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Research Article

Effects of ATP-$MgCl_2$ on Myocardial Ischemia-Reperfusion Injury: An In Vivo Experimental Study

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Abstract: We investigated the effect of ATP-$MgCl_2$ on myocardial hemodynamics and metabolism.

The study included 12 mongrel dogs that were randomly divided into 2 groups: the control and the ATP-$MgCl_2$ (study) groups. Following a right thoracotomy, the left anterior descending artery was ligated for 15 min and then reperfusion was performed. After the ischemic period, cardiopulmonary bypass was initiated and the aorta was cross-clamped. ATP-$MgCl_2$ (70+70 mmol/l) 0.25 ml/kg per h was given to the study group at the start of the ischemic period. Hemodynamic and metabolic measurements were obtained at basal, and then after 60, 120, and 180 min of reperfusion

Cardiac output values were significantly higher in the study group; at the end of the third hour of reperfusion they were 2370 ± 31 ml/min in the study group and 900 ± 45 ml/min in the control group ($P < 0.05$). Myocardial Oxygen Extraction (MOE) and Myocardial Lactate Extraction (MLE) values were significantly higher in the control group; at the end of the third hour of the reperfusion MOE values were 50 ± 2 ml in the study group and 55 ± 3 ml in the control group, while MLE values were -0.06 ± 0.02 mmol/ml in the study group and -0.12 ± 0.07 mmol/ml in the control group ($P < 0.05$).

Hemodynamic and metabolic parameters revealed that the use of ATP-$MgCl_2$ might be beneficial in reducing ischemia-reperfusion damage.

Key Words: In vivo experimental study, ischemia-reperfusion injury, myocardium, ATP-$MgCl_2$

ATP-$MgCl_2$ Kullanımının Myokard Hasarına Etkisi; İn Vivo Deneysel Çalışma

Özet: Bu çalışmada iskemi-reperfüzyon hasarında ATP-$MgCl_2$ kullanımının myokard üzerine etikisi hemodinamik ve metabolik parametreler yardımı ile araştırıldı.

Oniki adet sokak köpeği, ATP-$MgCl_2$ ve kontrol olmak üzere 2 eşit gruba rastgele olarak ayrılarak çalışmada kullanıldı. Bu işlemi izleyerek hayvanlara sağ torakotomi yapıldı ve perikard açıldı. Sol anterior desendens arteri 15 dakika süre ile ligatüre edilerek iskemik bırakıldı ve daha sonra ligatür açılarak reperfüzyon yapıldı. Daha sonra kardiyopulmoner bypasa girildi. Aortaya kros-klemp konulmasını takiben, aorta asendense konulan bir kanül yardımı ile soğuk kristaloid kardioplejik verilerek kardiak arrest sağlandı. Çalışma grubuna perfüzyon başlangıcından itibaren ATP-$MgCl_2$ (70+70 mmol/l) 0,25 ml/kg/saat hızında verildi, kontrol grubunda hiçbir sağaltım yapılmadı. Hemodinamik ve metabolik ölçümler bazal, 60 dakika, 120. dakika ve 180. dakikalarda alındı.

Çalışmamızda; kardiak output değerleri çalışma grubunda anlamlı olarak yüksekti. Çalışma ve kontrol grubunda 3. saatte 2370 ± 31 ml/dk ve 900 ± 45 ml/dk ($P < 0,05$) idi. MOE (miyokardial oksijen ekstraksiyonu) ve MLE (miyokardial laktat ekstraksiyonu) kontrol grubunda anlamlı olarak yüksekti. MOE perfüzyonun 3. saati sonunda çalışma grubunda 50 ± 2 ml, kontrol grubunda 55 ± 3 ml ve MLE ise çalışma grubunda - 0,06 ± 0,02 mmol/ml, kontrol grubunda - 0,12 ± 0,07 mmol/ml idi ($P < 0,05$).

Bu çalışma sonucunda; ATP-$MgCl_2$ kullanımı iskemi-reperfüzyon yaralanmalarında hemodinamik ve metabolik parametreleri düzeltmesi açısından faydalı bulunmuştur.

