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Kurian GA, Murugan M, Paddikkala J

Krause & Pachernegg GmbH · VERLAG für MEDIZIN und WIRTSCHAFT · A-3003 Gablitz/Austria

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Effect of Magnesium on Modulating the Activity of $Na^{+}K^{+}$-ATPase, Ca^{2+}-ATPase, Mg^{2+}-ATPase and 5'-Nucleotidase in South Indian Patients Undergoing Coronary Artery Bypass Graft Surgery

G. A. Kurian[1], M. Murugan[1], J. Paddikkala[2]

Objectives: *Cardiac surgery performed with cardiopulmonary bypass is associated with extensive overproduction of reactive oxygen species which is characterized by cell membrane damage that leads to impairments of membrane-bound ionic pumps. Changes in myocardial enzyme activity correlated with the progression of myocardial morphological changes and increased permeability of myocyte micro vessels.*
Aim of the Study: *The present study was designed to assess the status of plasma lipid peroxides and erythrocyte membrane activities of $Na^{+}K^{+}$-ATPase, Ca^{2+}-ATPase, Mg^{2+}-ATPase and 5'-nucleotidase of South Indian patients undergoing coronary artery bypass graft (CABG) surgery. This study also focused on the role of intra-operative magnesium supplementation to preserve membrane permeability.*
Patients and Methods: *51 South Indian patients who had undergone CABG surgery (with intra-operative magnesium supplementation) and 35 controls (without magnesium supplementation) were selected and matched. The activities of the above-mentioned enzymes and cardiac enzymes were measured in the erythrocyte membrane and plasma, respectively.*
Results: *The activities of erythrocyte ATPase were found to be significantly ($p < 0.05$) decreased in patients who were not administered Mg^{2+} during revascularization procedure and were improved by magnesium supplementation. However, the plasma TBARS concentration in magnesium-treated and -untreated patients did not show any significant ($p < 0.05$) change even though the level of TBARS was lower in the first group. The cardiac marker enzymes were found to be improved in their activities by Mg^{2+}supplementation.*
Conclusion: *These results predict increased oxidative stress and ionic imbalance during CABG procedure, both in plasma and erythrocyte membrane. The extensive administration of magnesium before release of the aortic cross clamp can protect cardiomyoctes by preserving the ionic status of the cell.* ***J Clin Basic Cardiol 2006; 9 (online): 10–6.***

Key words: *$Na^{+}K^{+}$-ATPase, Ca^{2+}-ATPase, Mg^{2+}-ATPase, CABG, reperfusion injury, magnesium*

Coronary artery bypass grafting (CABG) is one of the effective methods to improve the life expectancy and quality of life of patients with ischemic heart disease. However, coronary revascularization during CABG can elicit a number of adverse reactions that may limit its beneficial effects [1]. The pathogenesis of reperfusion injury is probably multifactorial and includes intracellular calcium overload and generation of oxygen-derived free radicals. Elevated cytosolic-free calcium levels can activate proteases that compromise plasma-membrane integrity, allowing calcium overload and irreversible damage to mitochondria. Previous studies reported the impairments of membrane-bound ionic pumps during heart surgeries as a result of membrane damage and changes in myocardial enzyme activity [2]. ATPase enzymes in the membrane are responsible for ionic pump regulation. Erythrocyte ATPases like $Na^{+}K^{+}$-ATPase, Mg^{2+}-ATPase and Ca^{2+}-ATPase are critical for normal erythrocyte functions such as exchanges of intra- and extra-cellular electrolyte homeostasis and membrane integrity. It has been reported that anesthesia and surgical trauma can lead to a significant decrease in the activities of these ATPases [3].

