Turk. J. Vet. Anim. Sci.
30 (2006) 449-456
© TÜBİTAK

Research Article

The Effects on Myocardial Improvement Using ATP-$MgCl_2$ during the Ischemia Reperfusion Period; An In Vitro Study*

Nusret APAYDIN[1], Zülfikar Kadir SARITAŞ[2], Kamuran PAMUK[2], Eser ÖZGENCİL[3], Fulya OCAK[4], Tulga ULUS[5], Baran BUDAK[5], Selçuk SÜRÜCÜ[6], Salih Fehmi KATIRCIOĞLU[5]

[1]Department of Surgery, Faculty of Veterinary Medicine, University of Erciyes, Kayseri - TURKEY

[2]Department of Surgery, Faculty of Veterinary Medicine, University of Afyon Kocatepe, Afyon - TURKEY

[3]Department of Surgery, Faculty of Veterinary Medicine, University of Ankara, Ankara - TURKEY

[4]Department of Microbiology, Faculty of Veterinary Medicine, University of Erciyes, Kayseri - TURKEY

[5]Department of Cardiovascular Surgery, Türkiye Yüksek İhtisas Hospital, Ankara - TURKEY

[6]Department of Anatomy, Faculty of Medicine, University of Hacettepe, Ankara - TURKEY

Received: 19.07.2004

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

In the in vitro study, left ventricular systolic and diastolic pressures and dp/dt values were significantly high in the ATP-$MgCl_2$ group beginning from 0.5 h of the reperfusion period. In the ATP-$MgCl_2$ group, at the end of the third hour, systolic and diastolic pressure values were 80.0 ± 6.2 and 75.2 ± 7.2 mmHg, while in the control group they were 18.4 ± 7.7 and 7.5 ± 6.8 mmHg ($P < 0.05$). In the ultrastructural analysis, myofibrillary damage was observed in both groups, but the mitochondrial pathologic changes were clearer in the control group. The electron microscopic score was 0.40 ± 0.061 in the ATP-$MgCl_2$ group and 1.2 ± 0.039 in the control group ($P < 0.001$).

The hemodynamic and ultrastructural parameters of the specimens investigated in this study revealed that the usage of ATP-$MgCl_2$ may be beneficial in coping with ischemia-reperfusion damage. In the near future, with the help of more comprehensive studies, ATP-$MgCl_2$ is expected to be used routinely because of its potential benefits.

Key Words: In vitro perfusion, ischemia-reperfusion damage, myocardium, ATP-$MgCl_2$

İskemi-Reperfüzyon Döneminde ATP-$MgCl_2$ Kullanımının Myokardiyal Düzelmeye Etkisi; İn Vitro Çalışma

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

İn vitro perfüzyon çalışmasında; sol ventrikül sistolik ve diastolik basınçları ve dp/dt değerleri reperfüzyon ölçümlerinin 0,5 saatinden başlayarak anlamlı şekilde ATP-$MgCl_2$ grubunda kontrol grubuna göre yüksek bulunmuştur. ATP-$MgCl_2$ grubunda 3. saatin sonunda sistolik ve diastolik basınç değerleri 80,0 ± 6,2 mmHg ve 75,2 ± 72 mmHg, kontrol grubunda ise 18,4 ± 7,7 mmHg ve 7,5 ± 6,8 mmHg idi ($P < 0,05$). Dokuların analizinde, her iki grup da da myofibriller yapıda hasar gözlendi. Ancak kontrol grubunda mitokondrial patoloji belirgin olup ATP-$MgCl_2$ grubunda hemen hemen normale yakındı. Elektron mikroskobik skor kontrol grubunda 1,2 ± 0,039, ATP-$MgCl_2$ grubunda 0,40 ± 0,061 olarak hesaplandı ($P < 0,001$).

Bu çalışma sonucunda; ATP-$MgCl_2$ kullanımı gerçekleştirilen iskemi-reperfüzyon yaralanmalarında, ultrastrüktürel analizlerin incelenmesi sonucunda hemodinamik ve ultrastrüktürel parametrelerin düzeltmesi açısından faydalı bulunmuştur. Bu çalışmayı takip edecek daha kapsaplı ve ileri çalışmaların yapılması ile ATP-$MgCl_2$ 'ün faydalı bir ilaç olma olasılığı kaçınılmazdır.