Anahtar Sözcükler: İn vivo deneysel çalışma, iskemi-reperfüzyon hasarı, myokard, ATP-$MgCl_2$

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Introduction

Open-heart surgery has been successfully performed for a long time as a result of technological developments in the application of cardiopulmonary bypass (CPB). In spite of the improvements in cardiovascular surgery, cardiopulmonary bypass systems have not yet reached an ideal level. Despite the great developments in the application of CPB during the last 30 years and presently available technical and pharmacological possibilities, patients that have undergone coronary artery bypass grafting (CABG) surgery have experienced insufficient myocardial protection (1,2).

As stated above, myocardial damage occurs mostly during the ischemia-reperfusion period. Therefore, studies dealing with new drugs have been conducted, and technical methods for shortening myocardial ischemia duration, for increasing the tolerance of ischemia, and consequently reducing reperfusion damage have been investigated (3-5). Murry et al. (6) showed that brief periods of myocardial ischemia protect the myocardium from ischemia-reperfusion damage and endogenous adenosine rising up in the preconditioning period is a protective factor during the ischemia-reperfusion period.

Myocardial ischemia is a pathology characterized by decreased myocardial energy storage and ventricular function. Unless sufficient blood flow is re-established, myocardial tissue necrosis occurs. The important determinants of cell death are the duration and extent of myocardial ischemia, as well as the absence of supportive collateral flow and the acceleration of myocardial tissue damage (7).

In the literature, it has been determined that the amounts of interruption of myocardial blood flow and ischemia duration are correlated with myocardial damage (8,9).

Adenosine triphosphate (ATP) sources rapidly decrease in the 3-4 min following ischemia. At the same time, the oxidative phosphorylation process, the fuel source of cell metabolism, completely stops. As a result, intracellular ion hemostasis breaks down. Impaired calcium transportation results in cell damage. Liquid peroxidation, which occurs during the reperfusion period following ischemia, as a result of radical free oxygen formation, causes cell membrane damage and microvascular spasm (10-12).

Adenosine triphosphate-magnesium chloride (ATP-$MgCl_2$), which has been investigated for a long time, is a valuable drug for treating the hazardous effects of organ ischemia-reperfusion. Its use in organ ischemia and shock continues to be investigated (13,14). The amount of magnesium decreases in ischemic injuries, which results in damage to cell membrane integrity, ATPase function, and, especially, production of co-factors involved in energy formation. Magnesium is an important cation that plays key roles in cell metabolism, including membrane synthesis, membrane integrity, protein synthesis, calcium transportation, and aerobic respiration. High doses of magnesium should be used to obtain these benefits (15-17).

The use of ATP-$MgCl_2$ results in increased uptake of magnesium into the cell. In addition, the use of ATP-$MgCl_2$ during the reperfusion period corrects micro-circulatory dysfunction, decreases cell edema, improves organ blood flow, refreshes the intracellular ATP level, and regulates endothelial function after ischemia or shock (18-21).

In this study, we investigated the use of ATP-$MgCl_2$ for the prevention of ischemia-reperfusion injury, and to determine whether it has any effects on myocardium hemodynamic and metabolic parameters.

Materials and Methods

The study included 12 mongrel dogs (24 ± 3 kg); 6 were in the study group (ATP-$MgCl_2$) and 6 were in the control group. The study was conducted in the surgery department of Ankara University, Veterinary Faculty, Ankara, Turkey. Before starting our investigation, all of the dogs were quarantined for 15 days, and routine clinical examinations and antiparasite therapy were administered. Ethical Committee approval was given for this study and the animal care and use complied with the "Principles of Laboratory Animal Care" and the "Guide for the Care and Use of Laboratory Animals".

The animals received no food during for 12 h before the operation, and were premedicated with subcutaneous atropine sulfate (0.04 mg/kg) 30 min before the operation and with intramuscular xylazine hydrochloride (2 mg/kg) 15 min before the operation. Anesthesia was induced with intravenous fentanyl citrate and sodium pentothal. After respiratory depression was maintained, all animals were intubated for ventilatory support. A

thermodilution catheter was introduced into the pulmonary artery via the external jugular vein. Heart rate and rhythm were recorded.

