Cardioplegia with Adenosine and Adenosine Triphosphate in the Isolated Guinea Pig Heart

Halim Soncul, M.D., Ali Ersöz, M.D., Levent Gökgöz, M.D., Çimen Karasu, M.D., Kamil Ayrancioglu, M.D., Volkan Singi, M.S., and Melih Altan, M.D.

SUMMARY

In order to determine the effect of adenosine triphosphate (ATP) and adenosine in cardioplegic solutions, a comparative study has been undertaken in isolated guinea pig hearts using the Langendorf perfusion technique as a model of cardiopulmonary bypass.

The hearts (n=10 in each group) previously being perfused by Krebs-Henseleit solution, were arrested by one of the following cardioplegic solutions: 1) Potassium 20 mM/L (Plegisol), 2) Potassium 20 mM/L + ATP 10 mM/L, 3) Adenosine 10 mM/L, 4) Adenosine 10 mM/L + ATP 10 mM/L. After 45 min of hypothermic ischemia, postischemic recovery of heart rate, ventricular contractility, heart work and postischemic changes in tissue enzymes (LDH, SGOT, SGPT) were compared among the 4 different cardioplegic solutions. Arrest time and number of arrest beats were also recorded and compared among the groups.

Although similar beneficial results on postischemic recovery were achieved with adenosine cardioplegia and with ATP supplemented potassium cardioplegia, ATP supplemented adenosine cardioplegia did not show any beneficial effects on postischemic recovery.

Key Words:
Cardioplegia    Adenosine    Adenosine triphosphate

Despite extensive investigations of cardioplegic solutions and additives, the problems of myocardial protection during ischemia have not been fully eliminated.

The idea of adding high energy phosphates to the cardioplegic solution to sustain the metabolic demands was attempted with positive results. Adenosine triphosphate (ATP), which is an energy-rich substrate itself, has been shown to improve postischemic myocardial recovery when administered

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From the Department of Thoracic and Cardiovascular Surgery of Gazi University Medical School and Department of Pharmacology, Faculty of Pharmacy of Ankara University, Ankara, Turkey.

Mailing address: Halim Soncul, M.D., Hurriyet Caddesi, 134/6, Dikmen, 06460, Ankara, Turkey.

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during reperfusion or added to a cardioplegic solution. On the other hand adenosine has cardiovascular effects similar to ATP such as the ability to antagonize calcium channels, inhibit noradrenaline release and inhibit sinoatrial and atrioventricular nodes. It was recently repopularized as a cardioplegic additive. Several experimental studies suggest that a high concentration of adenosine (10 mM/L) also induces rapid cardiac arrest when administered in cardioplegic solutions.

It has long been known that nearly all administered exogenous ATP hydrolyzes to adenosine and other adenine nucleotides in a single passage through the isolated perfused heart. Despite some investigation showing that poorly hydrolyzed ATP analogues were less effective than ATP, theoretically adenosine should be responsible for most of the cardiovascular effects of ATP.

In this study, we compared the cardioplegic effects of ATP and adenosine with classical high potassium cardioplegia (Plegisol) and sought to answer the following questions:

1. Does ATP have the same effect as adenosine on cardiac arrest and postischemic myocardial recovery when used in cardioplegia?
2. Can ATP and adenosine produce a different result when used in combination?

**MATERIALS AND METHODS**

**Animals**

Hearts were obtained from male guinea pigs (n=40) (Strain: albino purebright) weighing 330-430 g. All animals received human care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health (NIH Publication No. 80–23, revised 1978).

The animals were anesthetized by ether and given 200 units of heparin into the femoral vein. The hearts (1.8–2.5 g) were rapidly removed and cannulated via the aorta. Perfusion techniques and plegic solutions:

The hearts were mounted on a modified Langendorf perfusion apparatus and perfused by a gassed (oxygen 95%, carbon dioxide 5%) Krebs-Henseleit solution at a rate of 2 ml/min at 37°C. The composition of the perfusate was NaHCO₃ 25 mM/L, NaCl 118 mM/L, KH₂PO₄ 1.2 mM/L, KCl 4.8 mM/L, MgSO₄ 1.2 mM/L, CaCl₂ 1.2 mM/L and glucose 11.1 mM/L.