Several investigations have shown that peri-operative serum magnesium levels may be reduced in 70 % of patients undergoing conventional CABG [4] which can lead to coronary artery spasm [5]. Magnesium is a principal cofactor in more than 300 intracellular enzymatic processes, many of them are integrally involved in mitochondrial function, energy production, and maintenance of trans-sarcolemmal ionic gradients, cell-volume control, and resting membrane potential. Magnesium (Mg^{2+}), given early in the reperfusion period, has shown promising results in both clinical [6] and experimental studies [7]. There is, however, no consensus regarding the beneficial effects of Mg^{2+} on infarct size and mortality or how such actions should be explained. Two factors assumed to be of importance in ischemia-reperfusion-induced cardiomyocyte damage and death are cellular calcium (Ca^{2+}) overload and oxidative stress, both can alter the activities of erythrocyte ATPase. Both factors may be possible targets for the protective effect of Mg^{2+}.

The aim of the present study was to investigate the effect of extra-cellular Mg^{2+} on erythrocyte ATPase activity which may be altered under the pathological condition mediated by ROS and Ca^{2+} accumulation during CABG procedure. For this purpose, we chose South Indian patients assigned to undergo CABG procedure. They were randomly administered Mg^{2+} during revascularization.

Patients and Methods

Population

We studied patients undergoing CABG in which full revascularization was expected. Ethical approval was provided by the ethical committee of the Institute of Cardiovascular Diseases, Madras Medical Mission. Written consent was obtained from each patient.

92 south Indian patients (72 male, 20 female; mean age: 62.6 ± 11.2 yrs) as a total were included in this study. Patients were randomly assigned to magnesium-treated and magnesium-untreated groups. 52 patients (42 male, 10 female) re-

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From the [1]MMM-Academy of Medical Sciences, Institute of Cardiovascular Diseases, Madras Medical Mission, Jayalalithanagar, Mogappair, Chennai-37, India, and the [2]Department of Biotechnology, Amala Cancer Research Center, Amalanagar, Trichur, Kerala, India.
Correspondence to: Gino A. Kurian, Senior Lecturer Medical Biochemistry, School of Chemical and Biotechnology, SASTRA Deemed University, Thirumalaisamudram, Thanjavur, Tamil Nadu, India; e-mail: ginokurian@hotmail.com

ceived magnesium, 40 patients (30 male, 10 female) did not. Patients who used antioxidants such as captropil and allopurinol were excluded from the study. Patients who received blood transfusion or blood products during operation were also excluded since the antioxidant properties of such products are not as yet established. None of the patients were taking vitamins or dietary supplements with established antioxidant properties before the study. None of the controls had a history of cerebrovascular disease.

Anesthesia Technique

On the day of surgery, patients were pre-medicated with morphine (0.2 mg/kg) and promethazine (0.5 mg/kg) i. m. about 30–45 minutes prior to induction of anesthesia. Anesthesia was induced with thiopentone (5 mg/kg), and vecuronium was used to accomplish endotracheal intubation with an appropriately sized tube (generally 9.0 mm for males; 7.5 mm for females). Anesthesia was maintained with 50 % nitrous oxide (N_2O) along with halothane 0.5–1 %, morphine (0.05 mg/kg) was given before incision and 0.15 mg/kg was added to the pump prime.

Surgical Technique

A standard cardiopulmonary bypass technique with normothermia (> 32 °C) was used throughout the study. The extracorporeal circuit was primed with Ringer's lactate solution 1.5 l and mannitol 100 ml. In 52 patients, myocyte preservation was effected with magnesium (2 g/kg) administration just before release of the aortic cross clamp. Perfusion pressure was maintained between 50 mmHg and 70 mmHg during bypass. Cardiopulmonary bypass operation was instituted using ascending aortic cannulation and two-stage venous cannulation in the right atrium. The extracorporeal circuit consisted of a membrane oxygenator and a roller pump primed with crystalloid solution. Cardioplegia was given retrogradely except for the first two-thirds of crystalloid cold cardioplegia, which was given anterogradely. All distal and proximal anastomoses were completed before the aortic crossclamp was removed. At the end of CABG, heparin was neutralized by protamine chloride until the activated clotting time was less than 180 s. In the CABG group, hematocrit was kept more than 20 % during CPB.

