The Protective Effect of Lisinopril on Membrane Bound Enzymes in Myocardial Preservation

MYOKARD KORUNMASINDA LİSİNOPRİLİN MEMBRANA BAĞLI ENZİMLER ÜZERİNE KORUYUCU ETKİSİ

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Summary

The present study was done to evaluate the protective effect of lisinopril on membrane bound enzymes; Na+-K+/Mg^{2+} ATPase and Ca^{2+}/Mg^{2+} ATPase. There is substantial evidence that these enzymes can effect membrane integrity. We hypothesized that if we could protect the membrane in ischeinia-reperfusion there might be a chance to augment contractility. Guinea-pig hearts (n=24) were studied in isolated, Krebs-Henseleit perfused Langendoiff cardiac model. In group 1, control hearts (n=8) were arrested with St.Thomas Cardioplegic Solution (STHCS) alone. In group 2, hearts (n=8) were arrested with lisinopril (1 pmol/L) added STHCS and in group 3 (n=8) animals were pretreated with oral lisinopril (0.2ing/kg) for seven days and arrested with STHCS. Hearts were subjected to hypothermic global ischemia for 90 minutes and than were reperfused at 37°C. The addition of lisinopril improved both the levels of membrane bound enzymes and recoveiy of cardiac functions. On the basis of these results it can be concluded that lisinopril protects the membrane integrity and plays a role in restoring the contractility in ischeniia-repeifusion injury.

Key **Words:** Myocardial protection, Cardioplegia, Lisinopril, Na^+-K^+/Mg^{2+} ATPase, Ca^{2+}/Mg^{2+} ATPase

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Ozet

Bu çalışma lisinoprilin membrana bağlı enzimler olan Na^+-K^+/Mg^{2+} ATPaz ve Ca^{2+}/Mg^{2+} ATPaz üzerine koruyucu et/asini araştırmak için yapılmıştır. Bu enzimlerin membran bütünlüğünü koruduğuna dair pek çok gözlem bulunmaktadır. Bu çalışmanın hipotezi, iskemi-reperfüzyondu menıbrcının korunabilmesi halinde kontraktilitenin sağlanmasının daha kolay olacağıdır. Kobay kalpleri (n=24) izole edilerek Krebs-Hanseleit çözeltisi ile perfüze edilmiş ve Langendorff kalp modeli oluşturulmuştur. Birinci grupta kontrol kalpleri (n=8), yalnızca St. Thomas kardiyoplejik çözeltisi (STCHS) ile durdurulmuştur. İkinci gruptaki kalpler (n=8), lisinopril (I pM/L) eklenen STCHS ile durdurulmuştur. Üçüncü gruptaki kalpler ise (n=8), 7 gün boyunca 0.2 mg/kg ora! lisinopril uygulamasından sonra STHCS ile durdurulmuştur. Kalpler 90 dakika boyunca hipolermik global iskemiye maruz bırakılmış ve daha sonra 37°C'da reperfüze edilmişlerdir. Lisinopril eklenmesi hem membrana bağlı enzim seviyelerinin yüksek olmasını hem de kalp fonksiyonlarının geri dönmesini sağlamıştır. Bu sonuçlara göre lisinoprilin membran bütünlüğünü koruduğu ve iskemi-reperfüzyon hasarında kontraktiliteıiin geri dönmesinde rol oynadığı söylenebilir.

Anahtar Kelimeler: Myokard korunması, Kardiyopleji, Lisinopril, Na^+-K^+/Mg^{2+} ATPaz, Ca^{2+}/Mg^{2+} ATPaz

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Myocardial protection can be provided by a range of physiological or therapeutic processes. Ischemia can be due to atherosclerosis or thromboembolism or can be produced during interventions such as percutaneous transluminal coronary angioplasty, coronary artery bypass or transplantation. Whatever the source of ischemia, the consequences are always the same; lack of oxygen to the myocardium and lack of suitable substrates for metabolism. Within the first minutes of ischemia the glycolytic pathway is greatly stimulated but it is eventually inhibited by the devolopment of nicotinamide adenine dinucleotide hydrogen (NADH), citrate and lactate (1-4). The available oxygen is insufficient to support oxidative phosphorylation and