Anahtar Sözcükler: İn vitro perfüzyon, iskemi-reperfüzyon hasarı, myokard, ATP-$MgCl_2$

* This research was supported by TÜBİTAK VHAG and was carried out in the Experimental Surgery Research Centre, which was established in 1998 under the authority of Ankara University Veterinary Faculty Surgical Department. Project number: DECAR 4.

Introduction

Although knowledge concerning heart surgery has advanced, studies dealing with myocardial protection are still crucial. It is not possible to monitor all the events happening in cells during ischemia-reperfusion, especially those related with their functions and dysfunctions (1).

In addition to such myocardial protection techniques as hypothermia and crystalloid cardioplegia, which have been proved to be effective, studies have attempted to develop the ideal myocardial protection using retrograde cardioplegia infusion through the coronary sinus, cold and warm blood-cardioplegia, simultaneous antegrade and retrograde continuous cold blood cardioplegia and several pharmacological protective substances (2-5).

Magnesium is a necessary co-factor for both effective energy usage and enzymatic reactions. These enzymatic reactions include the following: mitochondrial functions, provision of necessary energy for transsarcolemmal ionic gradients, control of cell volume, and maintaining the resting membrane potential (6). Magnesium is essential for regular function of the Na-K ATPase pump. It regulates the potassium balance in the cell. Moreover, calcium ATPase and proton pumps need magnesium (6-10).

In studies investigating myocardial metabolism and hemodynamics, several distinct models and test animals have been used. Of these models, the most commonly used one is the isolated perfused heart model (Langendorff). Since its introduction in 1895, it has been modified and is considered the most preferred model worldwide. In this system, the heart is perfused at a certain pressure or flow level from the aorta retrogradely. Closing of the aortic leaflets as a result of the perfusion enables all perfusate to reach the coronary arteries (11).

ATP depots begin to decrease quickly in a 3-4 min interval following the ischemia. As a result of this decrease, oxidative phosphorilation completely stops. Thus, intracellular ion homeostasis is corrupted, and this leads to cell injury. Lipid peroxidation, which occurs as a result of free oxygen radicals formed during the reperfusion period following ischemia, causes damage to the cell membrane and microvascular spasms (12-14).

Magnesium treatment has been used in various cardiovascular diseases such as myocardial infarction and arrhythmias. Furthermore, decreases in intracellular or extracellular magnesium levels may lead to hypertension, congestive heart failure, and arrhythmias related to myocardial infarction (15,16).

Intracellular magnesium prevents deficiencies in potassium and high-energy phosphates in the cell. It may also prevent intracellular overloading of calcium and sodium. Decreasing the metabolic activity and calcium entrance, magnesium indirectly regulates the cellular free-energy level (15). High levels of magnesium improve intracellular ATP production and glucose usage. Magnesium is known as a physiological calcium blocker and it protects the cells from calcium overload during ischemia (17).

Pearson et al. (18) showed that hypomagnesemia damages the nitric oxide (NO) release from the coronary endothelia in a dog model. Hypomagnesemia may increase vasoconstriction and thrombosis. Dickens et al. (19) proved that, in the case of magnesium deficiency, those tissues exposed to oxidative stress are extremely damaged as a result of cellular lipid peroxidation. In a dog gracilis-muscle ischemia-reperfusion model, it was indicated that superoxide release from active neutrophils can be decreased with ATP-$MgCl_2$ and its protective effect is partially based on this fact (20). In the case of magnesium deficiency, oxidative damage is increased after ischemia as well, and this damage can be avoided with the help of antioxidants (21).

Magnesium has been investigated for a long time in relation to organ ischemia and shock. The magnesium level decreases in ischemic injuries of nerve tissues. This decrease damages the cell membrane integrity, ATPase function and particularly the production of co-factors that play an important role in the production of energy. Magnesium treatment improves the microcirculatory dysfunction observed in reperfusion, increases total blood flow through vital organs and decreases cell edema. Regulating the calcium transport, magnesium helps to decrease the tissue damage based on calcium. It raises the ATP levels, which are depleted during the ischemic period (22-24).