Sampling and Analysis

Paired coronary sinus and arterial blood samples were taken according to the following time scheme: just before the induction of anesthesia – group 1; 10 minutes after aortic cross clamp on – group 2; 30 minutes after aortic cross clamp on – group 3; 10 minutes after aortic cross clamp off – group 4; during re-warming – group 5. Groups 2 and 3 referred to the ischemic state of the heart while group 4 referred to the ischemic reperfused (revascularization) state.

Erythrocyte Membrane Preparation

Erythrocyte membranes or ghosts were prepared as described [8]. Briefly, the washed erythrocytes were lysed with 15 vol of 5 mmol/l of phosphate buffer (pH 8.0) and subsequently washed five additional times with the same lysing buffer. Membranes were collected after each wash by centrifugation at 4 °C for 10 min. at 10,000 g. This procedure yielded approximately 1 mg of protein/ml blood, as measured by the Lowry method [9], using bovine serum albumin as standard.

Biochemical Parameter

Na^+K^+-ATPase activity was estimated by the method of Nakao [10] in which 0.2 ml of the erythrocyte membrane or enzyme preparation was incubated with 1.0 ml of the reaction mixture containing NaCl: 140 mM, KCl: 14 mM, $MgCl_2$: 3 mM, Tris-HCl buffer (pH 7.4) Na_2-ATP: 30 mM (Sigma). Two controls were kept of which one was a blank specimen which contained the heat-inactivated enzyme/membrane preparation (boiled for 30 min. in a water bath). The second had the inhibitor of Na^+K^+-ATPase, ouabain, at a concentration of 10 μM/l. The samples were incubated for 2 hrs in a 37 °C water bath and reaction was terminated by adding 2 ml of 10 % (w/v) trichloroacetic acid. Inorganic phosphate content was measured by the method of Fiske and Subba Row [11] and total protein by the method of Lowry [9].

Ca^{2+}-ATPase activity in red cells was determined by the method of Shami and Radde [12]. The following procedure was that the enzyme preparation was incubated in 1.0 ml of reaction mix containing $CaCl_2$: 5 mM, Tris-HCl: 20 mM (pH 8.2), NaCl: l0 mM and Na_2 ATP: 5 mM. Ethacrynic acid, the inhibitor of Ca^{2+}-ATPase, was added at a concentration of 5 mM (Sigma). Inorganic phosphate content was measured by the method of Fiske and Subba Row [11] and total protein by the method of Lowry [9].

Mg^{2+}-ATPase was determined by Ohnishi's method [13] where the reaction mixture consisted of 0.1 ml each Tris-Hcl buffer (375 mM; pH 7.6), $MgCl_2$ (25 mM), ATP (10 mM) and the membrane suspension obtained. The content was thoroughly mixed and incubated at room temperature for 15 minutes. The reaction was arrested by adding 1 ml of 10 % TCA. The liberated inorganic phosphate is measured by the Fiske/Subba Row method [11].

Thiobarbituric acid reactive substances (TBARS) released from endogenous lipoperoxides, reflecting the lipid peroxidation process, were assayed in serum as described by [14] and in erythrocytes as described by [15]. The 5'-nucleotidase [16] activity was measured in serum. Serum concentration of troponin I was measured by commercially available enzyme immunoassays developed by Larue et al. (ERIA Diagnostics Pasteur). Lactate dehydrogenase activity and CPK MB mass concentration were analyzed using Sigma diagnostic kits.

Statistics

Data are presented as mean ± standard deviation. Data analyses were performed using SPSS software version 12.0. Comparisons within groups were made using repeated measures using one-way ANOVA. Comparisons between groups (pre-operative and surgical data) were carried out using chi-square-test. Continuous, normally distributed data was analyzed by t-test (single comparisons). Continuous non-normal data was analyzed with the Mann-Whitney-U-test.

