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

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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 minute 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

During open heart surgery, it is usually necessary to cross-clamp the aorta, with the risk of subsequent ischaemic injury to the heart. Major reduction of metabolic rate by cardioplegic arrest reduces detrimental sequelae to the myocardium, and accelerated resynthesis of high-energy phosphates could enhance recovery. Exogenous adenosine could preserve myocardial ATP during ischaemia and provide a substrate for subsequent ATP recovery. Addition of 50 μmol·L⁻¹ adenosine to a cold crystalloid cardioplegic solution has been reported to improve functional recovery in isolated rat hearts [1], while in one of the first reported trials of adenosine-potassium cardioplegia in humans, it was demonstrated that up to 25 μmol·L⁻¹ adenosine could be safely administered, both during induction of arrest, and after ischaemia with the last dose of cardioplegic solution, though higher doses caused systemic hypotension; dual delivery was intended to improve cardioprotection and reduce infarct size, although neither effect was assessed [2]. Another clinical study using dosages up to 2 mmol·L⁻¹ has revealed dose-dependent protection during bypass surgery [3]. It might also be possible to protect the myocardium before arrest, one promising possibility being ischaemic preconditioning, but the mechanism of action is not yet fully understood and use in the operating room remains controversial: adenosine and its analogues have yielded promising results in the search for pharmacological substitutes. It has been reported that in a canine model, pre-ischaemic intracoronary administration of adenosine at 50 μg·kg⁻¹·min⁻¹ significantly attenuates stunning [4] and benefits have already been demonstrated in patients [5]. Maintenance of arrest during reperfusion has been shown to reduce arrhythmias and to improve functional recovery [6].

A working heart model is more sensitive to ischaemic damage than a Langendorff [7] and more accurately determines the ability of the heart to support a physiological workload [8]. The use of an erythrocyte suspension as perfusate rather than a buffer solution has the advantage that the heart is well supplied with oxygen and has a large coronary flow reserve [9], which may be important when testing a potent vasodilator like adenosine. Most isolated heart studies published before this study began reported use of 10–100 μmol·L⁻¹ adenosine [1, 10, 11]. An ischaemic insult of 10 min causes a moderate amount of damage at normothermia (38 °C in rat) in unprotected hearts, while 12 min causes severe and less reproducible damage [12]. A potassium concentration of 30 mmol·L⁻¹ is necessary to maintain arrest in normothermic rat heart [13].

In our ejecting isolated rat heart model perfused with erythrocyte suspension, we tested the hypothesis that functional recovery after normothermic global ischaemia may be improved by a combination of adenosine pretreatment, and maintenance of arrest and administration of adenosine during reperfusion.

**Methods**

All animal procedures were reviewed by the Faculty Ethical Committee to ensure compliance with European standards. Differences were tested using Student’s t-test, taking a p-value less than 0.05 to indicate statistical significance. Errors are reported as standard error of the mean (SEM).

**Model**

The model used was that described previously [14], with changes and improvements found necessary during pilot experiments as detailed below. Briefly, a male Wistar rat of nominal weight 450 g was anaesthetized, the aorta was cannulated and perfusion in Langendorff mode was started at a pressure of 13 kPa (100 mmHg), using modified Krebs-Henseleit buffer (constituents in mmol·L⁻¹: NaCl 118, CaCl₂ 3.0, KCl 4.7, KH₂PO₄ 1.2, NaHCO₃ 25, MgSO₄ 1.2, sodium EDTA 0.5, and glucose 11.1).

After cannulating the left atrium, the setup was switched to working mode with a preload pressure of 2 kPa (15 mmHg) using erythrocyte suspension consisting of the same buffer.
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 to 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. 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 2.0 %, 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).
Discussion

Numerous studies have investigated the effects of adenosine and its analogues on ischemic 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 ischemic 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 ischemia in working rat hearts, with the suggestion that the effectiveness of pretreatment may be altered by a washout period before ischemia [10], while 50 µmol·L⁻¹ adenosine with potassium arrest has been shown to increase work output recovery after 30 min ischemia 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 and 20 min ischemia without washout and during reperfusion, significantly depresses rate-pressure product, an important indicator of sub-endocardial ischemia in the rat myocardium. Basic Res Cardiol 1992; 87: 548–58.

Adenosine receptors also have different structures and densities between species [21]. Activation of the human A1 and A2 receptors is certainly cardioprotective [22], though the A2 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.

Acknowledgements

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