Four different cardioplegic solutions at 4°C (10 experiments in each
group) were used to arrest the hearts. The compositions of these solutions were as follows:

Group 1 (Potassium group): KCl 20 mM/L, NaCl 123 mM/L, MgCl 8 mM/L, NaHCO₃ 9 mM/L and glucose 11.1 mM/dl (Plegisol).

Group 2 (ATP group): The same solution as in group 1 + ATP (bisodium salt) 10 mM/L.

Group 3 (Adenosine group): Adenosine 10 mM/L, NaCl 123 mM/L, MgCl 8 mM/L, NaHCO₃ 9 mM/L and glucose 11.1 mM/L.

Group 4 (Adenosine + ATP group): The same solution as in group 3 + ATP 10 mM/L.

Protocol
Ten min after the heart had begun to work we recorded the ventricular contractile force, heart rate and also collected perfusate samples from the right atrium to determine tissue enzymes. The hearts were arrested by introduction of one of the cardioplegic solutions from the aortic root at a rate of 2 ml/min for 3 min at 4°C. During this 3 min period of plegic infusion the arrest time and number of beats were assessed. Following cardiac arrest the cardioplegic solution was removed by infusion of 2 ml isotonic saline from the aortic root. During the ischemic period the hearts were kept at 8-10°C by topical cooling with isotonic saline. After 45 min of ischemia, reperfusion was begun using the same buffer at 37°C. At the tenth minute of reperfusion, ventricular contractility and heart rate were recorded again and perfusate samples were collected (Fig. 1).

Calculations
The following calculations were made.

Arrest time: Time (seconds) from the onset of cardioplegic infusion until the

![Fig. 1. Experimental protocol.](image-url)
heart arrests.

Arrest beats: Number of heart beats during the 3 min of cardioplegic infusion.

Percentage recovery of heart rate = \( \frac{\text{Postischemic heart rate}}{\text{Preischemic heart rate}} \times 100 \)

Percentage recovery of ventricular contractile force
\[ = \frac{\text{Postischemic contractions (mm/g)}}{\text{Preischemic contractions}} \times 100 \]

Percentage recovery of heart work
\[ = \frac{\text{Postischemic heart rate} \times \text{Postischemic contractions}}{\text{Preischemic heart rate} \times \text{Preischemic contractions}} \times 100 \]

Percentage change of tissue enzymes
\[ = \frac{\text{Postischemic enzyme (LDH, SGOT, SGPT) concentrations}}{\text{Preischemic enzyme concentrations}} \times 100 \]

**Data and statistics**

Ventricular contractile force (g) and heart rate (beats/min) were recorded through an isometric force transducer (Ugo Basile 7004) connected to a microdynamometer (Ugo Basile 7050). The biochemical parameters (LDH, SGOT, SGPT) were calculated by a "Technican RA-1000" autoanalyzer using "chromatest" kits. For statistical work, Student's t-test and one way analysis of variance were done using a "Microstat" PC program.

**Results**

**Effect of plegic solutions on cardiac arrest:**
The best results were achieved with adenosine and adenosine+ATP groups for both arrest time and arrest beats.

Time to cardiac arrest was reduced from 52.6±12.1 sec with the potassium group to 5.2±1.8 with the adenosine group, 38.7±8.2 with the potassium+ATP group and 7.4±1.5 with the adenosine+ATP group (Fig. 2).

The number of beats during the cardioplegic infusion (3 min) was reduced from 38.7±8.3 with the potassium group to 5.2±1.1 with the adenosine group, 23.2±7.4 with the potassium+ATP group and 7.8±0.9 with the adenosine+ATP group (Fig. 2).

**Effect of plegic solutions on myocardial functions:**
There were no statistically significant differences among the groups for postischemic percentage recovery of heart rate.

As for percentage recovery of postischemic ventricular contractile force,
EFFECTS OF PLEGIC SOLUTIONS
ON CARDIAC ARREST

Fig. 2. Arrest time: Time between the onset of cardioplegic infusion and cardiac asystole. Arrest beats: Number of heart beats during cardioplegic infusion. (**) indicates significant difference (p<0.01) between indicated group and potassium group (control group). Values are mean± standard error of the mean.