Results

There were no hospital mortalities or peri-operative myocardial infarctions in either group. The initial post-operative serum magnesium level was higher in patients receiving magnesium (Tab. 1). In patients who did not receive intra-operative magnesium, the incidence of post-operative hypomagnesemia (< 1.8 mEq/l) was 35 %, compared with 9 % in patients who received intra-operative magnesium.

Table 2 shows the activities of Na^+K^+-ATPase, Ca^{2+}-ATPase, Mg^{2+}-ATPase, and 5'-nucleotidase in the erythrocyte membranes of patients who received and did not receive intra-operative magnesium during CABG. Comparing the values of the above enzymes in patient samples who did not receive Mg^{2+}, a significant improvement was observed in the erythrocyte ATPase activity during revascularization in Mg^{2+}-supplemented patents. However, there was no great difference in the activity of 5'-nucleotidase in both groups during ischemic reperfusion.

Table 1. Pre-operative clinical data

Variables	Magnesium	No magnesium
N	52	40
Sex		
• Male	42	30
• Female	10	10
Mean age ± SD (yrs)	63.7 ± 12.7	62.1 ± 11.4
Hypertension	25	18
Diabetes	20	12
Angina class		
• I	12	10
• II	33	30
• III–IV	07	10
Coronary lesions (stenosis ≥ 70)		
• Left anterior descending artery	49	38
• Left circumflex artery	27	23
• Right coronary artery	30	13
Posterior descending artery	20	15
Pre-operative medicines		
• Beta blockers	36	24
• Calcium channel blocker	10	9
• Diuretics	2	2
• ACE inhibitors	4	5
Post-operative magnesium level		
• Initial magnesium	2.37 ± 0.54	1.86 ± 0.40
• Initial potassium	4.17 ± 0.50	4.22 ± 0.40
CPB time	84.4 ± 25.5	83.8 ± 24.7
Aortic cross clamp time	58.8 ± 18.9	57.2 ± 19.5

Table 2. The activity of erythrocyte membrane ATPase in CABG patients who received magnesium and who did not

Antioxidant enzymes	Magnesium	No magnesium
Na^+K^+ ATPase (nM Pi/mg protein/min.)		
• During CPB	[a]3.745 ± 0.76	[a]3.645 ± 0.63
• 10 min. after aortic cross clamp on	[b]2.557 ± 0.86	[b]2.507 ± 0.65
• 30 min. after aortic cross clamp on	[b]2.657 ± 0.90	[b]2.617 ± 0.76
• 10 min. after aortic cross clamp off	[b]2.931 ± 0.92	[b]2.961 ± 0.71
• 30 min. after aortic cross clamp off	[b]3.052 ± 0.91	[a]3.152 ± 0.84
• During re-warming	[c]4.55 ± 0.80	[c]4.75 ± 0.96
Ca^{2+}-ATPase (nM Pi/mg protein/min.)		
• During CPB	[a]32.71 ± 3.17	[a]33.71 ± 3.27
• 10 min. after aortic cross clamp on	[b]21.62 ± 3.06	[b]22.62 ± 3.46
• 30 min. after aortic cross clamp on	[c]29.25 ± 3.98	[c]29.55 ± 3.78
• 10 min. after aortic cross clamp off	[b]21.82 ± 3.34	[d]17.72 ± 2.64
• 30 min. after aortic cross clamp off	[b]22.18 ± 3.92	[b]20.85 ± 2.32
• During re-warming	[c]28.64 ± 3.18	[e]25.64 ± 3.91
Mg^{2+}-ATPase (nM Pi/mg protein/min.)		
• During CPB	[a]5.05 ± 1.76	[a]5.15 ± 1.36
• 10 min. after aortic cross clamp on	[b]3.40 ± 1.77	[b]3.52 ± 1.47
• 30 min. after aortic cross clamp on	[a]5.06 ± 1.61	[a]5.09 ± 1.32
• 10 min. after aortic cross clamp off	[b]3.51 ± 1.22	[b]3.21 ± 1.22
• 30 min. after aortic cross clamp off	[c]4.52 ± 1.21	[b]3.82 ± 1.43
• During re-warming	[d]6.24 ± 1.17	[a]5.64 ± 1.57
5'-nucleotidase (nM Pi/mg protein/min.)		
• During CPB	[a]0.8974 ± 0.07	[a]0.8951 ± 0.06
• 10 min. after aortic cross clamp on	[b]0.8138 ± 0.05	[b]0.8115 ± 0.05
• 30 min. after aortic cross clamp on	[a]0.9013 ± 0.06	[a]0.8901 ± 0.06
• 10 min. after aortic cross clamp off	[b]0.8234 ± 0.06	[b]0.8222 ± 0.05
• 30 min. after aortic cross clamp off	[b]0.8142 ± 0.05	[b]0.8129 ± 0.05
• During re-warming	[a]0.8919 ± 0.07	[a]0.8709 ± 0.06