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pyruvate, instead of passing into Krcbs' cycle is converted to lactic acid (5). The anaerobic production of adenosine 5' triphosphate (ATP) is insufficient to meet the tissues demands. With continuing ischemia the tissue levels of ATP fall and this initiates a scries of events which are deleterious to the cell which prevents it to maintain a membrane ionic balance. This results in an intake of sodium into the ischemic cell, which on reperfusion is believed to exchange with calcium. On reperfusion calcium readily enters the cell and calcium -sodium ion exchange may be the only one of the mechanisms by which calcium enters the cell. The net increase in calcium is due to an increased influx rather than a reduction of the calcium efflux (6)

The sodium-potassium activated-magnesium dependent adenosine 5'-triphosphatase activity (Na^+-KVMg^{2+} ATPasc) is the enzymatic basis of the sodium (Na^+)-potassium (K^+) pump of the plasma membrane. The major function of this pump is to assist in the creation of transmembrane gradients of Na' and K^+. The ability of calcium to inhibit Na+-K+/Mg^{2+} ATPasc has been established by previous studies (7). Kim and Akera reported that Na+-K+/Mg^{2+} ATPase activity was depressed in isolated guinea-pig heart following ischemia-rcperfusion and some of the free radical scavengers partly prevented the enzyme dysfunction (8). Krause et al. demonstrated that myocardial stunning is associated with damage to the calcium transport system of the sarcoplasmic reticulum (9).

Therefore the deleterious effect of ischemia-rcperfusion injury in membrane bound enzymes such as Na^+-K^+/Mg^{2+} ATPase and Calcium-Magnesium activated adenosine 5' triphophatase (Ca^{2+}/Mg^{2+} ATPase) have been established but their reversibility was not known.

The aim of the present study is to evaluate the effects of lisinopril, an angiotensin converting enzyme inhibitor, which has been proved to be effective in myocardial protection (10-13) on membrane bound enzymes which might affect contractility.

Materials and Methods

Preparation

Hearts were obtained from male Duncan-Hartley guinea-pigs weighing 300-430g. The animals were anesthetized by ether inhalation after intraperitoneal administration of heparin (200 TU) whole hearts were rapidly removed and quickly mounted on a non-recirculating Langendorff perfusion column.

The perfusion buffer was a modified Kiebs-Henseleit bicarbonate medium that consisted of: 118 mM NaCl, 4.7 mM KCI, 25 mM $NaHC0_3$,1.2 mM KH_2P0_4, 1.2 mM $MgS0_4$,1.2 mM $CaCl_2$ and 11.1 mM glucose. The solution was equilibrated with 95% oxygen and 5% carbon dioxide to achieve a pH of 7.4 at 37°C. A perfusion pressure of 60 mmHg was used in the experiment.

Five minutes after, the preparations were installed into the organ baths, the left ventricular contractile forces and the heart rates were recorded. Global ischemic cardiac arrest was induced by clamping the aortic cannula. Then the hearts were arrested by introducing one of the cardioplegic solutions, using a reservoir located 60 cm above the heart attached to a side arm of the aortic cannula (mean rate of 2,5 ml/min) for 3 min at 4°C. During the period of cardioplegic infusion the arrest time and the number of beats were recorded. The hearts were kept at 8-10°C by topical cooling with isotonic saline through the ischemic arrest period. After 90 min of ischemia the tissues were rcperfused for 10 minutes with the same buffer at 37°C. At the end of reperfusion, left ventricular free walls were dissected and tissue samples were frozen for biochemical detenninations.

