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QIAO, JIAN HUA

(Citation)
The Kobe journal of the medical sciences,34(6):285-302

(Issue Date)
1988-12

(Resource Type)
departmental bulletin paper

(Version)
Version of Record

(URL)
https://hdl.handle.net/20.500.14094/0100488694

Kobe J. Med. Sci. 34, 287/304, December 1988

AN EXPERIMENTAL STUDY ON THE ADDITIONAL MYOCARDIAL PROTECTIVE EFFECT OF ADENOSINE TRIPHOSPHATE AND NIFEDIPINE IN CARDIOPLEGIC SOLUTION

JIAN HUA QIAO M.D., KEIJI ATAKA, IKUSHI NISHIKAWA AND RAN ZHAO

Department of Surgery, Division II
Kobe University School of Medicine

INDEXING WORDS

adenosine triphosphate; nifedipine; isolated and perfused working rat heart model

SYNOPSIS

The potential protective effect on the ischemic myocardium of adenosine triphosphate (ATP) and nifedipine added to St.Thomas's Hospital cardioplegic solution was investigated by using isolated and perfused working rat heart model. Dose-response study with ATP ranging between 2.0 and 8.0 mmol/L showed 4.0 mmol/L to be the optimal concentration for recovery of aortic flow after 35 minutes of normothermic (37°C) ischemic arrest. Aortic flow after reperfusion remained at 50.3±5.3% of preishemic value in the ATP-free control group, whereas it recovered to 87.7±1.9% of preischemic value ($p<0.001$) and CPK leakage was reduced by 84% ($p<0.01$) in the ATP group. At its optimal concentration (4.0 mmol/L), ATP as well as ATP-$MgCl_2$ (1:1) as an additive to the K cardioplegic solution showed improved recovery of aortic flow to 71.5±1.5% ($p<0.05$) and to 80.3±1.4% ($p<0.001$) of the preischemic value, respectively. The myocardial protection of ATP-$MgCl_2$ was better than ATP alone ($P<0.001$). Dose-response study with nifedipine ranging between 0.2 and 1.0 mg/L showed 0.4 mg/L to be the optimal concentration for

Received for publication : December 28, 1988
Authors' names in Japanese : 喬　建華, 安宅啓二, 西川育志
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recovery of aortic flow after 35 minutes of normothermic (37°C) ischemic arrest. Aortic flow after reperfusion remained at 50.3±5.3% of preischemic control value in the nifedipine-free control group, but it recovered to 89.0±1.2% of preischemic control value in the nifedipine group ($p<0.001$). CPK leakage was reduced by 81% ($p<0.01$) by adding nifedipine to the ordinary St. Thomas' Hospital cardioplegic solution. The combined effects of ATP and ninedipine were evaluated by adding them to the St. Thomas' Hospital cardioplegic solution as well as K cardioplegic solution. The result showed that ATP and nifedipine added to the K cardioplegic solution in combination resulted in a greater protection; namely, while the recovery of aortic flow after reperfusion remained at 87.8±1.9% of preischemic value in the ATP alone group and at 89.0±1.2% in the nifedipine alone group, it increased to 93.7±1.1% in the combination group. In conclusion, ATP and nifedipine markedly improved the myocardial protecting properties of St. Thomas' Hospital cardioplegic solution during 35 minutes of normothermic (37°C) ischemic arrest.

INTRODUCTION

Ischemic contracture of the left ventricle ("stone heart") occurs in the rat heart after a period of global ischemia at 37°C. The onset of contracture may be delayed by various interventions that can reduce myocardial energy demand, increase myocardial energy supply or reduce cellular calcium influx.

Many studies have demonstrated that there is a clear correlation between structural preservation of ischemic cells and cellular contents of high-energy phosphates, particularly adenosine triphosphate (ATP). Although ATP has been administered with enhanced mytocardial protection,[10,7] there is a great variation in the optimal concentration of ATP used in different cardioplegic solutions. Hearse et al. [8] reported that the optimal concentration of ATP in potassium cardioplegic solution was 10 mmol/L. Robinson et al..[14] reported that the optimal concentration of ATP in the St. Thomas' Hospital cardioplegic solution (hereafter, referred as STHCS) was 0.1 mmol/L. On the other hand, several investigators still have negative viewpoint, because they could not get good result from experiment by adding ATP to a cardioplegic

solution . They speculated that an exogenous ATP could not penetrate the membrane of cell, therefore, it could not play a protection for ischemic myocardium. Whether the exogenous ATP can penetrate the cell membrane or not is a point of controversy, and further experiment seems to be necessary for clarifying the mechanism of ATP to enhance the myocardial protection. Another way to delay the development of ischemic contracture of the left ventricle is to reduce cellular calcium influx. The nifedipine is a new calcium antagonist. Combined addition of ATP and nifedipine to the STHCS may be an effective way to enhance the myocardial protection, but, up to date, we can not find any reports for confirming this presumption. The present study was designed for four purposes, namely, 1) to determine, if exogenous ATP, added to cardioplegic solution exogenously, improves myocardial tolerance to ischemia and to define the dose-response characteristics of ATP and its optimal concentration, 2) to define the mechanism of possible protective effect of ATP, 3) to determine, if nifedipine, added to a cardioplegic solution, improves myocardial tolerance to ischemia and to define the dose-response characteristics of nifedipine and its optimal concentration, and 4) to evaluate the effect of combination of exogenous ATP and nifedipine on myocardial protection.