Pi = inorganic phosphate; all values differ significantly from group 1 ($p < 0.05$); values not sharing a common superscript (a–e) differ significantly at $p < 0.05$ when compared between the groups

Table 3. Comparison of cardiac enzyme activity in total bypass patients who received magnesium and who did not

Cardiac enzymes	Magnesium	No magnesium
CPK MB (ng/ml)		
• Just before induction of anesthesia	[a]0.90 ± 0.10	[a]1.60 ± 0.50
• 10 min. after aortic cross clamp on	[a]0.70 ± 0.10	[a]1.40 ± 0.30
• 30 min. after aortic cross clamp on	[b]4.07 ± 1.40	[b]9.94 ± 2.10
• 10 min. after aortic cross clamp off	[c]17.28 ± 2.10	[c]15.43 ± 3.14
• 30 min. after aortic cross clamp off	[d]21.10 ± 3.60	[d]25.66 ± 4.50
• During re-warming	[d]20.60 ± 3.13	[d]24.80 ± 5.13
• 24 hr. post-operative	[e]11.80 ± 2.17	[d]21.27 ± 5.11
• 48 hr. post-operative	[b]2.90 ± 0.80	[e]4.32 ± 1.12
Troponin I (ng/ml)		
• Just before induction of anesthesia	[a]0.09 ± 0.10	[a]0.02 ± 0.10
• 10 min. after aortic cross clamp on	[a]0.07 ± 0.20	[a]0.04 ± 0.10
• 30 min. after aortic cross clamp on	[b]0.85 ± 0.20	[b]0.92 ± 1.20
• 10 min. after aortic cross clamp off	[c]3.21 ± 1.20	[c]7.5 ± 2.20
• 30 min. after aortic cross clamp off	[c]3.22 ± 1.10	[c]7.8 ± 2.10
• During re-warming	[c]2.81 ± 0.90	[c]7.4 ± 2.50
• 24 hr. post-operative	[d]1.9 ± 0.70	[d]6.5 ± 2.20
• 48 hr. post-operative	[b]1.1 ± 0.60	[e]5.6 ± 2.10
LDH (U/L)		
• Just before induction of anesthesia	[a]595 ± 132	[a]613 ± 213
• 10 min. after aortic cross clamp on	[a]485 ± 130	[a]598 ± 256
• 30 min. after aortic cross clamp on	[b]716 ± 231	[b]734 ± 287
• 10 min. after aortic cross clamp off	[b]786 ± 243	[c]833 ± 312
• 30 min. after aortic cross clamp off	[c]931 ± 345	[d]996 ± 333
• During re-warming	[c]1080 ± 376	[d]1166 ± 398
• 24 hr. post-operative	[d]1741 ± 413	[e]1954 ± 399
• 48 hr. post-operative	[d]2314 ± 412	[f]2561 ± 411

All values differ significantly from group 1 ($p < 0.05$); values not sharing a common superscript (a–f) differ significantly at $p < 0.05$ when compared between the groups

The changes in the activities of cardiac enzymes are shown in Table 3. The enzymes showed a similar pattern of changes in patients who received and did not receive magnesium. Even though cardiac enzyme activities were elevated in Mg^{2+}-treated patients, their increase was not as prominent as in the samples from Mg^{2+}-non-treated patients.

