APROTININ IMPROVES Ca^{2+} - Mg^{2+} / ATPase LEVELS IN ISCHEMIA-REPERFUSION INJURY[1,2]

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ABSTRACT

Objective: The aim of this study is to determine the effect of aprotinin, on calcium activated and magnesium dependent adenosine 5' triphosphatase (Ca^{2+}-Mg^{2+}/ATP ase) levels.

Methods: Twenty patients who had elective coronary artery bypass grafting (CABG) were randomly divided into two groups (n= 10, in each). Group A patients received full dose aprotinin (20 000 IU/kg as pretreatment followed by 7500 IU/kg for 6 hours) and Group B patients received normal saline as control group. Erythrocyte membrane Ca^{2+}-Mg^{2+}/ATP ase levels were measured.

Results: Ca^{2+}-Mg^{2+} /ATPase levels were protected in aprotinin group, 807.60+32.62 vs 634.28+22.91 ($p<0.05$) at post-operative 24th hour. ($p<0.05$).

Conclusion: Aprotinin protected the erythocyte membrane Ca^{2+}-Mg^{2+} ATP/ase from oxidant stress thus it might affect the cell membrane stabilization.

Key Words: Aprotinin, Ischemia-Reperfusion injury, Ca^{2+}-Mg^{2+}/ATPase.

INTRODUCTION

Aprotinin is a low molecular weight, broad spectrum protease inhibitor (1). It has been widely used in patients undergoing cardiac operations, those with an increased risk of blood loss (2). Several studies have also confirmed that aprotinin may have beneficial effect on the myocardium before and after prolonged cardioplegic ischemia followed by reperfusion (3, 4). However the exact mechanism responsible from this effect is yet unknown.

The rapid biochemical, functional and structural changes that occur in the heart during early ischemia and reperfusion have been fully described (5). Ischemia leads to the rapid reduction of high-energy phosphates and to an increase in membrane permeability to calcium (6,7). Briefly, calcium overload could activate variety of enzymes such as proteases, lipases phospholipases and ATPase (8, 9). Increased

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activity of proteases causes protein fragmentation which might end up with the inhibition of membrane bound Ca^{2+}-Mg^{2+} /ATPase (10). Because proteolytic enzymes play an important role in ischemic myocardial damage, one may expect a protective effect of aprotinin on the ischemic heart. The aim of the present study is to evaluate whether aprotinin treatment reduces the membrane damage by protecting the Ca^{2+}-Mg^{2+} /ATP ase.

MATERIAL AND METHODS

Twenty patients undergoing elective coronary artery bypass grafting (CABG) were randomly divided into two groups (n= 10 in each group). Group A patients received aprotinin (Trasylol, Bayer A.G Leverkusen Germany) in a dose of 20 000 IU / kg as pre-treatment followed by 7500 IU / kg for 6 hours. Group B patients received normal saline (Eczacıbası-Baxter Istanbul, Turkey) as the same dose. Patients who had low Ejection Fraction (EF<30%), high left ventricular end diastolic pressure (LVEDP>25 mmHg), hereditary thrombopathy and who had received aprotinin priorly were excluded from the study. The study protocol was approved by local ethical committee. All patients received standard anesthesia and surgical protocols for CABG. In brief, patients received anesthetic consisting of induction with fentanyl, propofol and vecuronium bromur, followed by isoflurane and N_2O+O_2 mixture. Cardiopulmonary bypass was established with a roller pump (Sarns 3M, MN, USA) and membrane oxygenator (Dideco SPA, Milan, Italy) with arterial line filtration. Moderate hypothermia (28°C core temperature) was used in all patients with flow rates between 2-2.4 l/m^2 and mean arterial pressures of 40 to 50 mmHg. Myocardial protection was achieved by antegrade cold crystalloid cardioplegia (Plegisol, Abbot Lab., USA) and topical cooling. Blood samples from the patients were drawn at different time points; 1- Baseline (before anesthesia induction but after placement of the arterial and intravenous catheters) 2- Before heparinization 3- After five minutes of cross clamping the aorta 4- After aortic cross clamp removed and cardiopulmonary bypass terminated 5- Ten minutes after protamine given 6- At the end of the operation after skin closure 7- Post-operative 24th hour.

These samples were analyzed for erythrocyte membrane Ca^{2+}-Mg^{2+}/ATP ase levels to show the drugs effect on membrane enzyme system.

Biochemical Determination

Calcium activated and magnesium dependent adenosine 5' triphosphatase determination was measured in membrane suspension according to the method of Atkinson, after incubation in media of Reading (11, 12). The standard incubation solution for determining the $Ca^{2+}Mg^{2+}$ ATPase activity at 37°C consisted of 6 mM $MgCl_2$, 2.5 mM $CaCl_2$, 0.1 mM Na-EDTA 0.1 and 135 mM Tris buffer pH 7.4.

