The additive protective effects of cardioplegia with slow-channel blockers during ischemic cardiac arrest in guinea pig heart: a comparative study of Nifedipine, Verapamil and Diltiazem

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This study was designed to compare the effects of calcium channel blocking agents nifedipine (0.075 mmol/L), verapamil (1.1 mmol/L) and diltiazem (0.03 mmol/L) on myocardium after global ischemia and reperfusion in the modified Langendorff model. Thirty-two isolated guinea pig hearts were divided into four groups (n:8) and subjected to 90 min of normothermic global ischemia, followed by 30 min of reperfusion. Cardioplegic arrest was achieved by adding one of the three Ca^{2+} channel blockers to St. Thomas' Hospital cardioplegic solution (CTHCS). The percent recovery of cardiac function was improved by the addition of Ca^{2+} channel blockers to STHCS. Decreased lipid peroxidation and adenosine triphosphate (ATP) catabolism, protected total glutathione levels and ATP content of mycocardium was observed with diltiazem, verapamil and nifedipine when compared STHCS group. These results confirmed that addition of Ca^{2+} channel blockers, in especially diltiazem can enhance cardioplegic protection. [Turk J Med Res 1997; 15(2):49-55]

Key Words: Myocardial protection, Cardioplegia, Nifedipine, Verapamil, Diltiazem, Calcium channel blocker

Nowadays, cardiac surgery is safe and effective with the current myocardial protection techniques. Reduction of myocardial ischemia is the most important factor for the success of the operation. Although, cold cardioplegia yields excellent outcome in myocardial protection, sometimes poor functional recovery is encountered. In order to maintain basic cellular metabolism, ionic equilibrium and membrane integrity, myocardium has been shown to be associated with exacerbation of cellular injury: Reperfusion occasionally potentiates the release of intracellular enzymes, influx of Ca^{2+}, breakdown of sarcolemmal phospholipids, and disruption of cell membranes, which either alone or in combination result in ultimate cell death. Events known as reperfusion injury; rather than, a result of biochemical changes during ischemia, specifically occur during reperfusion (1-5).

Current evidence leads to three major hypotheses concerning the mediators of reperfusion injury. These are (1) free radical hypothesis (2), the loss of sarcolemmal phospholipids hypothesis and (3) the calcium overloading hypothesis (1,4,5).

The role of calcium ion in the pathophysiology of myocardial ischemia and reperfusion was first hinted at by Shen and Jennings (6). Myocardial ischemia is characterized by a rise of cystolic hydrogen ion and a depletion of high-energy phosphates. The degree of calcium overload, induced by ischemia has been correlated with mitochondrial dysfunction and impaired ATP (adenosine triphosphate) generating capacity (7,8).

Many previous reports have shown that, calcium antagonists, as an additive to cardioplegic solutions (9-16) or administered intravenously before the onset of ischemia (17,18) can improve cardiac functional recovery after reperfusion.

Since calcium ion accumulation is believed to be one of the primary factors that participate in myocardial injury, we proposed to test the protective effects of calcium channel blockers, such as nifedipine, verapamil and diltiazem, as cardioplegic additives.

The aim of the present study was to evaluate the effects of calcium channel blockers on (I) heart protection and myocardial recovery after 30 min of global ischemia in Langendorff perfused guinea pig hearts; (II) lipid peroxidation, lactate, glutathione, hypoxanthine and ATP levels in myocardial tissue; and (Mi) creatine kinase release in the coronary effluent.

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MATERIALS AND METHODS

Experimental Protocol

Thirty-two male Duncan-Hartley guinea pigs weighing 250-320 gr were used in this study. All animals received humane 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.86-23, revised 1985).

The animals were anesthetized by ether and after intravenous administration of heparin (200 U) hearts were rapidly removed and quickly mounted on a non-circulating Langendorff perfusion column. Retrograde perfusion was established at a pressure of 100 cm H_20 with an oxygenated normothermic, modified Krebs-Henseleit bicarbonate buffer. The perfusion buffer consisted of: 118 mM/L NaCl, 4.7 mM/L KCl, 25 mM/L $NaHC0_3$, 1.2 mlWL, KH_2P0_4, 1.2 mM/L $MgS0_4$, 1.2 mM/L $CaCl_2$ and 11.1 mM/L glucose. The solution was equilibrated with 95% oxygen and 5% carbondioxide to achieve a pH of 7.4 at 37°C.