Basic St. Thomas' Hospital cardioplegic solution (STHCS) was used to arrest the hearts. The composition of the solution is shown in Table 1. Hearts of Group 1 (n=8) were arrested with STHCS. In group 2 (n=8,) hearts were arrested with lisinopril (1 umol/L) added STHCS and in group 3 (n=8) guinea-pigs were treated with oral lisinopril

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

Compound	Concentration (mmol *IL)*
Sodium chloride (NaCl)	110.0
Potassium chloride (KCI)	16.0
Magnesium chloride ($MgCl_2$)	16.0
Calcium chloride ($CaCl_2$)	1.2
Sodium bicarbonate ($NaHC0_3$)	10.0

pH adjusted to 7.8
Osmolality = 324 mOsm / kg H_20

(0.2mg/kg) by gastric lavage for seven days and then arrested with STHCS.

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. The biochemical determinations were done on this homogenate. Tissue lipid peroxide levels (MDA) were determined by the method of Uchiyama and Mihara (14). The thiobarbituric acid reactive substances (TBARS) were calculated as nanomol per gram wet tissue, and tetramethoxypropane was used as standard.

Total glutathione levels were determined according to the procedure of Tietz (15), using glutathione reductase and NADPH. Total glutathione levels were expressed as milimolar (ITIM).

Determination of Na^{+}-K^{+}/Mg^{2+} ATPase and Ca^{2+}/Mg^{2+} ATPase activities were done according to the method of Reading and Isbir (16). Ten percent homogenates of the tissue were prepared in 0.3M sucrose containing 1 pm magnesium by homogenizing for 90 seconds, using a teflon pestle clearence of 0.25 to 0.38 mm at lOOOrpm. ATPase activities were determined on the resulting supernatants by measuring the rate of liberation of inorganic phosphate (Pi) from disodium ATP (17). Incubation media were made up as described previously (18).The final concentrations in mM for the constituent for the different ATPases were as follows:

Na^{+}-K^{+}/Mg^{2+} ATPase; $MgCl_2$ 6, KCL 5, NaCl 100, EDTA 0.1 and Tris buffer pH 7.4.135.

Ca^{2+}/Mg^{2+} ATPase; $MgCl_2$ 6,$CaCl_2$ 2.5, EDTA 0.1, Tris buffer pH 7.4. 135.

The reaction was started by the adition of ATP to final concentration of 3mM after 5 minutes of preincubation at 37°C for comparison, a blank sample and standards were processed simultaneously. After incubation at 37°C for 30 minutes, the reaction was terminated by putting the sample on ice. For determination of the liberated inorganic phosphate (Pi),1 ml of the incubated mixture was mixed with 2ml lubrol-molybdate solution which was prepared according to the method of Atkinson et al.(18). Extinction at 390 nm was measured in a Schimadzu spectrophotometer. A11 assays were done in triplacate and KH_2P0_4 was used as a standard and specific activities were expressed as nmol Pi/hr/ng protein. Protein content was determined according to the method of Lowry et al.(19) and bovine serum albumin was used as a standard.

Expression of Results

During the pre-ischemic working control period heart rate, arrest beat, contractile force was recorded. During the recovery period these parameters were again measured and calculated as a percentage of their pre-ischemic control values as shown below:

A11 animals received human care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and Published by the National histitutes of Health (NIH Publication No.86-23, revised 1985).

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

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

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

Data and Statistics

The mechanical data (heart rate, contractility) was recorded by using Grass® Force-displacement transducer (FT 03C Grass Instrument Co. Quincy, Mass., USA) and calculated from the polygraph (Grass model 7; Volts 230, Freq 50; serial D531 V3 Grass Instrument Co. Quincy, Mass., USA).

For statistical analysis; analysis of variance, Mann-Whitney U, and Duncan tests were used where appropriate. A p value < 0.05 was considered to be statistically significant. A11 values are expressed as the mean±standard error of the mean (SEM).

Results

The effects of the addition of lisinopril to the STHCS and pretreatment with lisinopril upon postishemic recovery of cardiac function are shown in Table 2.

In group 1 cardiac arrest time was 42.7±3.1 sec. There was no significant difference between the group 1 and group 2 and 3. The number of arrest beats in group 1 was 58.3±7.1. Similar to arrest time there was no significant difference between the groups for arrest beats.

