfusion and subsequent infarct size.

2. Experimental method

2.1. The isolated heart model

Male New Zealand White rabbits (2.0–3.0 kg) were anesthetized with intravenous sodium pentobarbital (40 mg/kg) following cannulation of a marginal ear vein. A tracheostomy was performed under local anesthetic (3 ml of 2% lidocaine) and the rabbit was ventilated mechanically with 100% oxygen. The chest was opened through a left thoracotomy and the pericardium incised to expose the heart. A 3–0 silk suture on a small round-bodied needle was passed through the myocardium underneath a proximal branch of the left coronary artery and the ends were threaded through a small vinyl tube to form a snare which was used to occlude the artery as required. The rabbits were given 1000 units heparin intravenously, then the heart was rapidly excised by cutting the great vessels, placed in room temperature saline, and mounted on the Langendorff apparatus within 1 min.

The hearts were perfused retrogradely at a constant flow rate of 65 ml/min in a non-recirculating system using a roller pump. The perfusate was Krebs Henseleit buffer equilibrated with 95% O2 and 5% CO2 at 37°C which contained (mM) NaCl 118.0, KCl 4.7, MgSO4·7H2O 1.2, CaCl2·2H2O 1.8, NaH2PO4 14.6, KH2PO4 1.2 and glucose 11.0, pH 7.4. All hearts were electrically paced at 180 beats per minute via the right atrium. Left ventricular pressures were measured by means of a fluid-filled latex balloon, connected by polyethylene tubing to a pressure transducer and inserted into the left ventricle via an incision in the left atrial appendage. The balloon volume was adjusted to give an initial diastolic pressure of 10 mmHg. Coronary perfusion pressure was measured via a side arm in the aortic cannula using a pressure transducer. The heart was allowed to stabilize for at least 30 min before the experiment was begun.

2.2. Experimental protocols

There were seven experimental groups with rabbits being assigned sequentially to each. Group 1, the control group, was monitored for an additional 10 min before being subjected to ischemia and reperfusion. The remainder of the hearts (Groups 2 to 7) were perfused with Krebs Henseleit buffer containing adenosine at a fixed concentration (3 μM, 6 μM, 10 μM, 20 μM, 50 μM or 100 μM) for 5 min and then with Krebs Henseleit buffer alone for 5 min prior to ischemia and reperfusion. In each experiment, ischemia lasted 45 min and was followed by 180 min of reperfusion.

2.3. Measurement of infarct and risk area

At the end of each experiment, the heart was flushed with room temperature saline for 1 min. The coronary branch was then reoccluded and 1–10 μm fluorescent zinccadmium sulfide particles infused into the perfusate until the risk area could be visualized with UV light. The ligature was then released and 2–3 ml of 1% triphenyltetrazolium chloride (TTC) were injected through the side arm and the heart immersed in Krebs Henseleit buffer at 37°C for 2 min. (TTC stains viable tissue deep red.) The heart was weighed and frozen. While frozen, the heart was cut into 2 mm transverse slices. Tracings were made of the risk zones (lacking fluorescence under ultraviolet light) and the infarct zones (TTC-negative tissue) and the areas were determined by planimetry of the tracings. The volume of infarct and risk tissue was calculated as the product of area (cm²) and 0.2 cm (thickness of slice). This experimental method is well established [4,6].

2.4. Chemicals

Adenosine was purchased from Sigma Chemicals Ltd., UK.

All studies were in accordance with the United Kingdom Home Office regulations for the care and use of laboratory animals (Project Licence No. 70/01759).

2.5. Data analysis

All results are expressed as mean ± standard error (s.e.m.). Statistical analysis between groups was performed using ANOVA followed by Bonferroni’s multiple comparisons test.

3. Results

3.1. Infarct size data

Pre-treatment with adenosine led to concentration-dependent reduction in infarct/risk ratio which was highly significant (P < 0.01) at concentrations of 6 μM and above. Mean risk volumes were not significantly different between groups (see Fig. 1 and Table 1).
150

Fig. 1. Ratio of infarct volume to risk volume expressed as percentages for 7 groups of isolated perfused rabbit hearts. Hearts were perfused for 5 min with buffer + adenosine (0-100 μM) followed by 5 min washout, 45 min ischemia and 180 min reperfusion.

3.2. Hemodynamic data

Hemodynamic data relating to ventricular performance for all experiments are presented in Table 2. There were no differences between groups in the hemodynamic variables measured following the period of stabilization. However, 5 min perfusion with buffer plus adenosine led to a dose-dependent reduction in coronary perfusion pressure (significant at concentrations of 20 μM and greater) which had disappeared 5 min later. Adenosine had no effect on ventricular performance as judged by left ventricular systolic and diastolic pressures (see Table 2).

At the end of reperfusion, coronary perfusion pressure was the same in all groups. Left ventricular systolic pressures were significantly greater in hearts treated with higher concentrations of adenosine. Conversely, left ventricular systolic pressures tended to be lower and diastolic pressures higher in groups which had sustained larger infarcts (see Table 2).

4. Discussion

This study demonstrates that pre-treatment with adenosine leads to a concentration-dependent reduction in infarct size in the rabbit isolated perfused heart. These results support the findings of an earlier study in which we had reported a correlation between adenosine concentration in

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Table 1
Infarct/risk ratios and risk volume data for 36 isolated perfused rabbit hearts exposed to 45 min ischemia and 180 min reperfusion. Seven groups were studied: hearts perfused with buffer for 10 min prior to ischemia-reperfusion (Control) and hearts perfused with buffer + adenosine (3 μM-100 μM) for 5 min, then buffer for 5 min prior to ischemia-reperfusion.