Apical force displacement was used in order to measure the cardiac contractile force. A 7% silk ligature was attached to the left ventricular apex and connected to the Grass® FT 03C force displacement transducer (Grass Instrument Co, Quincy, Mass., USA). The transducer output was displaced continiously on a Grass® model 5 polygraph (Serial 7D531 V3, Grass Instrument Co, Quincy, Mass, USA). After waiting 15 minutes of stabilization period the preischemic heart rate and ventricular contractile force were recorded.

Ischemic cardiac arrest was induced by clamping the aortic cannula. Then the hearts were arrested by introducing one of the cardioplegic solutions, using reservoir located 60 cm above the heart and attached to a side arm of the aortic cannula for 3 min. Through thé ischemic arrest period the hearts were kept at 37°C with isotonic saline-jacketed heart chamber. At the end of 90 min global ischemia the hearts were reperfused with Krebs-Henseleit solution for 30 min at 37°C. The heart rate and ventricular contractile force were recorded every five minutes of reperfusion period. Coronary effluent was collected before cardioplegia and throughout the reperfusion period for cumulative creatine kinase (CK) release as a tissue damage marker. In all instances the left ventricular free wall was resected and stored until the tissue lactate, total glutathion, lipid peroxides (expressed by malondialdehyde-MDA-), hypoxanthine (Hpx), adenosine triphosphate (ATP) measurement were carried out.

Four different cardioplegic solutions were used to arrest the hearts. Hearts of Group I (control group) were arrested with the basic St. Thomas' Hospital cardioplegic solution (STHCS). The composition of the solution is shown in Table 1. In groups II, III and IV, Ca^{2+} channel blockers nifedipine (0.075 mmol/L), verapamil (1.1 mmol/L), and diltiazem (0.03 mmol/L) were added to the STCHS, respectively. Each group contained eight hearts.

Table 1. St Thomas' Hospital cardioplegic solution (STHCS)

Compound	Concentration (mmol/L)
Sodium chloride	110.0
Potassium chloride	16.0
Magnesium chloride	16.0
Calsium chloride	1.2
Sodium bicarbonate	10.0
PH adjusted to 7.8	
Osmolarity=324 mOsm/kg H_20	

Biochemical Determination

Frozen tissues were immediately weighed and homogenized in 10 volumes of ice-cold phosphate buffer (50 mM, pH:7.4), using a glass-glass homogenizer. All the biochemical determinations were done on this homogenate. Tissue lipid peroxide levels, expressed by malondialdehyde (MDA) were determined by the method of Uchiyama and Mihara (19).

The thiobarbituric acid reactive substances (TBARS) were calculated as nanomol per gram ^{1}/et tissue, and tetramethoxy-propane was used as standard.

One ml homogenate was deproteinized with equal volume of cold 8% (v/v) perchloric acid. After centrifugation the supernatant was saved for the determination of lactate, hypoxanthine and glutathione. Tissue lactate concentrations were determined from this supernatant as described (20). One ml of supernatant was neutralized with 0.65 ml of K_3P0_4 (0.7 M) for hypoxanthine and glutathione determinations. The precipitate was removed by centrifugation. Hypoxanthine concentrations were determined by measuring xanthine oxidase-catalyzed conversion of hypoxanthine into uric acid (21). The hypoxanthine levels were calculated taking the molar absorbtivity of uric acid as 12.200 M^ cm..,. In these determinations hypoxanthine standard was also used. Standard and samples were studied under the same conditions. Both calculations gave the same results. Tissue hypoxanthine levels were calculated as nanomol per gram of wet tissue.

Total glutathione levels were determined according to the procedure of Tietz (22), using glutathione reductase and NADPH. Total glutathione levels are expressed as milimolar (mM).