Unlike erythrocyte ATPase activity, TBARS activity in serum did not vary significantly in its concentration in different time intervalls of the surgery procedure. However, there was a significant change between the groups as the TBARS level in serum was more elevated in Mg^{2+}-non-treated CABG patients. On the contrary the TBARS level in erythrocyte membrane significantly changed between the groups and within the group.

Cardiac risk factors like hypertension and diabetes mellitus do have influence on the impact of injury due to reperfusion (Figs. 1, 2). When compared to diabetic patients, hypertensive patients who had undergone CABG showed increased activities of Mg^{2+}-ATPase during early and later phases of reperfusion. However, in diabetic patients, 5'-nucleotidase activity was observed to be higher as compared to hypertensive patients. The activities of blood ATPase are shown in Figures 3–5. Significant ($p < 0.05$) changes in the activities of Ca^{2+}- and Mg^{2+}-ATPase in the blood samples were observed during revascularization.

Discussion

Generally, on-pump cardiac surgery is a frequently used method for coronary artery bypass grafting. Evidence suggests that during cardiopulmonary bypass calcium overload and subsequent reactive oxygen species production by activated neutrophils or by tissue reperfusion injury may be in-

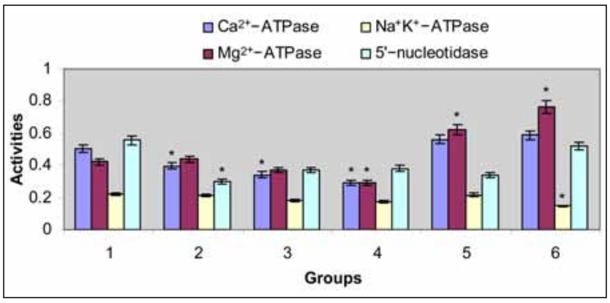

Figure 1. Influence of hypertension in the activities of blood ATPase during revascularization in CABG procedure[1]

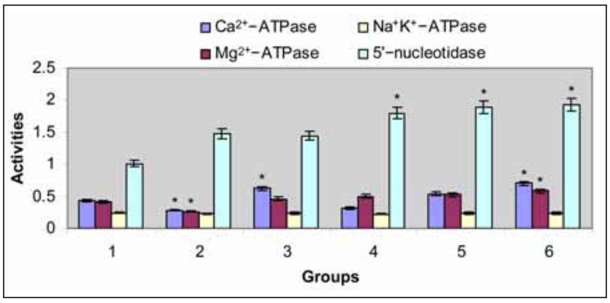

Figure 2. Influence of diabetes mellitus in the activities of blood ATPase during revascularization in CABG procedure[1]

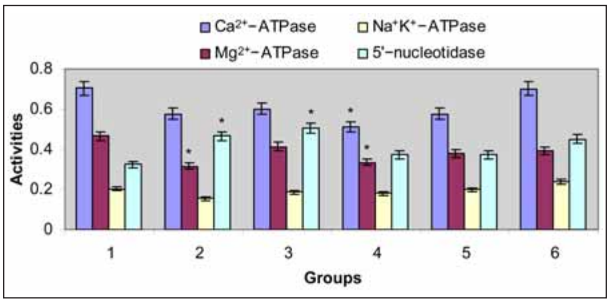

Figure 3. Activities of blood ATPase of patients who had less than 40 minutes aortic cross-clamp time[1]

volved in the pathogenesis of diffuse damage in patients undergoing cardiac surgery [17].