The present study investigated the effect of ATP-$MgCl_2$ on myocardium, using an in vitro perfusion system with the help of hemodynamic and ultrastructural parameters.

Materials and Methods

In the in vitro perfusion study, the model of isolated Langendorff rabbit heart was employed. This research was supported by TÜBİTAK and was carried out in the Experimental Surgery Research Center, which was established in 1998 under the authority of Ankara University Veterinary Faculty Surgical Department.

The study used control and study groups, each consisting of 6 New Zealand rabbits. The rabbits weighed 2-2.5 kg and they were 2-3 months old. The study was conducted with the approval of the Ethical Committee of the same institution.

For 12 h before the research, the rabbits were not fed. A premedication was applied firstly using atropine sulfate (0.5 mg/kg) and 10 min later using xylasine hydrochloride (5 mg/kg). Five minutes after premedication, anesthesia was achieved intramuscularly using ketamine hydrochloride (50 mg/kg) and phentanyl (0.15 mg/kg). After injecting heparin (100 U) through the vena auricularis magna, median sternotomy was performed and the pericardium was exposed. Following pericardiectomy, the aorta and pulmonary artery were ligated as distally as possible and the heart was taken out. The heart was immediately put into Krebs solution. The solution was put into a pot with water (+4 ºC) for transportation and after that the heart was integrated into the Langendorff system (Figure 1). The Krebs solution used for the in vitro perfusion consisted of 118 mmol/l NaCl, 5.4 mmol/l KCl, 1.2 mmol/l $MgCl_2$, 25 mmol/l $NaHCO_3$, 2.4 mmol/l $CaCl_2$, 1 mmol/l $Kh2PO_4$ and 10 mmol/l glucose. A 5% CO_2 and 95% O_2 gas combination was also used in the Krebs solution.

The heart, integrated into the Langendorff system, was perfused for stabilization and adaptation for 20 min. After the stabilization period, a catheter was placed in the left ventricle through the left atrium. In this way, left ventricle pressures and pressure alterations were recorded on a computer simultaneously using software (MP 100 version 3.2 for Windows, Biopac System, Inc. Santa Barbara CA). Using the same program, left ventricle pressure diagrams and its first derivative, dp/dt, were also calculated (Figure 2). After the determination of baseline measurements, absolute global ischemia was performed for 1 min. During the next period, the hearts were reperfused for 3 h using the Krebs solution, which included a gas mixture (5% CO_2 and 95% O_2), at 37 ºC. The pH level of the solution was 7.35-7.45. The flow rate was 8 ml/min and the heart rate was 230 beats/min. In the control group, only perfusion solution was used, while in the study group 30 cc/l ATP-$MgCl_2$ was used. ATP-$MgCl_2$ was prepared containing equal amounts of the 2 elements. ATP, in powder form, was weighed in 0.9% NaCl solution and the solution amount was set as 140 µmol/ml. The pH value of the solution was 7.4. Similarly, the solution of $MgCl_2$ was prepared and it was also 140 µmol/ml. These 2 solutions were equally mixed. Each milliliter of the solution contained 70 µmol/ml ATP and $MgCl_2$. Heart rates of the perfused hearts were stimulated using a computer-controlled stimulator to be 230 beats/min.