After preincubation for five minutes at 37°C, disodium ATP (Boehringer, Mannheim Corp. USA) was added to give a final concentration of 3 $mmol.l^{-1}$. The sample blank containing no enzyme standards and unknowns were incubated at 37°C for 30 minutes. The reaction was stopped by putting the samples on ice. Inorganic phosphate (Pi) liberated was determined on 1ml aliquots of the incubated mixtures by the addition of 2 ml of lubrol-molybdabet solution prepared according to the method of Atkinson et al. (13), followed by vortexing and standing at ambient temperature for 10 minutes. Extinction at 390 nm was measured in a Shimadzu spectrophotometer. Samples were compared for phosphate content with standards of KH_2PO_4. Specific activities were expressed as nmol/Pi/hr/mg protein. Protein content was determined according to Lowry et al (14). Bovine serum albumin was used as standard. All chemicals were of analar grade, unless stated otherwise. Solutions were made up in glass distilled deionised water.

Statistical Analysis

Values expressed as means $\pm$ SD. All comparisons were made by one way analysis of variance. Unpaired students t test was used to identify statistical significance between groups. P values less than 0.05 were accepted as showing statistical significance.

RESULTS

There were no significant differences between groups in age, body surface area, CPB time, aortic cross clamp time, left ventricular end

diastolic pressure and graft number. There were no peri-operative and post-operative complications in either group. Blood loss in the aprotinin treated group significantly reduced ($p<0.05$).

Ca^{2+}/Mg^{2+} / ATP ase levels were decreased following ischemia and reperfusion period in both groups. However gradual increase occurred in aprotinin group as reperfusion time was prolonged, and the difference was statistically significant ($p<0.05$), (921.48±47.69 to 634.28±22.91 versus 912.83±48.53 to 807.60±32.52) (Fig.1).

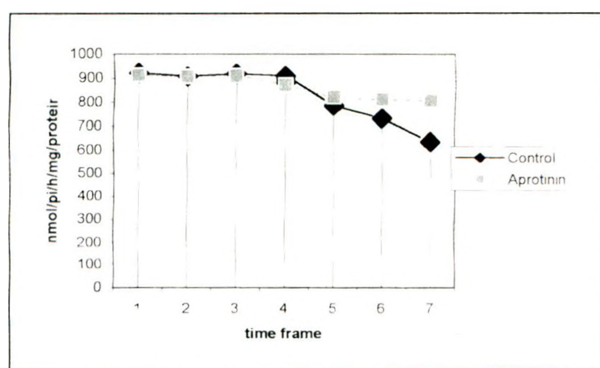

Fig.1 : Ca^{2+}-Mg^{2+}/ATP ase activity of the control and Aprotinin groups. 1- Baseline (before anesthesia induction but after placement of the arterial and intravenous catheters) 2- Before heparinization, 3- After five minutes of cross clamping the aorta 4- After cross clamp removed and CPB terminated 5- Ten minutes after protamine is given. 6- At the end of the operation following skin closure. 7- Post-operative 24th hour. *$p<0.005$ in comparison with control group.

DISCUSSION

Emerging evidence supports the concept that Ca^{2+} loading both during and after ischemia is primarily, but probably not exclusively, dependent on Na^{+} loading and Na^{+}-Ca^{+} exchange (15, 16). Cellular Na^{+} loading occurs during ischemia principally once myocytes are fully energy depleted. This may occur as a consequence of impaired Na^{+} efflux (inhibition of Na^{+}-K^{+}/ATP ase) and continued Na^{+} influx. Recent experiments have shown that depression of Ca^{2+}-Mg^{2+}/ATP ase activity has also been responsible for a significant increase in cellular Ca^{2+} (17). Analysis of the conformational state of Ca^{2+}-Mg^{2+}/ATP ase suggested that hydrophobic domains of the enzyme is responsible for a significant increase in calcium efflux and calcium pump uncoupling.

In fact, free radicals might be inducing the membrane defects which promote calcium entry. Thus reducing cellular Ca^{+} loading, during and after ischemia or minimising its intracellular actions would seem to be of great importance in preserving postischemic function of the heart.

Aprotinin treatment was able to protect the Ca^{2+}-Mg^{2+}/ATPase levels compared with the untreated control group ($p<0.005$). As one of the main transport systems for calcium efflux is Ca^{2+}-Mg^{2+} ATP ase we can say that aprotinin has a role in improvement of membrane calcium transport system. However the absence of intracellular calcium data is one of the drawbacks of this study. Therefore the membrane injury seems to be lowered by the aprotinin treatment.

Another drawback of this study is that the absence of left ventricular (LV) function in the post-operative period. As the study was not designed to define the precise mechanism of aprotinin's beneficial actions on preserving the LV function, further investigatons will be required to evaluate such a potential mechanism.

Therefore the following conclusion can be drawn from this study: The protective effect of aprotinin might be induced by myocardial protease inhibition and by protecting myocardial membrane from these protease attacks.

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