For the determination of myocardial ATP content, specimens obtained from myocardium were immersed in liquid nitrogen and then freeze-dried at 50°C. Specimens were analyzed by high-performance-liquid-chromatography using the techniques described by Hull-Ryde (23). Tissue ATP levels were calculated as pmol/gr dry weight Kreatine kinase (CK) enzyme was measured with an automated analyser using creatine kinase EC 2-7-3-2 (Boehringer, Mannheim) kits, and expressed as IU/min gr heart.

Expression of Results

The following calculations were made

Arrest Time: Time (seconds) from the onset of cardioplegic infusion until the heart arrests.

Table 2. The effects of the addition of nifedipine, verapamil and diltiazem to the STHCS upon post ischemic recovery of cardiac function

		Percent recovery of cardiac function			
	Arrest time (Sec)	Total pre Arrest beats	Heart rate	Contractile force	Heart work
STHCS (control)	63.4±18.0	72.2±8.3	96.1+7.8	36.2±4.5	38.5±6.2
STHCS+Nifedipine	50.2110.1	49.6+7.1*	83.4±9.1*	54.5±7.1*	52.3+5.0*
STHCS+Verapamil	50.6+7.9	42.1±9.6*	82.1±5.3*	56.2±4.0*	53.8±6.3*
STHCS+Diltiazem	49.1±6.5	44.3±7.8*	80.2±4.7*	58.1±2.6*	57.1±3.1*

STHCS: St Thomas' Hospital cardioplegic solution
The results are indicated meantSEM. Each group consisted of 8 hearts.
*(p<0.05) Indicates significant difference between the value indicated and STHCS group.

Table 3. Preischemic and reperfusion period contractile force (gr contractility/gr heart weight) values.

	Group I (Control)	Group II	Group III	Group IV
Preischemic (15th min)	21.443.1	22.1±1.8	20.8±2.4	22.4±1.6
Reperfusion period (min)				
5	14.2±2.4	18.8+2.7	17.611.1	19.1±1.7
10	9.9±1.7	15.3±2.0	15.2±1.6	16.0±2.0
15	8.4±2.4	13.211.1	12.1 ±1.9	14.1 ±1.5
20	7.9±4.5	11.9±2.4	12.0±0.8	13.8±1.2
25	7.8±3.9	11.9±1.6	11.6+1.2	13.1+1.8
30	7.7±2.8	12.0±2.1	11.6±1.4	13.0±1.8

Total pre arrest beats: Number of heart beats during the 3 min of cardioplegia infusion.

$$\text{Percentage recovery of heart rate (HR)} = \frac{\text{Post-ischemic heart rate}}{\text{Pre-ischemic heart rate}} \times 100$$

$$\text{Percentage recovery of ventricular contractile force} = \frac{\text{Post-ischemic concentrations (gr contractilitiy/gr. heart weight)}}{\text{Pre-ischemic contractions}} \times 100$$

$$\text{Percentage recovery of heart work} = \frac{\text{Post-ischemic HR} \times \text{Post-ischemic contractile force}}{\text{Pre-ischemic HR} \times \text{Pre-ischemic contractile force}} \times 100$$

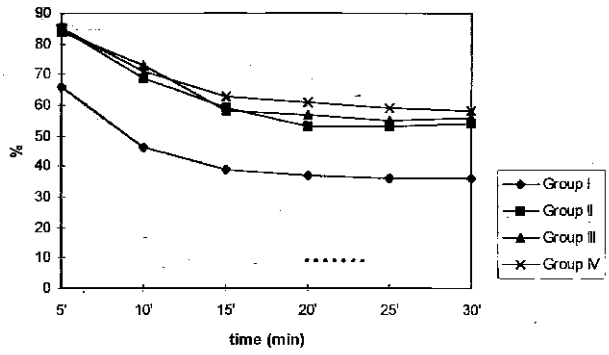


Figure 1. Contractile force-time graphy in the reperfusion period

Data and Statistics

All values are expressed as the meanistandard error of the mean (SEM). For statistical analysis; analysis of variance, Mann-Whitney U, and Kruskal-Wallis one-way anova test as were used where appropriate. A p value <0.05 was considered to be significant.