The experiments reported in the present study were designed to determine the effect of magnesium administration on the plasma lipid peroxidation and erythrocyte membrane ATPase changes during the revascularisation procedure in CABG patients. Our results indicate that the activity of erythrocyte membrane Na^+K^+-ATPase decreased significantly during revascularization procedure and recovered in the late phase. A similar pattern of changes was shown by other ATPase in the erythrocyte membrane like Ca^{2+}-ATPase and Mg^{2+}-ATPase. An increased concentration of TBARS in the plasma was observed during revascularization procedure, although it was not statistically significant when compared to group 1. However, patients pre-treated with magnesium showed improved activities of both enzymes during revascularization. The inhibition of red-cell membrane ATPase activity in the revascularization procedure reflects the probability of noxious factors released into the blood that

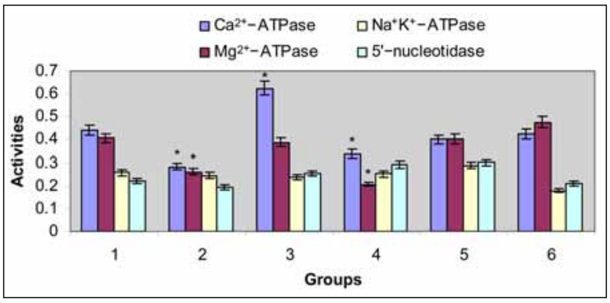

Figure 4. Activities of blood ATPase in patients who had 40–60 minutes aortic cross-clamp time[1]

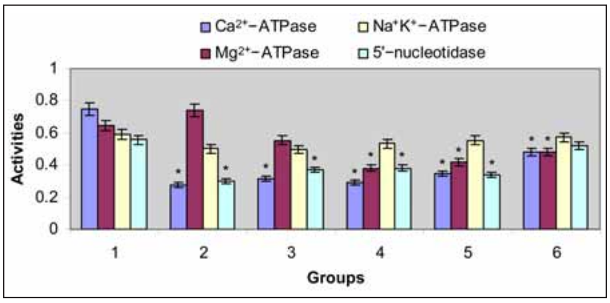

Figure 5. Activities of blood ATPase in patients who had more than 60 minutes aortic cross-clamp time[1]

may directly or indirectly destroy or inhibit cell-membrane function or ATPase activity.

Since Ca^{2+}-ATPase is an extrusion pump, diminished activity would lead to an intracellular calcium accumulation in vascular smooth muscle cells [18] and this may be of primary importance in the origin of increased peripheral vascular resistance, a characteristic feature of the ischemic reperfusion injury [19]. Magnesium inhibits calcium overload during initial phases of reperfusion through inhibition of calcium transport across most calcium channels [20]. Mg^{2+} increases calcium ATPase, which moves calcium back into the SR and into the extracellular space [21]. Numerous studies suggested that magnesium possesses class-IV (calcium channel-blocking activity) and class-I (sodium channel-blocking activity) antiarrhythmic properties, which result in an increase in conduction time through the atrioventricular node and accessory pathways as well as an increase in the refractoriness of the conducting system [22].

The decreased activity of Na^+K^+-ATPase might be correlated with direct destructive effects of some fluid factors such as complements, oxygen radicals and/or LPO on enzymatic molecular conformations and functions [23]. The suppressed activity of Na^+K^+-ATPase has also been reported by other workers in the early post-operative period [24]. Our data showed that decreased activities of Na^+K^+-, Mg^{2+}- and Ca^{2+}-ATPase were improved in the patient samples who received Mg^{2+} during revascularization procedure.