MATERIALS AND METHOD

Experimental preparation

Hearts were obtained from male S-D rats 300 to 400 gm in body weight. The isolated, perfused and working rat heart preparation was used in this study, and it has been described in detail elsewhere.[12] In this isolated heart preparation, oxygenated perfusion medium (Krebs-Henseleit bicarbonate buffer, [17] maintained at 37°C, pH 7.4, containing glucose 11.1 mmol/L and aerated with 95% oxygen and 5% carbon dioxide) enters the cannulated left atrium at a pressure of 18 cm H_2O. The perfusate passes into the left ventricle, from which it is ejected via an aortic cannula against a hydrostatic pressure of 80 cm H_2O. Coronary effluent, flowing from the pulmonary artery, can be sampled for biochemical analysis. The situation of total cardiopulmonary bypass with

coronary perfusion is simulated by clamping the left atrial cannula and introducing perfusion fluid at 37°C into the aorta from a reservoir located 80 cm above the level of the heart. This preparation allows the heart to continue to beat without any external work. Ischemic cardiac arrest can be induced by clamping the aortic cannula. During ischemia the heart is maintained at 37° C by using heating or cooling circuits supplying the water-jacketed heart chamber. Infusion of cardioplegic solution into coronary arteries is achieved by use of a reservoir (pressure 50 mmHg) attached to a side arm of the aortic cannula.

Cardioplegic solution

The composition of the STHCS and potassium (K) cardioplegic solution used in this study as the basic solutions are shown in Table I. The K cardioplegic solution is the STHCS without magnesium chloride. After addition of ATP or nifedipine, a small amount of sodium bicarbonate is added to cardioplegic solution for maintaining the pH of the solution at 7.8, but no significant change in sodium concentration of the basic solution occurs.

Experimantal time course

1) Immediately after excision of the heart, the aorta was cannulated and Langendorff perfusion was initiated for a 5-minute washout period. Left atrial cannulation was accomplished during 5 minutes. The perfusion fluid was Krebs-Henseleit bicarbonate buffer (37°C) during this period and subsequent perfusion period. 2) The heart was then converted to a working preparation by terminating retrograde aortic perfusion and initiating left atrial perfusion. In a 20-minute period of preischemic working control, values for aortic-and coronary flow, aortic pressure and heart rate were recorded as controls. 3) At the end of control period, atrial and aortic cannulas were clamped and heart was subjected to a 3-minute period of normothermic (37°C) coronary perfusion with various cardioplegic solutions. Then, perfusion was terminated and entire heart was maintained in a status of normothermic (37°C) ischemic arrest for 35 minutes. 4) After this period, the heart was reperfused initially in the Langendorff model for 10 minutes for collection of coronary effluent for CPK determination. 5)

After 10 minutes of non-working reperfusion period in Langendorff model, the hearts were converted to the working model for another 30 minutes and the recovery of aortic and coronary flow, heart rate and aortic pressure were recorded. Microscopic specimen was taken for checking change of the tissue, then hearts were heated for 24 hours for determination of the water content of the myocardium.

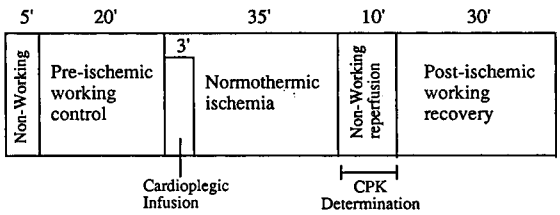

Fig. 1. Experimental time course: Hearts were perfused for 5 minutes in the Langendorff non-working model, thereafter, they were converted to working preparation in which control value for cardiac functional indices were recorded for 20 minutes. After 3 minutes of cardioplegic infusion, hearts were subjected to 35 minutes of normothermic (37°C) ishcemia. The hearts were then reperfused in the Langendorff model for 10 minutes for collection of the coronary effluent for CPK determination. Finally, recovery of hearts was monitored for 30 minutes.

Expression of results

During the preischemic working control period, the following variables were recorded: heart rate (HR),aortic pressure (AP), aortic flow (AF) and coronary flow (CF). Cardiac output (CO) was derived from the sum of aortic and coronary flow, and stroke volume (SV) was obtained by dividing cardiac output by heart rate. Minute work (MW) was derived by multiplying cardiac output by systolic pressure. The absolute values for the various indices of cardiac function obtained during working recovery period in individual hearts were expressed in terms of percent of those values obtained during the preischemic control period and compared with each other. CPK leakage was expressed as an international unit per 10-minute per gram of dry weight. At least, six hearts were used for each condition studied and all data were expressed

as mean± standard error. Statistical analysis of the results was made by unpaired Student's t test and statistical significance was assumed when P values were 0.05 or less.