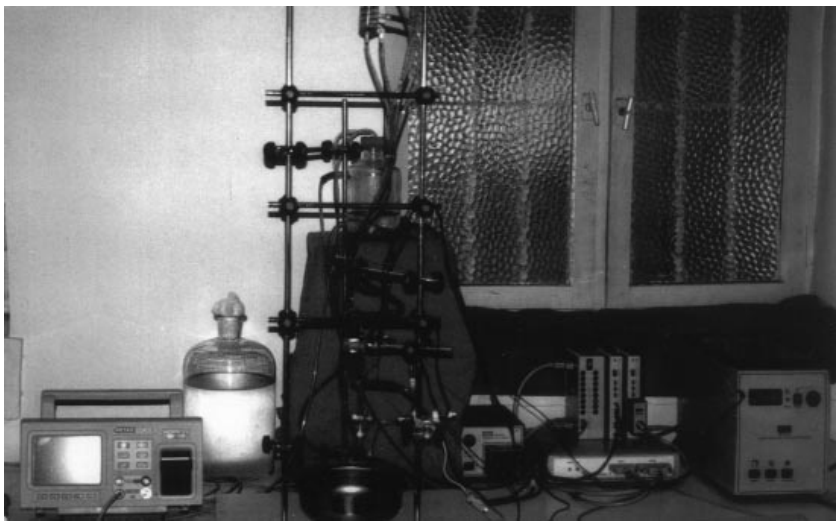

Figure 1. Langendorff system.

intergroup (+) dp/dt values (*P < 0.05).

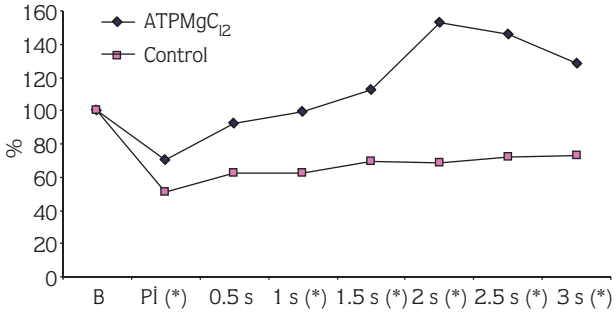

Figure 2. Values of in vitro perfusion (+) dp / dt.

Ultrastructural Analysis

The samples taken from the left ventricle muscle were fixed with 2.5% glutaraldehyde. Then they were refixed with osmium tetraoxide and dehydrated with alcohol series. Lastly the series were embedded into CY212. Crosscuts of 60-90 nm were prepared and contrasted with uranyl acetate. The samples were analyzed using a JEOL JEM 1200 electron microscope. The pictures were taken using a Nikon Optiphot microscope. The following classification method was used to analyze the findings:

Heart scoring for ultrastructural analysis

A. Intracellular edema (scored in 20 areas)
   - No edema 0
   - Mild edema 1
   - Intermediate edema 2
   - Extreme edema 3

B. Mitochondrial injury (40 mitochondria were scored)
   - Normal 0
   - Mild edema 1
   - Extreme edema 2

C. Glycogen exhaustion (scored in 20 areas)
   - Completely 0
   - Slightly 1
   - Intermediately 2
   - No glycogen depot 3

D. Myofibrillar structural damage (scored in 20 areas)
   - Normal I bands 0
   - Irregular I bands 1
   - No I bands 2

Statistical Analysis

All findings were compared using mean ± standard deviation. The value of $P < 0.05$ was regarded as the statistically significant level. In order to analyze the differences between groups, Student's t test was used.

Results

In the in vitro study, the left ventricle systolic and diastolic pressures in the study group (ATP-$MgCl_2$) were higher than those in the control group beginning from 0.5 h of the reperfusion period. In the ATP-$MgCl_2$ group, at the end of the third hour, systolic and diastolic pressure values were 80.0 ± 6.2 and 75.2 ± 7.2 mmHg, respectively. In the control group these values were 18.4 ± 7.7 and 7.5 ± 6.8 mmHg, respectively ($P < 0.05$) (Table).

Similarly, between the 2 groups, the values of dp/dt, which are the first derivative of the left ventricle pressure values, were different beginning from the start of the ischemia and such changes lasted until the third hour of the reperfusion. At the end of the third hour of the reperfusion period, the levels of change in +dp/dt and –dp/dt in the ATP-$MgCl_2$ group were 128.4 ± 28.0% and 138.5 ± 9.8%, respectively, in accordance with the baseline measurements. These values were 73.2 ± 12.6% and 67.6 ± 10.2%, respectively, in the control group ($P < 0.05$) (Figures 2 and 3).