After administration of adequate anesthesia, a right thoracotomy was made and the pericardium was opened. The left anterior descending artery was ligated to obtain ischemia for 15 min and than the ligature was removed to obtain reperfusion. After that, cardiopulmonary bypass was initiated using 2 venous cannulas placed in the caudal and cranial vena cava, and an arterial cannula placed in the femoral artery. Bypass flow was adjusted to approximately 100 ml/kg and the alpha-stat strategy was used to hold PCO_2 between 35 and 45 mmHg. Priming volume for the membrane oxygenator was prepared with ringer lactate (1000 ml), mannitol (1 mg/kg), heparin (5000 IU), and sodium bicarbonate (30 mEq). A 40 µm arterial filter was placed in the arterial line. After cross-clamping the aorta, antegrade cold crystalloid cardioplegia (St. Thomas no.2) was perfused from the ascending aorta via a cannula in order to maintain cardiac arrest. ATP-$MgCl_2$ (70+70 mmol/l) was given to the study group with an infusion rate of 0.25 ml/kg per h; no medication was administered to the control group. Hemodynamic and metabolic measurements were taken at basal, and then after 60, 120, and 180 min of reperfusion.

Hemodynamic measurements

Cardiac output was measured with the help of a thermodilution catheter. Heart rate and pressure were monitored and recorded.

Metabolic measurements

The HYDRAGEL ISO-CK kit (Sebia, Hydragel ISO-CK kit) was used for the separation and calculation of isoenzymes of creatine phosphokinase in serum by agarose gel electrophoresis. Following the determination of fractions, total activity of creatine phosphokinase was determined with a Hitachi 911 analyzer and Boehringer-Mannheim kits. Activities of the fractions (isoenzymes) are reported as percentage of total activity.

Myocardial oxygen extraction (MOE) was calculated using the equation: MOE = Ca – Cv/Ca, where Ca and Cv are the arterial oxygen content and coronary sinus oxygen content, respectively. The oxygen content of the aorta and coronary sinus was measured automatically with an OSM-3 Hemoximeter radiometer (Denmark).

The blood samples were immediately put into perchloric acid for lactate measurements and they were prepared for measurement by the enzymatic spectrophotometric method the same day. Myocardial lactate extraction (MLE) was calculated using the equation: MLE = AoL – CsL/AoL, where AoL and CsL show the aortic lactate level and the coronary sinus lactate level, respectively.

Statistical analysis

All data were compared with mean ± standard deviation. The level of significance was set at $P < 0.05$. Comparison between the groups was made with Student's t-test.

Results

There were no significant differences between the study and control groups with regard to mean arterial pressure, but cardiac output values were significantly higher in the study group after 60, 120, and 180 min of reperfusion. Cardiac output values in the study and control groups at 180 min were 2370 ± 31 ml/min and 900 ± 45 ml/min, respectively ($P < 0.05$). Measurements of mean pulmonary artery pressure and pulmonary capillary wedge pressure (PCWP) were significantly higher in the control group. PCWP values in the study and control groups after 180 min were 9.5 ± 0.7 mmHg and 15.4 ± 3.5 mmHg, respectively ($P < 0.05$) (Table 1).

MOE and MLE values were significantly higher in the control group. MOE was 50 ± 2 ml in the study group and 55 ± 3 ml in the control group after 180 min of reperfusion ($P < 0.05$). MLE was measured as –0.06 ± 0.02 mmol/ml in the study group and –0.12 ± 0.07 mmol/ml in the control group after 180 min of reperfusion ($P < 0.05$). CPK-MB values were significantly higher in the control group compared with the study group after 180 min of reperfusion (26 ± 3 IU/l vs. 44 ± 5 IU/l; $P < 0.05$) (Table 2).

Discussion

Myocardial damage occurs during the ischemia-reperfusion (IR) period. Studies about new drugs and

Table 1. Hemodynamic values of the study and control groups (* $P < 0.05$). MAP: mean arterial pressure; MPAP: mean pulmonary arterial pressure; PCWP: pulmonary capillary wedge pressure; CO: cardiac output.

