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Adenosine added to cardioplegic solution at low dose reduces functional recovery after normothermic ischaemia in isolated rat heart

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Adenosine and its analogues have emerged as promising candidates in the search for substances to improve functional recovery after intermittent cardioplegia, and the results of human trials are starting to be published, one study having demonstrated dose-dependent protective effects. Adenosine may improve recovery of high-energy phosphates, or mimic ischaemic preconditioning by stimulating adenosine A1 receptors.

In a working, isolated rat heart model, hearts which received 50 µmol·L⁻¹ adenosine in a warm “blood” cardioplegic solution administered before and after a 10 min normothermic ischaemic insult showed a lower post-ischaemic recovery of haemodynamic performance than control hearts, both immediately after resuming work (80 % (n = 5) compared with 92 % (n = 5), p = 0.014) and after 20 min (67 % against 88 % respectively, p = 0.042).

After discussing a number of possible reasons for this unexpected result, including exacerbated reperfusion injury, temperature, and species differences, we suggest that adenosine should be adopted cautiously as an additive to cardioplegic solutions, and that delivery protocol and dosage should be very carefully designed and administered. J Clin Basic Cardiol 1999; 2: 110–12.

Key words: adenosine; cardioplegia; working, isolated rat heart
solution as above with thrice-washed bovine erythrocytes added to give a haematocrit of 25 %, and bovine serum albumin fraction V at 15 g L⁻¹. The resulting free calcium concentration was 1.6 mmol L⁻¹. Perfusion gases were equilibrated using membrane oxygenators. The heart, paced slightly more rapidly than its intrinsic rate, pumped via a windkessel to simulate vascular compliance against an afterload of 13 kPa (100 mmHg), and the pumped fluid was recirculated, but not the coronary flow.

Changes to model
After sternotomy, heparin (500 IU kg⁻¹) was administered into the right atrium to reduce clotting. The membrane oxygenator for the buffer was supplied with carbogen (95 % O₂, 5 % CO₂) to maximize oxygen delivery, and that for the erythrocyte suspension changed to 18 % O₂, 8 % CO₂, and the rest N₂, to achieve physiological O₂ and CO₂ tensions (pO₂ 15 kPa, pCO₂ 6 kPa). The heart was arrested by adding isotonic KCl (154 mmol L⁻¹) from an infusion pump via a hypodermic needle into the perfusate (‘mini-cardioplegia’ [15]), fluid volume between the needle and the heart being only 4 mL to minimize adenosine consumption by erythrocytes. Delivery rate was controlled to keep delivered potassium concentration constant at 30 mmol L⁻¹. Precise temperature control is essential to achieve consistent results, so heart temperature was monitored with a sensor in the right ventricle (Model 511, Yellow Springs Instrument Co., Ohio, USA), and maintained by immersing the heart in a temperature controlled bath, into which the coronary flow drained. An additional heat exchanger was used to maintain perfusate temperature during Langendorff perfusion. Inorganic phosphate was included in the perfusate as it is washed out during ischaemia [16] and may also be necessary for post-ischaemic ATP recovery.

Protocol
Pilot experiments were conducted to verify the ideal ischaemic interval and potassium concentration. The protocol for the main experiments is shown in Figure 1. Two groups of five rats were used, a control and an adenosine group. After a 20 min period to assess work output, hearts were arrested by switching to Langendorff perfusion with erythrocyte suspension and using the infusion pump to raise potassium concentration to 30 mmol L⁻¹. In the adenosine group, adenosine (Adenocor, Sanofi Winthrop, Maassluis, The Netherlands) was added with the same pump to achieve a delivered concentration of 50 µmol L⁻¹. Pretreatment or sham arrest continued for 15 min, followed by a 10 min period of global normothermic ischaemia (38 °C), imposed by clamping the perfusion lines. All hearts were then reperfused in Langendorff mode using the same solution as before ischaemia, maintaining arrest during a 15 min posttreatment period. The infusion pump was then switched off, and following a 10 min recovery period of Langendorff perfusion, work output of paced hearts was again assessed, average values being calculated for the first and last 5 min of a 20 min period. Work output was taken as left ventricular output (aortic plus coronary flow) against constant pressure (13 kPa, 100 mmHg).

Results
In two hearts in each group, stable left ventricular output before ischaemia ranged from 43.9 to 48.7 mL min⁻¹, and due to a change of atrial cannula, outputs in the other three per group ranged from 67.8 to 76.2 mL min⁻¹. Recovery is expressed in percentage terms; we did not proceed with hearts achieving outputs less than 40 mL min⁻¹. All hearts spontaneously resumed beating with sinus rhythm during the recovery period, and while working, each heart was paced at a rate slightly higher than its intrinsic rate (300–360 beats min⁻¹), the same rate being used before and after ischaemia. Results are summarized in Figure 2. Adenosine-treated hearts (n = 5) recovered 80 % (SEM 3.3 %) of their pre-ischaemic left ventricular output, significantly less than control hearts (n = 5), which recovered 92 % (SEM 2.6 %, p = 0.014). Output during post-ischaemia assessment remained effectively stable in control hearts, while the adenosine-treated hearts tended to suffer a loss of function (difference between groups not significant, p = 0.11). During the last 5 min of the 20 min assessment period, outputs were 67 % (SEM 8.5 %) and 88 % (SEM 2.3 %) of control values, respectively (p = 0.042).