Ultrastructural Analysis

In the analysis of the tissue samples of both groups, no damage in the myofibrillary structure was observed. However, mitochondrial pathology was evident in the control group. The mitochondrial structure was almost regular in the ATP-$MgCl_2$ group. Intracellular edema was seen in the control group, whereas in the ATP-$MgCl_2$ group significant recovery was observed. The electron microscopic score was 1.2 ± 0.039 in the control group and 0.40 ± 0.061 in the ATP-$MgCl_2$ group ($P < 0.001$) (Figures 4 and 5).

Discussion

The model of isolated perfusion heart (Langendorff) is commonly used in studies concerning the myocardial metabolism and hemodynamics. In this technique, the heart is perfused at a certain pressure or flow from the aorta retrogradely (11). Many pharmacological protective

Table. Hemodynamic values of the in vitro study (*P < 0.05).

$ATP\text{-}MgCl_2$ Group								
mmHg	Baseline	P. ischemia	0.5 h *	1 h *	1.5 h *	2 h *	2.5 h *	3 h *
Systolic	30.6 ± 2.6	32.8 ± 3.1	61.5 ± 8.5	82.8 ± 14.4	85.8 ± 9.9	86.8 ± 6.9	85.2 ± 5.6	80.0 ± 6.2
Diastolic	20.3 ± 2.2	20.8 ± 1.7	49.7 ± 10.9	76.3 ± 15.9	79.7 ± 11.1	80.6 ± 8.6	79.9 ± 7.1	75.2 ± 7.2
Mean	24.4 ± 2.3	24.6 ± 1.6	53.6 ± 10.5	79.2 ± 15.2	82.6 ± 10.5	83.5 ± 7.7	82.5 ± 6.3	77.6 ± 6.7
Control Group								
mmHg	Baseline	P. ischemia	0.5 h *	1 h *	1.5 h *	2 h *	2.5 h *	3 h *
Systolic	28.5 ± 5.9	29.7 ± 2.1	24.3 ± 5.2	18.6 ± 4.7	22.6 ± 8.5	18.4 ± 3.7	24.8 ± 7.3	18.4 ± 7.7
Diastolic	19.9 ± 6.2	15.4 ± 6.0	9.2 ± 3.3	7.2 ± 3.5	12.4 ± 6.2	8.9 ± 4.4	13.5 ± 7.9	7.5 ± 6.8
Mean	23.3 ± 6.1	20.9 ± 2.8	14.3 ± 2.9	11.8 ± 3.5	16.8 ± 7.1	13.2 ± 3.8	18.4 ± 7.5	12.4 ± 6.9

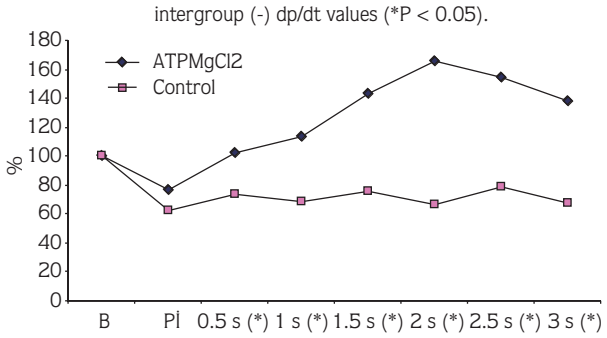

Figure 3. Values of in vitro perfusion (-) dp / dt.

