Effect of Magnesium on Modulating the Activity of Na⁺K⁺-ATPase, Ca²⁺-ATPase Mg²⁺-ATPase and 5´-Nucleotidase in South Indian Patients Undergoing Coronary Artery Bypass Graft Surgery

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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. 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**

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 assigned to magnesium-treated and magnesium-untreated groups. 52 patients (42 male, 10 female) re...
On the day of surgery, patients were pre-medicated with mor-
phine (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 appro-
priately sized tube (generally 9.0 mm for males; 7.5 mm for
females). Anesthesia was maintained with 50 % nitrous oxide
(N₂O) along with halothane 0.5–1 %, morphine (0.05 mg/kg)
given before incision and 0.15 mg/kg was added to the
circuit.

Anesthesia Technique
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(N₂O) along with halothane 0.5–1 %, morphine (0.05 mg/kg)
given before incision and 0.15 mg/kg was added to the
pump prime.

Surgical Technique
A standard cardiopulmonary bypass technique with normo-
thermia (> 32 °C) was used throughout the study. The extra-
corporeal circuit was primed with Ringer’s lactate solution
1.5 l and mannitol 100 ml. In 52 patients, myocyte preserva-
tion was effected with magnesium (2 g/kg) administration
just before release of the aortic cross clamp. Perfusion pres-
sure was maintained between 50 mmHg and 70 mmHg dur-
ing 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 antegrade. 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
maintained 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 in-
duction 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 reperfusion (revascularization) state.

Erythrocyte Membrane Preparation
Erythrocyte membranes or ghosts were prepared as de-
scribed [8]. Briefly, the washed erythrocytes were lysed with
15 vol of 5 mmol/l of phosphate buffer (pH 8.0) and subse-
quently washed five additional times with the same lysing
buffer. Membranes were collected after each wash by cen-
trifugation at 4 °C for 10 min. at 10,000 g. This procedure
yielded approximately 1 mg of protein/ml blood, as meas-
ured 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 reac-
tion mixture containing NaCl: 140 mM, KCl: 14 mM,
MgCl₂: 3 mM, Tris-HCl buffer (pH 7.4) Na₂-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. 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].
Ca²⁺-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 CaCl₂: 5 mM, Tris-HCl: 20 mM
(pH 8.2), NaCl: 10 mM and Na₂-ATP: 5 mM. Ethacrynic
acid, the inhibitor of Ca²⁺-ATPase, was added at a concentra-
tion 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].

Mg²⁺-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), MgCl₂ (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

Thiobarbituric acid reactive substances (TBARS) released
from endogenous lipoperoxides, reflecting the lipid per-
oxidation process, were assayed in serum as described by [14]
and in erythrocytes as described by [15]. The 5'-nucleotidase
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 mea-
ures 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 myocar-
dial infarctions in either group. The initial post-operative
serum magnesium level was higher in patients receiving mag-
nesium (Tab. 1). In patients who did not receive intra-oper-
avative magnesium, the incidence of post-operative hypomag-
nesia (< 1.8 mEq/l) was 35 %, compared with 9 % in pa-
tients who received intra-operative magnesium.

Table 2 shows the activities of Na⁺K⁺-ATPase, Ca²⁺-
ATPase, Mg²⁺-ATPase, and 5'-nucleotidase in the erythro-
cyte membranes of patients who received and did not receive
intra-operative magnesium during CABG. Comparing the
values of the above enzymes in patients who did not receive
Mg²⁺, a significant improvement was observed in the
erythrocyte ATPase activity during revascularization in
Mg²⁺-supplemented patients. However, there was no great
difference in the activity of 5'-nucleotidase in both groups
during ischemic reperfusion.
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 Mg²⁺-treated patients, their increase was not as prominent as in the samples from Mg²⁺-non-treated patients.

Unlike erythrocyte ATPase activity, TBARS activity in serum did not vary significantly in its concentration in different time intervals of the surgery procedure. However, there was a significant change between the groups as the TBARS level in serum was more elevated in Mg²⁺-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 CABC showed increased activities of Mg²⁺-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 Ca²⁺- and Mg²⁺-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-