RESULTS

Hemodynamic data

Two hearts in group I and one in group III developed irreversible ischemic contracture at the end of 90 min of normothermic global ischemia. As shown in Table 2, there were no significant difference in arrest time among the groups. The number of total pre-arrest beats were 72.20±8.30 in STHCS group. Although there were no significant difference between groups II-IV, the difference was found to be significant between the drug treated groups and control group (p<0.05).

The preischemic (15th min) and post ischemic left ventricular contractile force values obtained from each group was shown in Table 3. Contractile force-time graphy in the reperfusion period was shown in Fig. 1.

The hearts in study groups showed better preservation of left ventricular contractile function. At the 30th min

Table 4. The effects of calcium channel blockers on tissue lactate, MDA, Hpx, total glutathione and ATP content.

	Tissue Lactate umol/gr wet weight	MDA umol/gr wet weight	Hpx umol/gr wet weight	Total glutathion umol/gr wet weight	ATP Umol/gr wet weight
STHCS	0.66±0.11	125.31±20.08	0.55±0.22	3.31*0.62	9.0*0.8
STHCS+Nifedipine	1,49*0.12*	84.10±10.12	1.3910.12*	13.61*1.51*	11.2*0.5
STHCS+Verapamil	1.12*0,18*	86.75*11.9	1,48*0,13*	17.99*3.15*	13.2*0.6*
STHCS+Diltiazem	1.45*0.30*	52.02*3.01*	1.20+0.31*	28.54*5.63*	15.3*1.3*
Left ventricular tissue before hypoxia as control	0.65±0.04	46.38±5.51	0.31*0.27	27.58*0.19	19.1*0.9

All results are the mean and the standard error of the mean. Each group consisted of 8 hearts.
*p<0.05 indicates significant difference between the value indicated and STHCS group.
MDA: Malondialdehyde, Hpx: Hypoxanthine, ATP: Adenosine triphosphate

of reperfusion, contractile force was reduced to 54.5%±7.1%, 56.2%±4.0%, and 58.1%±2.6% of their control values for groups II, III and IV respectively (p<0.05 as compared to STHCS group).

Percentage recovery of postischemic heart work, were better in the groups in which nifedipine, verapamil and diltiazem were added to the STHCS. Although there were no significant difference between these groups, the differences were significant when compared to control.

Metabolic effects of global ischemia

Biochemical determinations of the reperfused myocardium were shown in Table 4. Tissue lactate and hpx concentrations were unexpectedly low in the STHCS group. This may be the sign of inhibited glycolysis (p<0.05 as compared the other groups).

Lipid peroxidation was significantly decreased in the fourth group (p<0.05 vs. control). Although MDA levels in group II and III were lower than the control, the difference was not found to be significant. Although, the difference between the study groups and control was found to be significant, according to the myocardial glutathione content, the best results were obtained in the last group (p<0.05 as compared the other groups).

Tissue MDA and glutathione contents showed that there was a strict correlation between the depletion of glutathione content and increased lipid peroxidation. As ATP concentration was significantly decreased in the control group, Ca^{2+} channel blockers were found to be effective for the maintenance of tissue ATP levels. According to the myocardial functional and biochemical data, there was a strict correlation between the tissue glutathione, ATP contents and postischemic contractile function.

Initial and reperfusion period CK relase and coronary flow data (Table 5) showed that nifedipine cardioplegia has no superority when compared with the control group.

Although, there was a significant increase in CK leakage as compared to the group III and group IV, best results for coronary flow was achieved in the last two groups.

Table 5. Preischemic and reperfusion period CK leakage and coronary flow values (*p<0.05 vs. control).