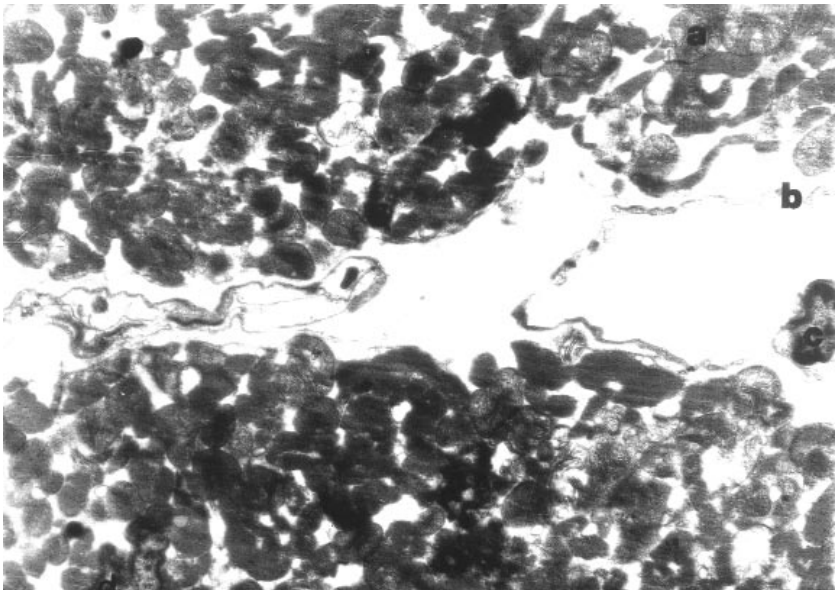

Figure 4. Electron microscopic view of the sample of in vitro perfusion $ATP\text{-}MgCl_2$ group.
a) mitochondrion b) capillary c) nucleus of endothelial cell. d) nucleus of the heart muscle cell
A slight degeneration in the ultrastructure of heart muscle can be observed. Most of the mitochondria are normal in structure. There is no intracellular edema.

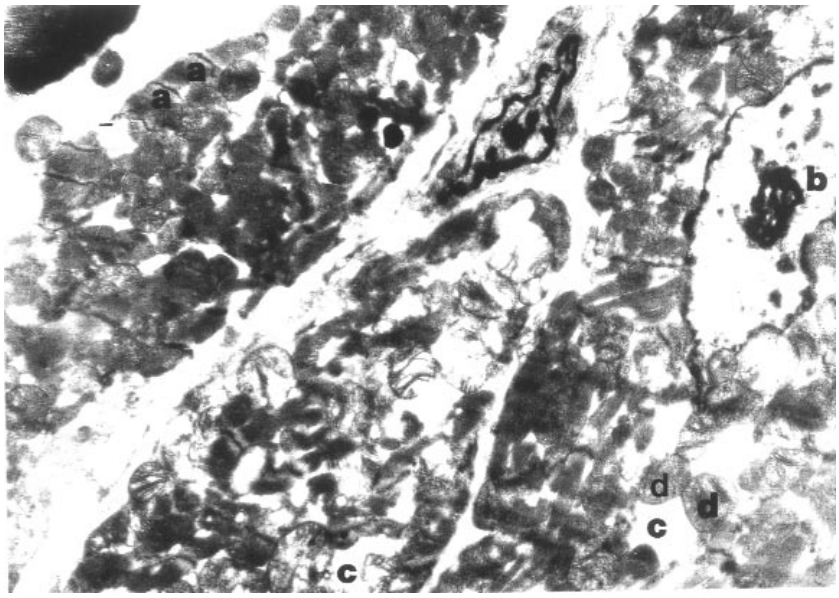

Figure 5. Electron microscopic view of the sample of in vitro perfusion control group.
a) I bands b) nucleus of the heart muscle cell c) areas of intracellular edema
d) edematous mitochondria
Edema can be observed in the most of the mitochondria while rupture can be observed in some. Intracellular edema is present but there is no degeneration in the general ultrastructure (I bands can be observed).

substances have been studied for myocardial protection (2-5). Our research was performed using the Langendorff technique, and the isolated heart was perfused using ATP-$MgCl_2$ at a certain pressure and flow level.

It was reported that magnesium is commonly employed for the treatment of cardiovascular diseases and that decreases in intracellular and extracellular magnesium levels may lead to several problems such as hypertension, congestive heart failure and arrhythmias due to myocardial infarction (15,16). The findings of our study performed using ATP-$MgCl_2$ in an in vitro system show that magnesium can be used in many cardiovascular diseases related with ischemia-reperfusion injury because of its ability to improve the hemodynamic parameters, as stated by previous studies. Thus, the findings of our study are parallel to the previous findings on this topic.