Table 1. Pre-operative clinical data

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

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

<table>
<thead>
<tr>
<th>Antioxidant enzymes</th>
<th>Magnesium</th>
<th>No magnesium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺-K⁺-ATPase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(nM Pi/mg protein/min.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>During CPB</td>
<td>2.37 ± 0.54</td>
<td>1.86 ± 0.40</td>
</tr>
<tr>
<td>Initial magnesium</td>
<td>4.17 ± 0.50</td>
<td>4.22 ± 0.49</td>
</tr>
<tr>
<td>CPB time</td>
<td>84.4 ± 25.5</td>
<td>83.8 ± 24.7</td>
</tr>
<tr>
<td>Aortic cross clamp time</td>
<td>58.8 ± 18.9</td>
<td>57.2 ± 19.5</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Cardiac enzymes</th>
<th>Magnesium (ng/ml)</th>
<th>No magnesium (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPK MB</td>
<td>485 ± 130</td>
<td>598 ± 256</td>
</tr>
<tr>
<td>10 min. after aortic cross clamp</td>
<td>716 ± 231</td>
<td>734 ± 287</td>
</tr>
<tr>
<td>30 min. after aortic cross clamp</td>
<td>768 ± 243</td>
<td>833 ± 312</td>
</tr>
<tr>
<td>10 min. after aortic cross clamp</td>
<td>931 ± 345</td>
<td>998 ± 333</td>
</tr>
<tr>
<td>During re-warming</td>
<td>1080 ± 376</td>
<td>1166 ± 398</td>
</tr>
<tr>
<td>48 hr. post-operative</td>
<td>1741 ± 413</td>
<td>1954 ± 399</td>
</tr>
<tr>
<td>48 hr. post-operative</td>
<td>2314 ± 412</td>
<td>2561 ± 411</td>
</tr>
</tbody>
</table>

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 Mg²⁺-treated patients, their increase was not as prominent as in the samples from Mg²⁺-non-treated patients.

Unlike erythrocyte ATPase activity, TBARS activity in serum did not vary significantly in its concentration in different time intervals of the surgery procedure. However, there was a significant change between the groups as the TBARS level in serum was more elevated in Mg²⁺-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 CABC showed increased activities of Mg²⁺-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 Ca²⁺- and Mg²⁺-ATPase in the blood samples were observed during revascularization.
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 revascularization 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 ATPases in the erythrocyte membrane like Ca²⁺-ATPase and Mg²⁺-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 may directly or indirectly destroy or inhibit cell-membrane function or ATPase activity.

Since Ca²⁺-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]. Mg²⁺ 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⁺-ATPase and Ca²⁺-ATPase were improved in the patient samples who received Mg²⁺ during revascularization procedure.

The improved 5'-nucleotidase activity in the erythrocyte membranes of Mg²⁺-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...
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.

References:

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

<table>
<thead>
<tr>
<th>Anti oxidant enzymes</th>
<th>Magnesium (µ moles/ml)</th>
<th>No magnesium (µ moles/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocyte membrane</td>
<td></td>
<td></td>
</tr>
<tr>
<td>During CPB</td>
<td>2.1213 ± 0.26</td>
<td>2.1230 ± 0.24</td>
</tr>
<tr>
<td>10 min. after aortic cross clamp on</td>
<td>2.5341 ± 0.27</td>
<td>2.4352 ± 0.27</td>
</tr>
<tr>
<td>30 min. after aortic cross clamp on</td>
<td>3.0764 ± 0.30</td>
<td>3.1791 ± 0.02</td>
</tr>
<tr>
<td>30 min. after aortic cross clamp off</td>
<td>3.0676 ± 0.30</td>
<td>3.1827 ± 0.29</td>
</tr>
<tr>
<td>During re-warming</td>
<td>2.1534 ± 0.21</td>
<td>2.1234 ± 0.20</td>
</tr>
<tr>
<td>Serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>During CPB</td>
<td>0.38 ± 0.01</td>
<td>0.41 ± 0.03</td>
</tr>
<tr>
<td>10 min. after aortic cross clamp on</td>
<td>0.56 ± 0.03</td>
<td>0.53 ± 0.03</td>
</tr>
<tr>
<td>30 min. after aortic cross clamp on</td>
<td>0.79 ± 0.07</td>
<td>0.76 ± 0.08</td>
</tr>
<tr>
<td>30 min. after aortic cross clamp off</td>
<td>0.19 ± 0.09</td>
<td>0.22 ± 0.09</td>
</tr>
<tr>
<td>During re-warming</td>
<td>0.89 ± 0.06</td>
<td>1.15 ± 0.07</td>
</tr>
</tbody>
</table>

All values differ significantly from control group (those who did not receive magnesium) (p < 0.05); *differ significantly from group 1 (p < 0.05); **differ significantly at p < 0.05 when compared between the groups.
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