	Preischemic	Reperfusior
CK leakage (IU/L min gr. Heart)		
Group I (Control)	20.8*3.1	312.5*47.8
Group II (Nifedipine)	24.6*3.8	264.6*34.2
Group III (Verapamil)	29.3*4.8	155.0*18.5
Group IV (Diltiazem)	25.2*4.0	140.6*35.0
Coronary Flow (ml/min gr heart)		
Group I	45.1*8.0	46.3*4.6
Group II	40.2*6.5	50.5*5.8
Group III	47.3*4.6	68.9*4.1*
Group IV	45.8*6.0	75.4*3.4*

DISCUSSION

The protection of myocardium so as to minimize the postishemic impairment of left ventricular function is a major concern during cardiac surgery. During the ischemic period, oxidative phosphorylation is impaired due to the lack of oxygen, and therefore high energy phosphates (primarly adenosine 5' thriphosphate (ATP) and creatine phosphate) are depleted (24-26). At the early stages of ischemia, glycolysis is stimulated to compansate the energy need. However, in prolonged ischemia, glycolysis is inhibited by the development of tissue acidosis and the accumulation of several metabolites including citrate (3,4,25).

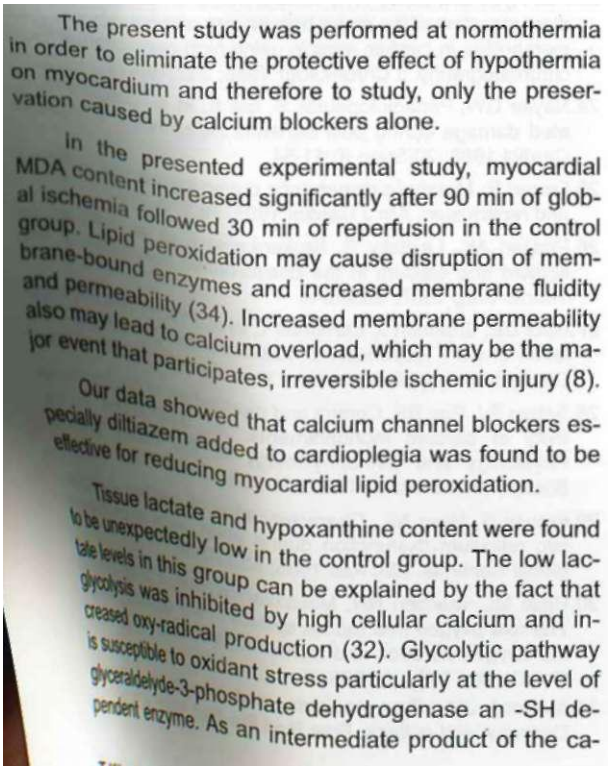
In physiologic conditions, cytoplasmic calcium concentration is maintained under $1fJ^{-7}$ M. When calcium concentration is elevated to micromolar levels, calcium-ATPase is activated to pump calcium in to the sarcoplasmic reticulum vesicles. In addition, excess cystosolic calcium is pumped out of the cell or into the mitochondria by other calcium-activated ATPases. Calcium transport against a concentration gradient is strictly dependent on ATP energy. During prolonged ischemia, calcium transport is blocked because of insufficient ATP production and a sharp increase in calcium concentration occurs (27-29).

On reperfusion, more calcium can accumulate in the cytoplasm (24,26). It is well known that the production of free oxygen radicals is increased with the resupply of

oxygen after ischemia (2,4,30,31). These radicals react with membrane phospholipids to initiate lipid peroxidation which in turn irreversibly inactivates calcium-ATPases (8). In addition to the inhibition of calcium-ATPases, the inhibition of glycolysis is also held responsible for calcium overload (32).

An increased level of calcium activates several metalloproteinases including calpains involved in proteolytic conversion of xanthine dehydrogenase to xanthine oxidase. Calcium can also activate phospholipase A_2, the enzyme that degrades membrane phospholipids (33). Without any doubt, reperfusion is the most effective way to treat the ischemic myocardium. Some authors believe that much of the injury is the consequence of events occurring at the moment of reperfusion, rather than as a result of changes occurring during the ischemic period (2,4).