Pearson et al. (18) showed that hypomagnesemia distorts NO release from the coronary endothelia in a dog model. Dickens et al. (19) proved that, in the case of magnesium deficiency, those tissues exposed to oxidative stress are extremely damaged as a result of cellular lipid peroxidation. In a dog gracilis-muscle ischemia-reperfusion model, it was indicated that superoxide release from active neutrophils can be decreased with ATP-$MgCl_2$ and its protective effect is partially based on this fact (20). In the case of magnesium deficiency, oxidative damage is increased after the ischemia period, and this damage can be avoided with the help of antioxidants (21). With respect to our findings on the ultrastructure of the study and control groups, no damage in myofibrillary structure was observed in either group. However, mitochondrial pathology was evident in the control group. It was not identified in the ATP-$MgCl_2$ group. We agree with the view expressed by Dickens et al. (19) that cellular degeneration occurs in hypomagnesemia.

Magnesium has been investigated for a long time in relation to organ ischemia and shock. In ischemic injury to the nerve tissue, the amount of magnesium decreases. Magnesium improves the microcirculatory distortion observed in reperfusion, increases the total blood flow to crucial organs and decreases the cellular edema. It decreases tissue damage, regulating calcium transport. The amount of ATP, which decreases during the ischemic period, increases after the application of magnesium

treatment (22-24). According to the results of the ultrastructural analysis of our study, the intracellular edema observed in the ATP-$MgCl_2$ group was significantly less than that in the control group. Based on the analysis of the hemodynamic findings in the ATP-$MgCl_2$ group during the reperfusion period of our study, it is possible to argue that magnesium can be applicable during shock.

In our research performed using an isolated rabbit heart ischemia-reperfusion model, both left ventricle pressure and dp/dt values were better than those of the control group. This finding is parallel to the values determined with an electron microscopic ultrastructural investigation. ATP-$MgCl_2$, which was proved to be efficient in various organ ischemia and shock models, was also efficient in our study (25-26).

In conclusion, the use of ATP-$MgCl_2$ in an in vitro system is useful for improving hemodynamic and ultrastructural parameters in ischemia-reperfusion injury. More comprehensive studies are needed to confirm that ATP-$MgCl_2$ is a useful medicine in this area.