EFFECTS OF PLEGIC SOLUTIONS
ON MYOCARDIAL RECOVERY

Fig. 3. Postischemic recovery of heart rate, ventricular contractions and heart work according to their preischemic values. (*) indicates significant difference (p<0.05) between value indicated and potassium (control) groups. Values are mean±SEM.

the results were best in the adenosine (77.4±5.2%) and potassium+ATP (71.5±4.7%) groups when compared with the potassium (53.7±6.7%) and adenosine+ATP (50.4±5.5%) groups.

For postischemic percentage recovery of heart work, again the results were significantly better in the adenosine (92.6±10.1%) and potassium+ATP (88.4±9.1%) groups compared to the potassium (45.7±2.6%) and adenosine+ATP (48.7±4.3%) groups (Fig. 3).
EFFECTS OF PLEGIC SOLUTIONS ON TISSUE ENZYME LEVELS

Fig. 4. Postischemic concentrations of lactic dehydrogenase (LDH), serum glutamic pyruvate transaminase (SGPT) and serum glutamic oxalic transaminase (SGOT) of the perfusate as percentage change of their preischemic values. (§) indicates significant difference (p<0.1), (*) indicates significant difference (p<0.05) between indicated group and potassium (control) group. Values are mean±SEM.

Effect of plegic solutions on postischemic myocardial injury:
Although there were no significant differences between the groups for postischemic percentage change in SGOT levels, the results of SGPT change were better in the adenosine (105±11%) and potassium+ATP (102±9%) groups compared with the potassium (147±8%) and adenosine+ATP (140±10%) groups. The postischemic elevation of LDH levels were significantly less in the adenosine (134±12%), potassium+ATP (117±10%) and adenosine+ATP (120±10%) groups when compared with the potassium (200±16%) group (Fig. 4).

DISCUSSION

This study demonstrated that supplementing potassium cardioplegia with ATP or using adenosine instead of potassium alone in the cardioplegic solution had nearly the same beneficial effect on postischemic myocardial recovery and on postischemic tissue injury.

If we agree with the concept that most of the exogenous ATP rapidly hydrolyzes to adenosine,8,9 it may be possible to explain our results by the following actions of adenosine which were studied by several investigators. 1) Adenosine inhibits generation of superoxide anion and hydrogen peroxide by activated neutrophils.10,11 2) It inhibits adherence of stimulated neutrophils to endothelial cells.12 3) It stimulates glycolysis and hence increases cellular ATP.13 4) It decreases oxygen demand, reduces the degradation of
ATP AND ADENOSINE IN CARDIOPLEgia

ATP during ischemia and improves repletion of ATP during reperfusion.\textsuperscript{13)–15)

One thing difficult to explain in our study is the fact that the addition of ATP to adenosine cardioplegia surprisingly had an unfavorable effect on post-ischemic myocardial recovery. Since there is no evidence of antagonistic mechanisms between ATP and adenosine, our result may be due to our use of high concentrations of both ATP and adenosine. So it can be argued that the combination of high concentrations of ATP and adenosine in cardioplegia induces poor myocardial recovery because of improved myocardial depression.

The other objective of our study was to examine the arrest effect of the solutions. Although we could achieve a more rapid cardiac arrest with adenosine cardioplegia and with ATP supplemented adenosine cardioplegia, no superior effects could be shown by the addition of ATP to classical potassium cardioplegia.

Our results with adenosine corroborate those of previous studies.\textsuperscript{5),6),16) Probably adenosine itself was also responsible for most of the effects in the ATP supplemented adenosine group. ATP seems not to have any role in promoting rapid cardiac arrest when it was added to classical potassium cardioplegia. If we did not have any experimental error, this means that adenosine which is hydrolyzed by ATP acts differently from exogenous adenosine, or ATP was not hydrolyzed to adenosine effectively and rapidly under our experimental conditions.

In conclusion, we can argue that rapid cardiac arrest and improved post-ischemic recovery can be achieved by using adenosine at 10 mM/L instead of potassium in cardioplegic solutions. Except for rapid cardiac arrest, nearly the same results can also be achieved by adding ATP 10 mM/L to classical potassium cardioplegia. On the other hand, ATP as an additive to high dose adenosine cardioplegia has a particularly poor effect on postischemic myocardial recovery.

\textbf{References}

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