Despite numerous experimental and clinical studies, ideal myocardial protection has not yet been found. Recent reports on the experimental (9-14) and clinical (15,16) use of calcium channel blockers to limit reperfusion injury have been encouraging. The protective properties of calcium channel blockers include reduction of the rate of extent of injury during ischemia together with combating coronary spasm, reduction of arrhythmia and hypertension, influence automaticity and slow conduction (9,11,14). The purpose of these experiments were to determine, if the addition of nifedipine, verapamil and diltiazem to potassium cryoplegic solution was synergistic in aiding restoration of cardiac function and myocardial protection.


The present study was performed at normothermia in order to eliminate the protective effect of hypothermia on myocardium and therefore to study, only the preservation caused by calcium blockers alone.

In the presented experimental study, myocardial MDA content increased significantly after 90 min of global ischemia followed 30 min of reperfusion in the control group. Lipid peroxidation may cause disruption of membrane-bound enzymes and increased membrane fluidity and permeability (34). Increased membrane permeability also may lead to calcium overload, which may be the major event that participates, irreversible ischemic injury (8).

Our data showed that calcium channel blockers especially diltiazem added to cardioplegia was found to be effective for reducing myocardial lipid peroxidation.

Tissue lactate and hypoxanthine content were found to be unexpectedly low in the control group. The low lactate levels in this group can be explained by the fact that glycolysis was inhibited by high cellular calcium and increased oxy-radical production (32). Glycolytic pathway is susceptible to oxidant stress particularly at the level of glyceraldehyde-3-phosphate dehydrogenase an -SH dependent enzyme. As an intermediate product of the catabolism of adenine nucleotides, hypoxanthine is considered to be a maker for ischemia. The reason why hypoxanthine levels were low is the STHCS group might be explained in the form that hypoxanthine to uric acid conversion might have been blocked in the step of xanthine oxidase or much of hypoxanthine might have been converted to uric acid before biochemical determination. According to this data it can be concluded that calcium channel blockers can block xanthine dehydrogenase to xanthine oxidase conversion.

Glutathione, as a cellular antioxidant, protects proteins and other biomolecules from oxidation. The levels of total glutathione were also very low in the control group, showing that this molecule was lost from the tissue. Since no calcium channel blocker was used in the STHCS group and glutathione was lost from the tissue, it was reasonable to suggest that xanthine dehydrogenase was converted into oxidised form by Ca^{2+} and/or by -SH modification. To clarify these statements, the inhibition of glycolysis and convention of xanthine dehydrogenase to its oxidase form must be demonstrated in the STHCS group.

Rosencrantz and collagues reported good myocardial function in hearts with endocardial ATP levels <1 mmol/kg wet weight after reperfusion (35). However, the ability to resynthesize ATP is more critical than the absolute level of these compounds. If ATP is low because of impaired mitochondrial oxidation phosphorilation, the viability of the myocyte is in jeopardy.

Our ATP data showed that the calcium antagonists are capable of preventing nucleotide depletion. Although Barnes and collagues stated that diltiazem is inferior in this regard (36); Vouhe and co workers suggested that returning of the left ventricular function on the whole, was superior when diltiazem was used as an additive to cardioplegic solution (10). In the presented study, it was shown that there was a clear relationship between the beneficial effects on contractile function and the maintainance of ATP levels after ischemia-reperfusion in the hearts treated with Ca^{2+} channel blockers.

Hearts treated with verapamil and diltiazem displayed a marked hyperemic response in the early period of reperfusion. This response has also been associated with better left ventricular preservation and less enzyme (CK) release. Result of the present study confirmed that, under normothermic condition, the protective effect of calcium channel blockers in cardioplegic solution were additive. Hemodynamic values and biochemical parameters in diltiazem group were found slightly better then verapamil and nifedipine groups.

Our study showed that, nifedipine, verapamil and especially diltiazem used as cardioplegic additives can enhance cardioplegic protection against ischemia reperfusion injury.