The final recovery of heart rate was 96.3±9.1 in group 1 where it was 100.1±9.2 in lisinopril cardioplegia (group 2), 99.8±5.1 in prctreated group with lisinopril (group 3). However there were no significant differences between groups.

The percentage recovery of contractile force was significantly better in group 2 (83.1±4.5) and in group 3 (80.2±6.1) than the group 1 (69.1±3.1). But no significant difference was found between the groups 2 and 3.

For percentage recovery of postischemic heart work the results were better in the lisinopril groups ($p<0.05$) as compared to STHCS group (Table 2). However, the difference was not found to be significant between the groups 2 and 3.

The results of biochemical determinations are shown in Table 3. Lipid peroxidation (measured as TBARS) was significantly high in group 1 (103.3±5.79 umol/gr wet weight). Lisinopril pro-

Table 2. The effects of the addition of Lisinopril to the STHCS and pretreatment with Lisinopril upon post-ischemic recovery of cardiac function

		PERCENT RECOVERY OF CARDIAC FUNCTION			
	ARREST TIME(SEC)	ARREST BEAT	HEART RATE	CONTRACTILITY	HEART WORK
GROUP1	42.7±3.1	58.3±7.1	96.3±9.1	69.U3.1	61.2±5.8
GROUP2	43.9±6.1	60.3±6.2	100.Ü9.2	83.H4.5 *	76.3±6.9*
GROUP3	45.U4.8	62.1±8.2	99.8±5.1	80.2±6.1 *	72.3±S.l*

'•"Indicates significant difference ($p<0.05$) between the value indicated and group
STHCS:Sl.Thomas Hospital Cardioplcgic Solution
The results arc indicated mean ± Standart error of the mean. Each group consisted of eight hearts

Table 3. The effects of Lisinopril on Myocardial MDA; Glutathione; Na^+-K^+/Mg^{++} ATPase and Ca^{++}/Mg^{++}ATPase

Tissue	GSH pmol/gr.wct weight	MDA pinol / gr wet weight	Na^+-K^+/Mg^{++} ATPase nmol/Pi/mg	Ca^{+}VMg^{++} ATPase nmol/Pi/mg
Group 1	Ü.15±Ü.Û1	1Ü3.30±5.79	466.38i5.99	884.69i9.128
Group2	0.44±0.1 1*	75.47il.29*	558.53i7.45*	1240.36i9.59*
Group3	0.35±0.01*	84.05±5.83*	538.96il7.04*	1142.77i7.78**

The results are indicated mean i SEM. Each group consisted of 8 hearts
* Indicates significant difference ($p<0.05$) between the value indicated and STHCS group
** Indicates significant difference between the value indicated and group2
MDA: Malondialdehyde
GSH: Reduced Glutathione

tected the myocardium toward lipid peroxidation. As a matter of fact that MDA levels in group 2 (75.47±1.29 umol/gr wet weight) and in group 3 (84.05±5.83 umol/gr wet weight) were found to be significantly lower ($p<0.05$) when compared to the control (group 1).

Similar observation was found for glutathione (GSH) levels. GSH concentration was significantly decreased in the STHCS group (0.15±0.01 umol/gr wet weight) when compared to group 2 (0.44±0.21) and group 3 (0.35±0.0T). In terms of glutathione loss and lipid peroxidation, application of lisinopril significantly protected the myocardial tissue. This protection was observed to be in the order of lisinopril cardioplegia > pretreatmcnt with lisinopril > STHCS group.

Ca^{2+}/Mg^{2+} ATPase activities were significantly better in groups 2 (1240.36±9.59 nmol/Pi/mg) and 3 (1142.77±7.78 nmol/Pi/mg) when compared to group 1 (884.69±9.128). Na^{+}-K^{+}/Mg^{2+} ATPase activities were also significantly better in group 2 (558.53±7.45 nmol/Pi/mg) and 3 (538.96±17.04 nmol/Pi/mg) when compared to group 1 (466.38±5.99 nmol/Pi/mg).These results were adjusted with lipid peroxidation levels.