Table I. St. Thomas' Hospital cardioplegic solution (without procaine)

Compound	Concentration (mmol/L)
Sodium chloride	110.0
Potassium chloride	16.0
Magnesium chloride	16.0
Calcium chloride	1.2
Sodium bicarbonate	10.0
pH adjusted to 7.8	
Osmolarity = 324 mOsm H_2O	

Potassium (K) cardioplegic solution is the St. Thomas' Hospital cardioplegic solution without magnesium chloride.

Table II. Effect of adenosine triphosphate (ATP) added to the basic STHCS upon the postischemic recovery of various indices of cardiac function and upon enzyme leakage after 35 minutes of normothermic (37°C) ischemia.

ATP concentration (mmol/L)	No. of experiment	Heart rate	Aortic pressure	Aortic flow	Coronary flow	Stroke volume	Minute work	CPK leakage (IU/10 min/gm dry weight)	Percent reduction of CPK
		Percent recovery after 40 min reperfusion	Percent recovery after 40 min reperfusion	Percent recovery after 40 min reperfusion	Percent recovery after 40 min reperfusion	Percent recovery after 40 min reperfusion	Percent recovery after 40 min reperfusion		
0.0	n: 8	84.9 ± 4.4	94.6 ± 2.6	50.3 ± 5.3	74.6 ± 3.2	64.3 ± 3.7	52.1 ± 3.6	1.14 ± 0.21	0
2.0	n: 6	85.2 ± 5.0	99.3 ± 0.5	65.0 ± 4.2	88.7 ± 4.1[a]	83.7 ± 5.4[b]	74.5 ± 3.8[c]	0.83 ± 0.41	27
4.0	n: 6	88.2 ± 3.0	97.3 ± 2.3	87.8 ± 1.9[c]	96.7 ± 2.2[c]	98.2 ± 1.8[c]	91.2 ± 2.4[c]	0.19 ± 0.12[b]	84[b]
6.0	n: 6	82.7 ± 3.8	97.0 ± 1.4	69.3 ± 7.0[a]	95.2 ± 2.8[c]	94.0 ± 4.1[c]	78.0 ± 6.0[b]	0.59 ± 0.41	48
8.0	n: 6	72.7 ± 3.8[a]	97.2 ± 2.3	61.7 ± 1.6	85.8 ± 2.3[a]	89.8 ± 2.5[c]	66.8 ± 2.7[b]	1.54 ± 1.23	0

Percent recovery in each concentration group for each variable was compared with the percent recovery in the control cardioplegic solution group and statistically analyzed by Student's t test. Statistically significant value are marked with a=$p<0.05$, b=$p<0.01$ and c=$p<0.001$.

RESULTS

Myocardial protective effect of ATP and its optimal concentration in normothermic (37°C) ischemia.

The results were shown in Table II, Fig. 2 and Fig. 3. Addition of ATP in the amount of 2.0, 4.0, 6.0 and 8.0 mmol/L to basic STHCS afforded additional protection with peak protection occuring at 4.0 mmol/L. At this optimum, postischemic recovery of

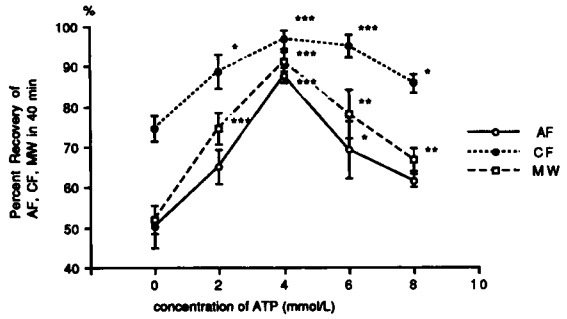

Fig. 2. The relationship between the concentration of ATP (mmol/L)in the basic STHCS and postischemic recovery of aortic flow (AF), coronary flow (CF) and minute work (MW) expressed as a percentage of their preischemic control values. Recovery was measured at the end of a 40-minute reperfusion period after 35 minutes of normothermic (37°C) ischemia. Statistically significant values compared with the ATP-free control group are marked with * p<0.05,**p<0.01, ***p<0.001.

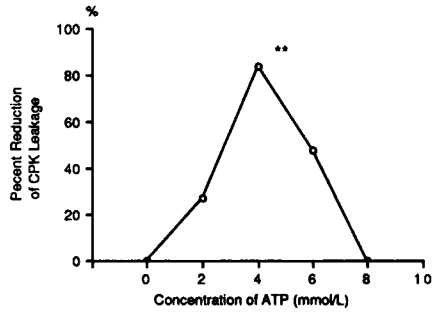

Fig. 3. The relationship between the ATP concentration in the basic STHCS and the percent reduction of APK leakage (the absolute amount of CPK released during reperfusion in each ATP concentration group was compared to the amount of enzyme released from the hearts of the ATP-free control group). The actual values of enzyme released were used for calculation and statistical comparisons with the ATP-free control group. *p<0.05, **p<0.01.