Kobay kalbinde iskemik kardiak arrest sırasında yavaş kanal blokerleri ile kardioplejinin ilave koruyucu etkileri; Nifedipine, Verapamil ve Diltiazem'in karşılaştırmalı incelemesi

Bu çalışma; modifiye Langendorff modelinde, kalsiyum kanal blokerleri olan nifedipin (0.075 mmol/L), verapamil (1.1 mmol/L) ve diltiazem (0.03 mmol/L)'in global iskemi ve reperfüzyon sonrası myokard üzerindeki etkilerini karşılaştırmak amacıyla planlanmıştır. İzole edilmiş 32 adet kobay kalbi, 4 gruba ayrıldı (n:8) ve 90 dk normotermik global iskemiyi takiben 30 dk reperfüzyona tabi tutuldu. Kardioplejik arrest St. Thomas' Hastanesi Kardioplejik Solüsyonu'na (STHCS) üç Ca^{+2} kanal blokerlerinden birinin eklenmesiyle elde edildi. SıHCS'e Ca^{+2} kanal blokerleri eklenmesiyle kardiyak fonksiyonun iyileşme yüzdesi arttırıldı. STHCS grubuyla karşılaştırıldığında diltiazem, verapamil ve nifedipin eşliğinde myokardda azalmış lipid peroksidasyonu ve adenozin trifosfat (ATP) katabolizması, korunmuş glutatyon seviyesi ve ATP içeriği gözlendi. Bu sonuçlar; Ca^{+2} kanal blokerlerinin özellikle de diltiazem'in eklenmesinin kardiyoplejik korunmayı arttırabileceğini doğruladı. [T Klin Araştırma 1997; 15(2):49-55]