The improved 5'-nucleotidase activity in the erythrocyte membranes of Mg^{2+}-treated CABG patients (Tab. 2) indicates the possible early recovery of coronary blood flow that will increase the supply of oxygen to the myocardium [25]. This is made possible by the release of adenosine (the product of 5'-nucleotidase) that causes the arteriolar dilation. This increases coronary flow, which provides more substrate and oxygen and enables increased phosphorylation and replenishment of ATP.

Increased plasma concentration of TBARS (Tab. 4) during revascularization predicts the release of free radicals from the myocardium. This may oxidize the sulfhydryl group of

[1]Values shown are mean ± SD. *p < 0.05 compared with group 1. Group 1: before aortic cross clamp; group 2: 10 min. after aortic cross clamp on; group 3: 30 min. after aortic cross clamp on; group 4: 10 min. after aortic cross clamp off; group 5: 30 min. after aortic cross clamp off; group 6: during rewarming.

Table 4. TBARS levels in CABG patients who received magnesium and who did not

Anti oxidant enzymes	Magnesium	No magnesium
Erythrocyte membrane		
TBARS (n moles/mg protein)		
• During CPB	[a]0.38 ± 0.01	[a]0.41 ± 0.03
• 10 min. after aortic cross clamp on	[b] 0.56 ± 0.03	[b]0.53 ± 0.03
• 30 min. after aortic cross clamp on	[c]0.79 ± 0.07	[c]0.76 ± 0.08
• 10 min. after aortic cross clamp off	[d]1.09 ± 0.09*	[d]1.22 ± 0.09
• 30 min. after aortic cross clamp off	[b]0.89 ± 0.06*	[e]1.15 ± 0.07
• During re-warming	[c]0.61 ± 0.07	[c]0.86 ± 0.04
Serum		
TBARS (n moles/ml)		
• During CPB	[a]2.2123 ± 0.26	[a]2.1230 ± 0.24
• 10 min. after aortic cross clamp on	[b]2.5341 ± 0.27	[b]2.4352 ± 0.27
• 30 min. after aortic cross clamp on	[c]2.7385 ± 0.25	[c]3.0764 ± 0.31
• 10 min. after aortic cross clamp off	[d]3.0876 ± 0.30	[c]3.1791 ± 0.23
• 30 min. after aortic cross clamp off	[a]2.2727 ± 0.23	[c]3.1827 ± 0.29
• During re-warming	[a]2.1534 ± 0.21	[c]3.1234 ± 0.20

All values differ significantly from group 1 ($p < 0.05$); *differ significantly from control group (those who did not receive magnesium) ($p < 0.05$); values not sharing a common superscript (a–e) differ significantly at $p < 0.05$ when compared between the groups

proteins and result in disturbances of the ion permeation or gating of ionic channels, decrease of transport capacity of carrier molecules and activation of enzymes [26]. The increased free radicals released may further lead to decreased activities in red blood cell membrane ATPases and other enzymes. This may result in disturbances in intra- and extra-cellular electrolyte homeostasis and in increased permeability of red blood cell membranes by various ions and small molecular substances, and then induce massive potassium efflux and sodium influx, thereby leading to a reduction of red-cell membrane fluidity and deformability and an increase in membrane fragility. Such alterations may directly affect gas exchanges between blood and tissues, and microcirculatory functions, resulting in failures in microcirculation and in multiple organ system.

Over the past several decades, serum levels of cardiac enzymes and isoenzymes have become the final arbiters by which myocardial damage is diagnosed or excluded. TnI measurements are highly sensitive in the diagnosis of myocardial injury and accurately detect even small amounts of myocardial necrosis [27]. For this reason, in our study, we measured their serum levels to detect the cytoprotective effect of magnesium during ischemic reperfusion. The significantly lower troponin I level along with other cardiac marker enzymes suggested the cardio-protective nature of Mg^{2+} during ischemic reperfusion.

The above results suggest that the administration of magnesium preserves the erythrocyte ATPase and thereby stabilizes the erythrocyte membrane from the oxidative stress due to ischemic reperfusion.

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