AF was 87.8±1.9% of preischemic control value. This is far better than the recovery of ATP-free control group which showed 50.3±5.3% of preischemic value. This substantial improvement in myocardial protection was also reflected by CF, SV and MW (Fig. 2). The dose-response curves for cardiac function were paralleled by the results of CPK leakage (Fig. 3), again indicating substantial additional protection of the ischemic myocardium expressed by 84% reduction ($p<0.01$) of CPK leakage at optimal ATP concentration (4.0 mmol/L). No significant beneficial effect in terms of enzyme leakage was observed at higher or lower concentrations.

Myocardial protective effect of ATP and ATP-$MgCl_2$ added to the K cardioplegic solution

As an optimal concentration, 4.0 mmol/L of ATP and ATP-$MgCl_2$ (1:1) are used in this study. The results are shown in Table III,

Table III. Effect of ATP and ATP-$MgCl_2$ (1:1) added to the K cardio plegic solution upon the postischemic recovery of various indices of cardiac function and upon enzyme leakage after 35 minutes of normothermic (37°C) ischemia.

Group	No. of experiment	Heart rate Percent recovery after 40 min reperfusion	Aortic pressure Percent recovery after 40 min reperfusion	Aortic flow Percent recovery after 40 min reperfusion	Coronary flow Percent recovery after 40 min reperfusion	Stroke volume Percent recovery after 40 min reperfusion	Minute work Percent recovery after 40 min reperfusion	CPK leakage (IU/10 min/ gm dry weight)	Percent reduction of CPK
control	n: 8	84.9 ± 4.4	94.6 ± 2.6	50.3 ± 5.3	74.6 ± 3.2	64.3 ± 3.7	52.1 ± 3.6	1.14 ± 0.21	0
ATP	n: 6	92.0 ± 3.4	92.1 ± 2.9	71.5 ± 1.5^{a}	98.7 ± 0.8^{c}	84.3 ± 3.8^{b}	76.8 ± 1.0^{c}	0.32 ± 0.2^{a}	72^{a}
ATP-$MgCl_2$	n: 6	97.3 ± 0.8^{a}	95.1 ± 3.3	80.3 ± 1.4^{c,C}	95.4 ± 2.2^{c}	87.9 ± 2.3^{c}	83.0 ± 1.4^{c,B}	0.18 ± 0.1^{b}	84^{b}

Control group : no ATP or ATP-$MgCl_2$ in the solution.
ATP group : ATP 4.0 mmol/L in the solution.
ATP-$MgCl_2$ group : ATP 4.0 mmol/L and $MgCl_2$ 4.0 mmol/L in the solution.
Percent recovery in ATP or ATP-$MgCl_2$ group for each variable was compared (Student's t test) with the values of the control group and each other. In comparison with control group, a=$p<0.05$, b=$p<0.01$, c=$p<0.001$. In comparison between ATP group and ATP-$MgCl_2$ group, B=$p<0.01$, C=$p<0.001$.

Fig. 4 and Fig. 5. Recovery of AF in the control group was 50.3±5.3%, but it reached to 71.5+1.5% in the ATP group ($p<0.05$).

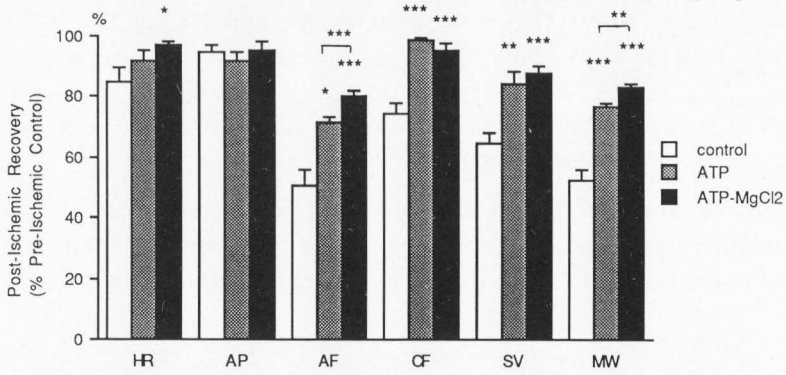

Fig. 4. Myocardial protective effect of ATP and ATP-$MgCl_2$(1:1) added to the K cardioplegic solution upon the postischemic recovery of various indices of cardiac function after 35 minutes of normothermic (37°C) ischemia. Comparison was made with the control group (ATP-free), or between ATP group and ATP-$MgCl_2$ (1:1) group. Statistically significant values in comparison among three groups are marked with *p<0.05, **p<0.01, ***p<0.001.