REFERENCES

1. Flitter WD. Free radicals and myocardial reperfusion injury. Br Med Bui 1993; 49:545-55.
2. Hearse DJ. Ischemia, reperfusion, and the determinants of tissue injury. Cardiovasc Drugs and Ther 1990; 4:767-76.
3. Opie LH. Myocardial ischemia-metabolic pathways and implications of increased glycolysis. Cardiovasc Drugs and Ther 1990; 4:777-90.
4. Ferrari R, Ceconi C, Curello S, et al. Myocardial damage during ischemia and reperfusion. Eur Heart J 1993; 14(Suppl G):25-30.
5. Opie LH. The mechanism of myocyte death in ischemia. Eur Heart J 1993; 14(Suppl G):31-33.
6. Shen AC, Jennings RB. Kinetics of calcium accumulation and acute myocardial ischemic injury. Eur Heart J 1972; 67:441-52.
7. Nayler WG, Ferrari R, Williams A. The protective effect of pretreatment with verapamil, nifedipine and propranolol on myocardial function in the ischemic and reperfused myocardium. Am J Cardiol 1980; 46:242-8.
8. Jimenez E, del Nido P, Feinberg H, et al. Redistribution of myocardial calcium during ischemia. Relationship to onset of contracture. J Thorac Cardiovasc Surg 1993; 105:988-93.
9. Yamamoto F, Manning AS, Braimbridge MV, et al. Cardioplegia and slow calcium blokers. Studies with verapamil. J Thorac Cardiovasc Surg 1983; 86:252-61.
10. Vouhe PR, Helias J, Grondin CM. Myocardial protection through cold cardioplegia using diltiazem, a calcium channel blocker. Ann Thorac Surg 1980; 30:342-8.
11. Yamamoto F, Manning AS, Crome R, et al. Calcium antagonists and myocardial protection: A comparative study of the functional metabolic and electrical consequences of Verapamil and Nifedipine as additives to the St. Thomas' cardioplegic solution. Thorac Cardiovasc Surgeon 1983; 33:354-9.
12. Clark RE, Ferguson TB, West PN, Shuchleib RC, et al. Pharmacological preservation of the ischemic heart. Ann Thorac Surg 1977; 24:307-14.
13. Magovern GJ, Dixon CM, Burkholder JA. Improved myocardial protection with nifedipine and potassium-based cardioplegia. J Thorac Cardiovasc Surg 1981; 82:239-44.
14. Yamamoto F, Manning AS, Braimbridge MV, et al. Nifedipine and cardioplegia: Rat heart studies with the St Thomas' cardioplegic solution. Cardiovasc Res 1983; 17:719-24.
15. Clark RE, Christlieb IY, Ferguson TB, et al. Laboratory and initial clinical studies of Nifedipine, a calcium antagonist for improved myocardial preservation. Ann Surg 1981; 193:719-32.
16. Clark RE, Christlieb IY, Ferguson TB, et al. The first American clinical trial of nifedipine in cardioplegia. J Thorac Cardiovasc 1981; 82:848-59.
17. Bourdillon PD, Poole-Wilson PA. The effects of verapamil, quiescence, and cardioplegia on calcium exchange and mechanical function in ischemic rabbit myocardium. Circ Res 1982; 50:360-8.
18. Watts JA, Koch CD, La Noue KF. Effects of calcium antagonism on energy metabolism: calcium and heart function after ischemia. Am J Physiol 1980; 223:H909-16.
19. Uchiyama M, Mihara M. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem 1977; 86:271-8.
20. Burtis CA, Ashwood ER. Determination of lactate in whole blood. In: Tietz Textbook of Clinical Chemistry. Philadelphia: WB Saunders, 1994:976-8.
21. Jong JW, Goldstein S. Changes in coronary venous inosine concentration and myocardial wall thickening during regional ischemia in the pig. Circ Res 1974; 35:111-6.
22. Tietz F. Enzymatic method for quantitative determination of nanogram amounts of total and oxidized glutathione. Anal Biochem 1969; 27:502-22.
23. Hull-Ryde EA, Lewis WR, Verone CD, et al. Simple step gradient elevation of the major high energy compounds and their metabolites in cardiac muscle using high performance liquid chromatography. J Chromotogr 1986; 337:165-74.
24. Nayler GW, Panagiotopoulos S, Ela K, et al. Calcium mediated damage during post-ischemia reperfusion. J Mol Cell Cardiol 1988; 20(Supp II):41-54.
25. Ferrari R. Metabolic disturbance during myocardial ischemia and reperfusion. Am J Cardiol 1995; 76:17B-24B.
26. Pridjian AK, Levitsky S, Krukenkamp I, et al. Intracellular sodium and calcium in the postischemic myocardium. Ann Thorac Surg 1987; 43:416-9.
27. Gool DE, Shannon J, Edmunds T, et al. Properties and regulation of Ca^{2+} dependent proteinases. In: Bernard BD, ed. Calcium binding proteins. New York: Elsevier, 1983:19-35.
28. Solaro RJ, Pan BS. Control and modulation of contractile activity of cardiac monofilaments. In: Speralakis N, ed. Physiology and pathophysiology of the heart, 2nd ed. Boston: Kluwer Acad, 1985:291-303.
29. Krause S, Hess ML. Characterization of cardiac sarcoplasmic reticulum dysfunction during short term normothermic global ischemia. Circ Res 1985; 55:176-84.
30. Hess ML, Manson NH. Molecular oxygen: Friend and foe. The role oxygen free radical system in the calcium paradox, the oxygen paradox and ischemia/reperfusion injury. J Mol Cell Cardiol 1984; 16:969-85.
31. McCord JM. Free radicals and myocardial ischemia: Overview and outlook. Free Rad Bio Med 1988; 4:9-14.

32. Coretti MC, Koretsune Y, Kusuoka H, et al. Glycolytic inhibition and calcium overload as consequences of exogenously generated free radicals in rabbit hearts. J Clin Invest 1991; 88:1014-25.

33. Cohen MV. Free radicals in ischemic and reperfusion myocardial injury: Is this the time for clinical trials? Ann Inter Med 1989; 111:918-31.

34. Prasad K, Kalra J, Chan WP, et al. Effect of oxygen free radicals on cardiovascular functions at organ and cellular level. Am Heart J 1989; 117:1196-202.

35. Rosenkranz ER, Okamoto F, Buckberg GD, et al. Studies of controlled reperfusion after ischemia. II.Biochemical studies: failure of tissue adenosine triphosphate levels to predict recovery of contractile function after controlled reperfusion. J Thorac Cardiovasc Surg 1986; 92:488-501.

36. Barnes HB, Jellinek M, Standeven JW, et al. Cold blood-diltiazem cardioplegia. Ann Thorac Surg 1982; 33:55-62.