References

1. Buckberg, D.G.: Updates on current techniques of myocardial protection. Ann. Thorac. Surg., 1995; 60: 805-814.
2. Solorzano, J., Taitelbaum, G., Chiu, R.C.: Retrograde coronary sinus perfusion for myocardial protection during cardiopulmonary bypass. Ann. Thorac. Surg., 1978; 25: 201-208.
3. Menasche, P., Kural, S., Fauchet, M., Lavergne, A., Commin, P., Bercot, M., Touchot, B., Georgiopoulos, G., Piwnica, A.: Retrograde coronary sinus perfusion; a safe alternative for ensuring cardioplegic delivery in aortic valve surgery. Ann. Thorac. Surg., 1982; 34: 647-648.
4. Lichtenstein, S.V., Abel, J.G., Panos, A., Slutsky, A.S., Salerno, T.A.: Warm heart surgery: experience with long cross-clamp times. Ann. Thorac. Surg., 1991; 52: 1009-1013.
5. Abd-Elfattah, A.S., Jessen, M.E., Lekven, J., Wechsler, A.S.: Differential cardioprotection with selective inhibitors of adenosine metabolism and transport: role of purine release in ischemic and reperfusion injury. Mol. Cell. Biochem., 1998; 180: 179-191.
6. Woods, K.L.: Possible pharmacological actions of magnessium in acute myocardial infarction. Br. J. Clin. Pharmacol., 1991; 32: 3-10.
7. Granger, D.N.: Role of xanthine oxidase and granulocytes in ischemia-reperfusion injury. Am. J. Physiol., 1988; 255: 1269-1275.
8. McGarrity, S.T., Stephenson, A.H., Webster, R.O.: Regulation of human neutrophil functions by adenine nucleotides. J. Immunol., 1989; 142: 1986-1994.
9. White, R.E., Hartzell, H.C.: Magnesium ions in cardiac function. Regulator of ion channels and second messengers. Biochem. Pharmacol., 1989; 38: 859-864.
10. Reinhart, R.A.: Clinical correlates of the molecular and cellular actions of magnesium on the cardiovascular system. Am. Heart J., 1991; 121: 1513-1521.
11. Süzer, Ö.: Langendorff İzole Perfüze Kalp Preperatı ve Kullandığımız Çalışma Modelleri. In: Bökesoy, T.A. Ed. Türk Farmakoloji Derneği Farmakoloji Eğitiminde Kuşaklararası Bilimsel Etkileşme Seminerleri Programı, Prof. Dr. Reşat Garan Toplantısı. Türk Farmakoloji Derneği Yayınları No: 14, Ankara. 1997; 40-47
12. Jennings, R.B., Reimer, K.A.: Lethal myocardial ischemic injury. Am. J. Pathol., 1981; 102: 241-255.
13. Demopoulos, H.B., Flamm, E.S., Pietronigro. D,D., Seligman, M.L.: The free radical pathology and the micro circulation in the major central nervous system disorders. Acta Physiol. Scand. Suppl., 1980; 492: 91-119.
14. McCord, J.M.: Oxygen derived free radicals in postischemic tissue injury. N. Engl. J. Med., 1985; 312: 159-163.
15. Headrick, J.P., McKirdy J.C., Willis, R.J.: Functional and metabolic effects of extracellular magnesium in normoxic and ischemic myocardium. Am. J. Physiol., 1998; 275: 917-929.
16. Rasmussen, H.S., McNair, P., Norregard, P., Backer, V., Lindeneg, O., Balslev, S.: Intravenous magnesium in acute myocardial infarction. Lancet, 1986; 1: 234-236.
17. Shechter, M., Sharir, M., Labrador, M.J., Forrester, J., Silver, B., Bairey Merz, C.N.: Oral magnesium therapy improves endothelial function in patients with coronary artery disease. Circulation, 2000; 102: 2353-2358.
18. Pearson, P.J., Evora, P.R., Seccombe, J.F., Schaff, H.V.: Hypomagnesemia inhibits nitric oxide release from coronary endothelium: protective role of magnesium infusiun after cardiac operations. Ann. Thorac. Surg., 1998; 65: 967-972.
19. Dickens, B.F., Weglicki, W.B., Li, Y.S., Mak, I.T.: Magnesium deficiency in vitro enhances free radical-induced intracellular oxidation and cytotoxicity in endothelial cells. FEBS Lett., 1992; 311: 187-191.
20. Korthuis, R.J., Grisham, M.B, Zimmerman, B.J., Granger, D.N., Taylor, A.E.: Vascular injury in dogs during ischemia-reperfusion: improvement with ATP-$MgCl_2$ pretreatment. Am. J. Physiol., 1998; 254: 702-708.

21. Kharb, S., Singh, V.: Magnesium deficiency potentiates free radical production asscociated with myocardial infarction. J. Assoc. Physicians India, 2000; 48: 484-485.

22. Chaudry, I.H., Clemens, M.G., Baue, A.E.: The role of ATP-magnessium in ischemia and shock. Magnesium, 1986; 5: 211-220

23. Chaudry, I.H., Stephan, R.N., Dean, R.E., Clemens, M.G., Baue, A.E.: Use of magnesium-ATP following liver ischemia. Magnesium, 1988; 7: 68-77

24. Simpson, J.I., Eide, T.R., Schiff, G.A., Clagnaz, J.F., Hossain, I., Tverskoy, A., Koski, G.: Intratechal magnesium sulfate protects the spinal cord from ischemic injury during thoracic aortic cross-clamping. Anesthesiology, 1994; 81: 1493-1499.

25. Katırcıoğlu, S.F., Ulus, A.T., Sarıtaş, Z., Gökçe, P.: Effects of ATP-$MgCl_2$ administration in hypovolemic dogs. Panminerva Med., 1999; 41: 323-330.

26. Ulus, A.T., Sarıtaş, Z., Yamak, B., Sürücü, S., Tuncer, M., Katırcıoğlu, S.F.: ATP-$MgCl_2$ utilization for spinal cord protection during experimental thoracic aortic occlusion. J. Cardiovasc. Surg., 1999; 40: 495-499.