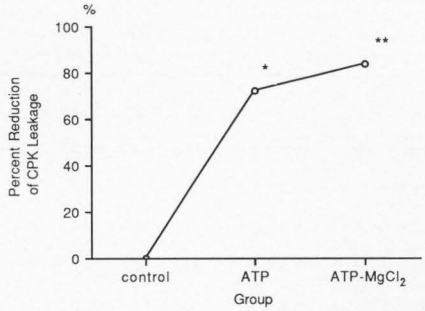

Fig. 5. Myocardial protective effect of ATP and ATP-$MgCl_2$ (1:1) added to the K cardioplegic solution upon CPK leakage after 35 minutes of normothermic (37°C) ischemia. The actual values of enzyme release was used to calculate the statistical as compared with control group: *p<0.05, **p<0.01.

Significant improvements in CF, SV and MW ($p<0.01$) were also observed. CPK leakage decreased from 1.14±0.21 IU/10 min/gm dry weight in control group to 0.32±0.2 IU/10 min/gm dry weight in the ATP group ($p<0.05$). Substantial improvements in the recovery of aortic flow (AF) was observed in the ATP-$MgCl_2$ group. It was 80.3±1.4%, in contrast to 50.3±5.3% in the control group, ($p<0.001$). Significant improvements in CF, SV and MW ($p< 0.001$) were clearly observed. CPK leakage decreased from 1.14±0.21 IU/ 10

min/gm dry weight in control group to 0.18±0.1 IU/10 min/gm dry weight in the ATP-$MgCl_2$ group. Comparison between ATP and ATP-$MgCl_2$ disclosed that the recovery of AF and MW in the ATP-$MgCl_2$ group was better than that of ATP alone group.

Myocardial protective effect of nifedipine and its optimal concentration in normothermic (37°C) ischemia

The results are shown in Table IV, Fig.6 and Fig.7. Addition of nifedipine to the STHCS in the range between 0.2 and 1.0 mg/L afforded substantial additional protection, with peak protection

Table IV. The effect of nifedipine added to basic STHCS on the postischemic recovery of various indices of cardiac function and upon enzyme leakage after 35 minutes of normothermic (37°C) ischemia.

Nifedipine concentration (mg/L)	No. of experiment	Heart rate	Aortic pressure	Aortic flow	Coronary flow	Stroke volume	Minute work	CPK leakage (IU/10 min/gm dry weight)	Percent reduction of CPK
		Percent recovery after 40 min reperfusion	Percent recovery after 40 min reperfusion	Percent recovery after 40 min reperfusion	Percent recovery after 40 min reperfusion	Percent recovery after 40 min reperfusion	Percent recovery after 40 min reperfusion		
0.0	n: 8	84.9 ± 4.4	94.6 ± 2.6	50.3 ± 5.3	74.6 ± 3.2	64.3 ± 3.7	52.1 ± 3.6	1.14 ± 0.21	0
0.2	n: 6	76.0 ± 3.9	98.7 ± 0.8	71.3 ± 3.7[a]	91.2 ± 2.4[b]	97.2 ± 1.9[c]	78.5 ± 4.3[c]	0.67 ± 0.46	42
0.4	n: 6	87.2 ± 1.7	98.7 ± 1.0	89.0 ± 1.2[c]	98.5 ± 1.0[c]	99.5 ± 0.5[c]	91.5 ± 1.7[c]	0.22 ± 0.15[b]	81[b]
0.6	n: 6	79.2 ± 4.4	97.5 ± 0.6	81.5 ± 2.0[c]	98.0 ± 0.9[c]	98.3 ± 1.7[c]	83.3 ± 2.0[c]	0.40 ± 0.18[a]	65[a]
0.8	n: 6	78.0 ± 3.2	98.0 ± 1.0	76.3 ± 4.3[b]	93.6 ± 3.3[b]	94.7 ± 1.8[c]	78.0 ± 4.0[c]	0.52 ± 0.38	55
1.0	n: 6	77.8 ± 2.0	99.5 ± 0.3	71.8 ± 2.9[b]	93.5 ± 1.5[b]	92.5 ± 2.7[c]	76.8 ± 2.2[c]	1.42 ± 0.73	0

Percent recovery in each concentration group for each variable was compared with the percent recovery in the Nifedipine-free control cardioplegic solution group and statistically analyzed by the Student's t test. Statistically significant values are marked with a=p<0.01, b=p<0.01, c=p<0.001.

occuring at 0.4 mg/L. At this optimum, postischemic recovery of the AF was 89.0±1.2% of preishcemic value whereas it was 50.3 ±5.3% in nifedipine-free control group (p<0.001). Thus, a marked

improvement in myocardial protection was also reflected by CF, SV and MW ($p<0.001$ see Table IV). Improvement in protective effect on cardiac function of nifedipine was paralleled by the result for CPK leakage (Table IV and Fig. 7), which showed almost 81% reduction ($p<0.01$) at optimal nifedipine concentration.