ATP-$MgCl_2$ Group:

	0 min	60 min	120 min	180 min
HR (/min)	104.8 ± 26.8	93.3 ± 15.6	75.3 ± 12.8 *	62.8 ± 11.8 *
MAP (mmHg)	84.6 ± 14.3	35.8 ± 22.6	36.3 ± 20.2	32.8 ± 22.4
MPAP (mmHg)	14.0 ± 5.6	13.5 ± 3.5*	14.5 ± 0.7 *	11.5 ± 0.7 *
PCWP (mmHg)	16.5 ± 2.1 *	10.5 ± 0.7*	11.5 ± 0.7 *	9.5 ± 0.7 *
CO (ml/min)	1285 ± 54	1893 ± 64*	1933 ± 63 *	2370 ± 31 *

Control Group:

	0 min	60 min	120 min	180 min
HR (/min)	112.4 ± 13.5	116.1 ± 11.6	115.2 ± 8.5 *	113.1 ± 6.8 *
MAP (mmHg)	92.3 ± 11.5	56.7 ± 10.2	50.2 ± 9.4	45.0 ± 13.6
MPAP (mmHg)	14.5 ± 5.2	22.5 ± 6.5 *	23.2 ± 5.4 *	18.3 ± 6.1 *
PCWP (mmHg)	9.5 ± 3.2 *	16.3 ± 3.5 *	15.1 ± 2.6 *	15.4 ± 3.5 *
CO (ml/min)	1300 ± 50	900 ± 40 *	925 ± 45 *	900 ± 40 *

Table 2. Metabolic values of the study and control groups (* $P < 0.05$). MOE: myocardial oxygen extraction; MLE: myocardial lactate extraction; CPK-MB: creatine phosphokinase-myocardial band.

	0.min	60.min	120.min	180.min
MOE (ml)		*	*	
Control	52 ± 3	61 ± 3	57 ± 2	55 ± 3
ATP-$MgCl_2$	50 ± 2	54 ± 3	51 ± 2	50 ± 2
MLE (mmol/ml)		*	*	*
Control	–0.01 ± 0.002	–0.16 ± 0.03	–0.13 ± 0.05	–0.12 ± 0.07
ATP-$MgCl_2$	–0.02 ± 0.001	–0.09 ± 0.02	–0.05 ± 0.03	–0.06 ± 0.02
CPK-MB (IU/l)			*	*
Control	26 ± 4	37 ± 9	48 ± 10	44 ± 5
ATP-$MgCl_2$	24 ± 3	28 ± 6	31 ± 4	26 ± 3

techniques that reduce this damage are ongoing (3-5). Murry et al. (6) declared that endogenous adenosine was a protective factor during the IR period. In the present study, the effect of ATP-$MgCl_2$ on myocardial damage during the IR period was investigated.

ATP-$MgCl_2$, which has been investigated for a long time, is reported to be a useful drug in overcoming the hazardous effects of organ IR. Its use during organ ischemia and shock continues to be investigated (13,14). Evaluating the results of our study, we can claim that use of ATP-$MgCl_2$ improves hemodynamic and metabolic parameters during the IR injury period. No statistical difference was seen between the control and study groups in terms of mean arterial blood pressure. The measurements of cardiac output in the study group were much higher compared to the control group values. As a result, it can be said that the use of ATP-$MgCl_2$ had a beneficial effect on hemodynamic parameters. Additionally, it was observed that pulmonary artery and pulmonary capillary wedge pressure results were very high in the control group.

The amount of magnesium decreases in ischemic injuries. Lack of magnesium results in damage to cell membrane integrity, ATPase function, and, especially, production of co-factors involved in energy formation. Magnesium is an important cation that plays key roles in cell metabolism, including membrane synthesis, membrane integrity, protein synthesis, calcium transportation, and aerobic respiration. High doses of magnesium should be used to obtain these benefits (15-

17). Since high-doses of magnesium may have adverse effects, ATP-$MgCl_2$ was used in our study. The use of ATP-$MgCl_2$ increases the cellular uptake of magnesium. The use of ATP-$MgCl_2$ during the reperfusion period corrects micro-circulatory dysfunction, decreases cell edema, improves organ blood flow, refreshes intracellular ATP level, and regulates endothelial function after ischemia or shock (18-21). It was noted that MOE and MLE values were high in the control group; therefore, it can be concluded that ATP-$MgCl_2$ improved intracellular functional damage in the study group.

Satisfactory results have been obtained in terms of hemodynamic and metabolic parameters using ATP-$MgCl_2$. ATP-$MgCl_2$, which has been previously found to be useful in different organ ischemia and shock models, was also found to be effective in our study (13,14,20).

In conclusion, the in vivo use of ATP-$MgCl_2$ was useful in improving hemodynamic and metabolic parameters during IR injury. Additional studies concerning ATP-$MgCl_2$ are needed to demonstrate its beneficial effects during IR injury.

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