The presence of erythrocytes in the perfusate ensured adequate oxygenation for the working heart with a coronary flow of 11 % (SEM 1.4 %) and 13 % (SEM 1.0 %) respectively of cardiac output during the 20 min assessment period in the control and adenosine groups (7.2 from 64.4, and 7.9 from 61.5 mL min⁻¹, respectively, with mean aortic pressure of 13 kPa = 100 mmHg), not different between groups (p = 0.34). The trend was for coronary flow during arrest with adenosine to be higher than that with potassium alone at 126 % (SEM 13 %) and 91 % (SEM 9.8 %), respectively, of the assessment value in each heart, though as observed in pilot experiments, the high potassium concentration suppressed the vasodilatory effect of adenosine, and the difference was not significant (p = 0.064).
Adenosine reduces post-ischaemic cardiac function

Discussion
Numerous studies have investigated the effects of adenosine and its analogues on ischaemic damage, many looking into adenosine A1 receptor activation or inhibition, with activation leading to improved protection against infarct, but not always improving functional recovery [10]. There are many possible reasons for the widely differing results: dose, temperature, delivery protocol differences, the duration of the ischaemic insult, and species effects. A clear dose-response relationship has been demonstrated in patients receiving adenosine in blood cardioplegic solution, with the highest dose group needing less postoperative drug treatment [3]. However, by the authors’ own reasoning, the patients in the 100 µmol L−1 group needed more of all drugs (except one) than the placebo group. Adenosine is more protective at 20 °C than at lower temperatures [17], but higher temperatures were not used. Regarding protocol, pretreatment with 10–100 µmol L−1 adenosine without arrest, followed by washout, does not improve functional recovery after 20 min ischaemia in working rat hearts, with the suggestion that the effectiveness of pretreatment may be altered by a washout period before ischaemia [10], while 50 µmol L−1 adenosine with potassium arrest has been shown to increase work output recovery after 30 min ischaemia in rat hearts [1], but in neither study was adenosine administered during reperfusion. It has been demonstrated that 100 µmol L−1 group needed more of all drugs (except one) than the placebo group. Adenosine is more protective at 20 °C than at lower temperatures [17], but higher temperatures were not used. Regarding protocol, pretreatment with 10–100 µmol L−1 adenosine without arrest, followed by washout, does not improve functional recovery after 20 min ischaemia in working rat hearts, with the suggestion that the effectiveness of pretreatment may be altered by a washout period before ischaemia [10], while 50 µmol L−1 adenosine with potassium arrest has been shown to increase work output recovery after 30 min ischaemia in rat hearts [1], but in neither study was adenosine administered during reperfusion. It has been demonstrated that 100 µmol L−1 adenosine, delivered both before 20 min ischaemia without washout and during reperfusion, significantly depresses rate-pressure product, an indicator of diastolic function, in an unpaced guinea-pig heart, a result more comparable to ours, while not sparing ATP [11]; the loss of ATP during our short ischaemic period was probably, as found in that study, too small for added adenosine to have much effect. Damage mechanisms which adenosine could exacerbate are reperfusion injury, by free radical generation in the presence of adenosine metabolites [18], and ionic imbalance by opening ATP-sensitive potassium channels, increasing permeability to potassium, and causing excessive depolarization. However, adenosine has been shown to protect guinea-pig myocytes against potassium-induced calcium overloading [19] and also reduce the time to potassium arrest, possibly by superpolarization and arrest of the sodium-potassium pump [20]. Adenosine receptors also have different structures and densities between species [21]. Activation of the human A2 and A3 receptors is certainly cardioprotective [22], though the A3 effect is doubtful in rat [10], and the A3 receptor shows a marked lack of homology among species [23].

To summarize, the cardioprotective effectiveness of adenosine pretreatment could be affected by protocol. Posttreatment could help ATP regeneration after a longer insult, but the presence of adenosine during reperfusion may worsen free radical damage, possibly aggravated in the rat by higher enzyme activity; conflicting study results could also be due to species-related receptor variations. The differences reported here are small, and the reasons speculative, but the adenosine-treated hearts nevertheless recovered significantly worse than the control hearts.

We conclude that caution should be exercised in the introduction of adenosine as a cardioprotective agent, and that delivery protocol and dosage should be carefully designed and administered to maximize the benefits reported by others and to minimize the possible risk we observed.

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References
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