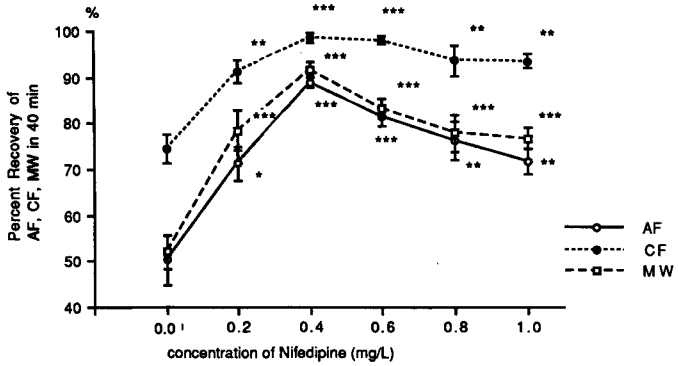

Fig. 6. The relationship between the concentration of nifedipine (mg/L) in the basic STHCS and the postischemic recovery of AF, CF and MW expressed as a percentage of their preischemic control values. Recovery of the hearts observed at the end of a 40-minutes reperfusion period after 35 minutes of normothermic (37°C) ischemia. Statistically significant values compared with the nifedipine-free control group are marked with *p<0.05, **p<0.01, ***p<0.001.

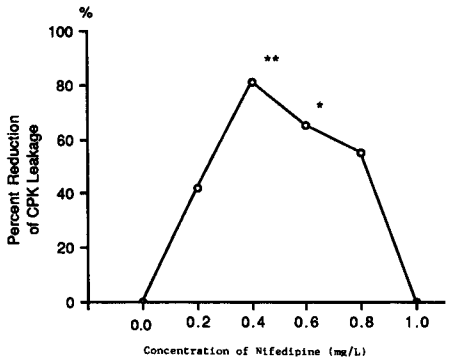

Fig. 7. The relationship between the nifedipine concentration in the basic STHCS and the percent reduction of CPK release (the absolute amount of CPK released during reperfusion in each nifedipine concentration group was compared to the amount of enzyme released from the heart of the control group). The actual values of enzyme relased were used for calculation and statistical comparisons with the Nifedipine-free control group: *p<0.05;**p < 0.01.

Comparison of myocardial protective effect of the various combination of ATP, ATP-$MgCl_2$ and nifedipine added to the basic St. Thomas' Hospital cardioplegic solution or the K cardioplegic solution

Myocardial protective effects of the ATP, ATP-$MgCl_2$ and nifedipine added o the STHCS or K cardioplegic solution in various combination were studied. Concentrations of ATP, ATP-$MgCl_2$ and nifedipine in this study were derived from the results in the experiment described above. The results are shown in Table V, Fig. 8 and Fig. 9.

Table V. Comparison of the myocardial protective effect of the various combination of ATP, ATP-$MgCl_2$ (1:1) and nifedipine added to the STHCS or K cardioplegic solution. Concentration of these additives was derived from the result of the experiment described in the previous paragraphs, namely, ATP in 4.0 mmol/L, nifedipine in 0.4 mg/L and ATP-$MgCl_2$ (1:1) in 4.0 mmol/L.

Group	No. of experiment	Heart rate Percent recovery after 40 min reperfusion	Aortic pressure Percent recovery after 40 min reperfusion	Aortic flow Percent recovery after 40 min reperfusion	Coronary flow Percent recovery after 40 min reperfusion	Stroke volume Percent recovery after 40 min reperfusion	Minute work Percent recovery after 40 min reperfusion	CPK leakage (IU/10 min/ gm dry weight)	Percent reduction of CPK
control(St)	n: 8	84.9 ± 4.4	94.6 ± 2.6	50.3 ± 5.3	74.6 ± 3.2	64.3 ± 3.7	52.1 ± 3.6	1.14 ± 0.21	0
A+St	n: 6	88.2 ± 3.0	97.3 ± 2.3	87.8 ± 1.9^{c}	96.7 ± 2.2^{c}	98.2 ± 1.8^{c}	91.2 ± 2.4^{c}	0.19 ± 0.12^{b}	84^{b}
N+St	n: 6	87.2 ± 1.7	98.7 ± 1.0	89.0 ± 1.2^{c}	98.5 ± 1.0^{c}	99.5 ± 0.5^{c}	91.5 ± 1.7^{c}	0.22 ± 0.15^{b}	81^{b}
A+N+St	n: 6	81.7 ± 3.9	99.8 ± 0.2	89.2 ± 5.9^{c}	96.5 ± 3.3^{c}	99.0 ± 2.5^{c}	95.3 ± 5.1^{c}	0.16 ± 0.16^{b}	86^{b}
A-M+N+K	n: 6	85.3 ± 3.1	100.0 ± 0.0	93.7 ± 1.1^{c,A}	100.0 ± 0.0^{c}	100.0 ± 0.0^{c}	97.3 ± 1.2^{c,A}	0.30 ± 0.21^{a}	74^{a}

Percent recovery in each combination group for each variable was compared with the percent recovery in the control or basic STHCS group and statistically analyzed by Student's t test. Statistically significant values are marked with a=$p<0.05$, b=$p<0.01$, c=$p<0.001$, and A=$p<0.05$.