Discussion

Protection of the myocardium to minimize the impairment of the ventricular function is a major concern during cardiac surgery. Repcrfusion without any doubt, is the most effective way to treat the ischemic myocardium. When ischemia is severe and prolonged, irreversible damage occurs and there is no recovery of contractile function. This entity has been called repcrfusion injury. Some authors believe that most of the injury is the consequence of events occurring during reperfusion, rather than the result of changes occurring during the ischemic period (1-4). The cellular mechanisms underlying reperfusion induced injury, calcium overload, and arrhythmias have yet to be definitively characterized. However, there is evidence to suggest that rcadmission of oxygen may play a central role. Shattock et al. reported that oxidant stress induces calcium overload and this cellular calcium overload causes the inhibition of Na^{+}-K^{+} pump activity (20). Therefore we suggest that this inhibition may lead to an accumulation of intracellular sodiurn, activation of the Na^{+}-Ca^{2+} exchange, and cellular calcium overload. Oxygen free radicals initiate and enhance lipid peroxidation of sarcolemmal membranes by attacking unsaturated fatty acid residues of phospholipids. The lipid peroxidation and the resultant biochemical changes may alter physical properties of membranes, such as changes in lipid microenviromcnts of membrane bound enzymes. These changes may cause inhibition of enzyme activities and may also increase membrane permeability (21,22,23). Krause et al. showed that sarcoplasmic reticulum isolated from stunned myocardium demonstrates a decrease in the ability to transport calcium, concomitant with a reduction in the activity of the associated Ca^{2+}/Mg^{2+} ATPase activity (9). In the present study tissue Na^{+}-IC7Mg^{2+} ATPase, Ca^{2+}/Mg^{2+} ATPase, MDA, and GSH concentrations were determined using homogenates instead of sarcolemmal preparations to circumvent possible changes in yield or purity of sarcolemma following ischemia and repcrfusion. The results, therefore, do not specifically show the degree of sarcolemmal lipid peroxidation, but indicate the level of tissue peroxidation or its reversal by lisinopril. Our data showed that lisinopril added St.Thomas' Hospital Cardioplegic Solution or oral prctreatment with lisinopril has increased the Na^{+}-KVMg^{2+} ATPase, Ca^{2+}/Mg^{2+} ATPase activities and decreased MDA. Good correlation was observed between inhibition of MDA production and the degree of enzyme protection caused by lisinopril. GSH measurements showed a significant loss of glutathione from the tissues in group 1 when compared to group 2 and 3. According to this we may say that lisinopril has a protective effect on glutathione, a natural free radical scavenger. The increase in Ca^{2+}/Mg^{2+} ATPase activity decreased free cytosolic calcium by increasing transsarcolcmmal calcium influx. Normal contractile activity depends on the proper functioning of the calcium release - uptake cycle. Intracellular free calcium concentrations rise transiently at the initiation of each contraction, principally from the release of calcium from sarcoplasmic reticulum stores (9). After the interaction of calcium with the contractile proteins for generation of contraction-relaxation is initiated by sequestration of calcium by the sarcoplasmic reticulum via an energy requiring process. Since the amount of calcium sequestered by the sar-

coplasmic reticulum determines the calcium availaiblc for release and thus activation, a defect in the function of the calcium pump would subsequently result in less releasable calcium and activation. Therefore any decrease in the amount of calcium stored in the sarcoplasmic reticulum could diminish contractility. Our results confirm the Krause's report (9). We hypothesized that if we could protect the integrity of the sarcoplasmic reticulum we might have a chance to augment contractility and myocardial performance. Angiotcsin converting enzyme inhibitors have been proved to be effective in myocardial protection and used extensively as free radical scavengers (10,11,12). There were no reports indicating whether they are effective in protecting membranes integrity.

Our results showed that angiotensin converting enzyme inhibitor, lisinopril has beneficial effect on membrane integrity besides its efficicacy in lipid peroxidation. Although we didn't find any significant differences between oral and intravenous routes this issue needs, further investigation. Because we have chosen our dosages arbitrarily we think different dosages must be tested.

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