<table>
<thead>
<tr>
<th>Group</th>
<th>Control</th>
<th>3 μM ADO</th>
<th>6 μM ADO</th>
<th>10 μM ADO</th>
<th>20 μM ADO</th>
<th>50 μM ADO</th>
<th>100 μM ADO</th>
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<td>n</td>
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<td>5</td>
<td>4</td>
<td>4</td>
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<tr>
<td>L/R (%)</td>
<td>58.5 ± 1.5</td>
<td>51.6 ± 3.0</td>
<td>44.1 ± 2.0</td>
<td>33.3 ± 1.9</td>
<td>26.6 ± 0.9</td>
<td>21.6 ± 3.5</td>
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<td>Risk volume (cm³)</td>
<td>1.0 ± 0.4</td>
<td>0.9 ± 0.5</td>
<td>0.9 ± 0.6</td>
<td>1.0 ± 0.4</td>
<td>1.1 ± 0.6</td>
<td>1.2 ± 0.2</td>
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</tr>
</tbody>
</table>

* P < 0.01; ** P < 0.001 vs. corresponding values from Control hearts.

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Table 2
Hemodynamic data for 36 isolated perfused rabbit hearts exposed to 45 min ischemia and 180 min reperfusion. Seven groups were studied: hearts perfused with buffer for 10 min prior to ischemia-reperfusion (Control) and hearts perfused with buffer + adenosine (3 μM-100 μM) for 5 min, then buffer for 5 min prior to ischemia-reperfusion.

<table>
<thead>
<tr>
<th>Group</th>
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<th>3 μM ADO</th>
<th>6 μM ADO</th>
<th>10 μM ADO</th>
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<th>50 μM ADO</th>
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<td>5</td>
<td>4</td>
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<tr>
<td>Time = 0 min (end of stabilization)</td>
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<tr>
<td>CPP</td>
<td>100 ± 3</td>
<td>98 ± 1</td>
<td>103 ± 2</td>
<td>100 ± 3</td>
<td>105 ± 1</td>
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<td>118 ± 3</td>
<td>116 ± 4</td>
<td>115 ± 3</td>
<td>122 ± 4</td>
<td>118 ± 5</td>
<td>115 ± 7</td>
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<td>7 ± 2</td>
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<td>6 ± 2</td>
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<td>7 ± 1</td>
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<td>101 ± 2</td>
<td>97 ± 3</td>
<td>95 ± 5</td>
<td>88 ± 1</td>
<td>82 ± 5**</td>
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<td>116 ± 3</td>
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<td>116 ± 3</td>
<td>120 ± 4</td>
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<td>DP</td>
<td>8 ± 2</td>
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<td>7 ± 2</td>
<td>12 ± 2</td>
<td>10 ± 1</td>
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<tr>
<td>Time = 180 min reperfusion (i.e. conclusion of experiment)</td>
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<tr>
<td>CPP</td>
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<td>90 ± 3</td>
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<td>22 ± 3</td>
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<td>14 ± 2</td>
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* P < 0.05; ** P < 0.01; *** P < 0.001 vs. corresponding values from Control hearts. L/R, infarct volume as a percentage of risk volume. CPP, coronary perfusion pressure (mmHg). LVDP, left ventricular developed pressure (mmHg). DP, diastolic pressure (mmHg).
the coronary perfusate and the degree of infarct size reduction [10].

Cardioprotection was noted following perfusion with adenosine concentrations of 6 μM and the effect was maximal at a concentration of 50 μM. Thus the graded cardioprotective response was present over a narrow dose range, only one order of magnitude. The degree of protection noted at higher doses was similar to that which we have previously reported following ischemic pre-conditioning in the rabbit isolated perfused heart and which could be abrogated by prior treatment with 8-p-sulfophenyl-theophylline (8-SPT), an adenosine receptor antagonist.

Although there is one report of "dose-dependency" of protection by ischemic pre-conditioning against ischemia-induced arrhythmias in the rat isolated heart, this has not been reported in other species [11]. Optimal protection against myocardial necrosis can be achieved in dogs and rabbits with a single pre-conditioning cycle of 5 min ischemia and 5 min reperfusion and there appears to be no additional protection from further cycles [12,13]. Although the detailed mechanisms which underly the anti-arrhythmic and anti-ischemic effects of ischemic pre-conditioning may well be different, it remains possible that a graded response between pre-conditioning and myocardial necrosis could be demonstrated in the rabbit or dog if the pre-conditioning cycles were sufficiently brief. This conclusion is consistent with our observation that graded adenosine-dependent cardioprotection can be demonstrated over a relatively narrow range of concentrations of adenosine.

Treatment with adenosine led to a brief fall in coronary perfusion pressure with no change in the indices of ventricular performance. No chronotropic effects of adenosine were identified because all hearts were paced electrically at 180 bpm. At the conclusion of reperfusion, we noted no differences in coronary perfusion pressure between the groups.

Progressive loss of myocardial function with falling ventricular systolic and increasing ventricular diastolic pressure is a feature of the isolated perfused heart preparation. However, the decreases in ventricular systolic pressure were more marked in Control hearts and there was a correlation between infarct size and systolic pressure in adenosine-treated hearts. Similarly, infarct size correlated with increases in ventricular diastolic pressure.

In conclusion, this study demonstrates that pre-treatment with adenosine leads to a concentration-dependent reduction in subsequent infarct size following ischemia-reperfusion in the rabbit isolated perfused heart. The graded response to adenosine occurs over a narrow concentration range (one order of magnitude) and this may account for the supposition that ischemic pre-conditioning has an "all-or-none" action.

References