A + St: Group with the STHCS combined with ATP.

N + St: Group with the STHCS combined with nifedipine.

A+N+St: Group with the STHCS combined with ATP and nifedipine.

A-M + N + K: Group with K cardioplegic solution combined with ATP-$MgCl_2$ and nifedipine.

It was clear that ATP and nifedipine added to the STHCS at its optimal concentration showed marked enhancement of the protective properties of the STHCS. Moreover, combination of these agents exhibited an effect equal to or superior to the group in which each agent was used alone. The best protective effect was obtained when ATP-$MgCl_2$ (1:1) and nifedipine were added to the K cardioplegic solution.

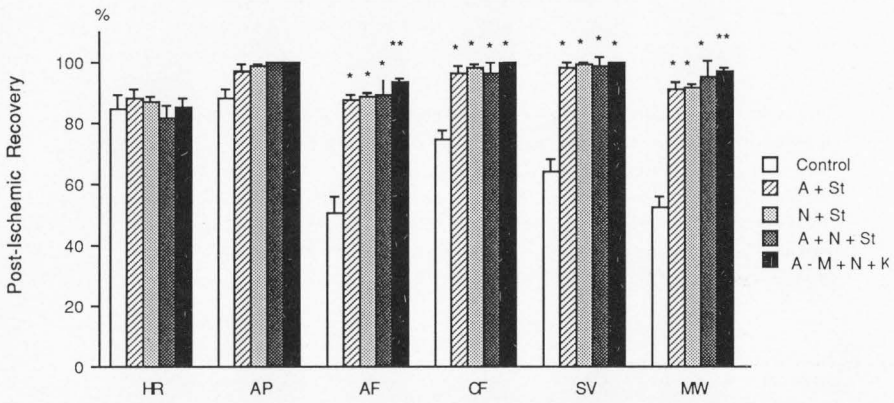

Fig. 8. The postischemic recovery of cardiac functions in the groups treated with various types of myocardial protective agents. (expressed as a percentage of the preischemic control value).

Legends:

Control : ATP-free and nifedipine-free, STHCS.

A + ST : SYHCS containing ATP in the amount of 4.0 mmol/L.

N + St : STHCS containing nifedipine in the amount of 0.4 mg/L.

A+N+St : STHCS containing ATP 4.0 mmol/L and nifedipine 0.4 mg/L.

A-M+N+K : Potassium (K) cardioplegic solution containing ATP-$MgCl_2$ (1:1) 4.0 mmol/L and nifedipine 0.4 mg/L.

Statistical analysis : comparing with control group : *$p<0.001$.

Statistical analysis : comparing A-M + N + K goup with A + St group or N + St group : ** $p<0.05$.

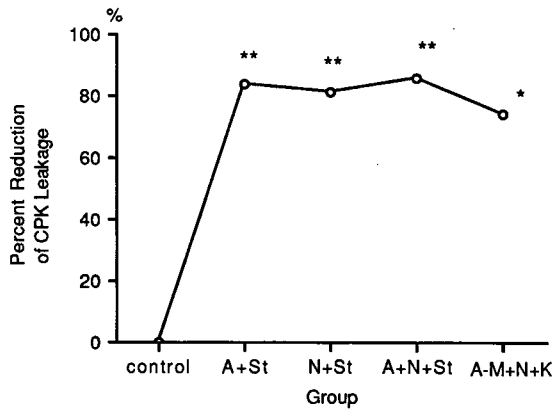

Fig. 9. Reduction of CPK leakage after a 35-minute normothermic (37°C) ischemia in the groups treated with various types of myocardial protective agents.
Legends : same as in Fig. 8.
Statistical analysis was based on the calculation using actual values of enzyme released, and compared with the values of the control group. *$p<0.05$, **$p<0.01$.

DISCUSSION

The ischemic contracture of the left ventricle after anoxic cardiac arrest in open-heart surgery has been attributed to several mechanisms[11]. One explanation is the development of ATP-sensitive, calcium-insensitive rigor complexes. Alternatively, a calcium-sensitive systolic arrest may occur when high concentration of calcium in the vicinity of the myofibrils may prevent normal relaxation. From the studies of myocardial carbon dioxide tensions during ischemia, MacGregor et al.[11] inferred that the onset of contracture was associated with a cessation of glycolytic anaerobic ATP production and they concluded that ischemic contracture could be adequately explained in terms of ATP depletion without resorting to calcium involvement.

In the global ischemia there is an effective cessation of oxydative metabolism and ATP production. Despite a rapid decline of contractile activity with decreased oxygen demand induced by potassium and magnesium infusion, cellular energy demand exceeds cellular energy supply and there is a rapid depletion of creatine

phosphate stores in the myocardium. It was suspected from the mechanisms described above that the onset of ischemic contracture may be delayed by conserving or maintaining cellular ATP content. Addition of ATP to cardioplegic solution may be the one to resolve the problem of ischemic contracture.

However, there are several other groups who have different opinion, because they could not get good result from experiment by adding ATP to cardioplegic solution. Forker, Einzig, and Wang[5] found that introcoronary adenosine triphosphate infusion showed no effect on myocardial ATP content after 20 minutes of normothermic ischemia in dog, though this may due to coronary blood adenosine deaminase that degrades adenosine to inosine. Reibel and Rovetto[13] also found no protective effect of adenosine in the ischemic rat heart. Someone thought that ATP could not penetrate the intact cell membrane, because of its polar nature, its instability in solution, its high reactivity and electrical charge and its large molecular size. Therefore, ATP added to cardioplegic solution can not be utilized by myocardial cells and can not play a role in the myocardial protection. Whether or not ATP can penetrate the cell membranes is a focal point of controversy. Taleat[16] indicated for the first time that ATP was useful in treating shock through protection of anoxic cells. But, Glynn[6] confirmed experimentally that ATP can not penetrate the cell membranes and could not be utilized by cells. Up to 1974, Chaudry[2] reported an experimental data indicating that if ATP was combined with magnesium chloride forming ATP-$MgCl_2$ energy complex, it could penetrate the cell membranes and provide energy to cells directly. At the same time, many investigators indicated through a series of experiments that ATP-$MgCl_2$ could obviously increase survival rate from the hemorrhagic shock[1], severe burns[18], postischemic hepatic failure[9] and endotoxin shock[4], and also promoted recovery of acute renal failure[15].

The present study disclosed a bell-shaped dose-response curve for ATP added to the STHCS with an optimal protective concentration at 4.0 mmol/L. This may be due to the presence of magnesium chloride in the STHCS. It combines with ATP forming ATP-$MgCl_2$ (1:1) energy complex which enhances the myocardial protection of the STHCS. Higher and lower concentrations of ATP-$MgCl_2$ energy complex may be less effective in myocardial

protection. High ATP concentration may depress postischemic recovery of the cardiac function by the known calcium-chelating effect of high ATP concentrations and high concentration of extracellular ATP may suddenly exert a severe harmful effection the matabolism of calcium in the myocardium, as seen in the calcium paradox[3]. In order to clarify the mechanism of myocardial protection of ATP, ATP and/or ATP-$MgCl_2$ (1:1) was added to the K cardioplegic solution at its optimal concentration. The results confirmed that the myocardial protective effect of ATP-$MgCl_2$ was better than ATP alone. If ATP can be combined with $MgCl_2$ forming ATP-$MgCl_2$ energy complex, then it can improve the microcirculation of the myocardium, the cell function as well as the permeability of cell membrane and can correct the hormonal imbalance. Thus, ATP-$MgCl_2$ can penetrate the cell membrane to provide the energy to cells directly.

Another way to prevent ischemic contracture of the left ventricle is to reduce cellular calcium influx during ischemia. For this purpose, nifedipine was added to St. Thomas' Hospital cardioplegic solution in this experiment. The present study showed a substantial additional protective effect of nifedipine, with peak protection occuring at the concentration of 0.4 mg/L. But when ATP and nifedipine was added together at their optimal concentrations to the St Thomas' Hospital cardioplegic solution no additional protective effect on the ischemic myocardium was observed. On the other hand, addition of ATP-$MgCl_2$ (4.0 mmol/L : 4.0 mmol/L) in combination with nifedipine (0.4 mg/L) to the K cardioplegic solution (potassium 16.0 mmol/L, without magnesium) brought about a better myocardial protective effect than that of St. Thomas' Hospital cardioplegic solution, which contained ATP alone or nifedipine alone or combination of ATP and nifedipine. It was suggested from these experimental results that extracellular concentrations of potassium (16.0 mmol/L), ATP(4.0 mmol/L), magnesuim chloride (4.0 mmol/L) and nifedipine (0.4 mg/L) do not decrease the myocardial contractility, and enhanced the myocardial protection of K cardioplegic solution.

In conclusion, the results of the present study suggested that exogenous ATP and nifedipine added to the St. Thomas' Hospital cardioplegic solution at their optimal concentrations could offer considerable myocardial protection during 35 minutes

of normothermic (37°C) ischemia, and the optimal concentrations of ATP and nifedipine were 4.0 mmol/L and 0.4 mg/L, espectively when ATP could be combined with $MgCl_2$ forming ATP-$MgCl_2$ energy complex, its myocardial protection was better than ATP alone, suggesting that ATP enhancement of the myocardial protective effect of the STHCS was due to a formation of ATP-$MgCl_2$ in it .But, further experimental study will be necessary to evaluate their effects on prolonged and hypothermic ischemia on the myocardium, and its protection.

ACKNOWLEGEMENTS

The author wish to express his sincere gratitude to Prof. K. Nakamura, the Department of Surgery, Division II, for his generous guidance, encouragement and continuous support throughout this work.

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