Oral Magnesium Supplementation Inhibits Platelet-Dependent Thrombosis in Patients With Coronary Artery Disease

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The use of magnesium in the treatment of acute myocardial infarction remains controversial despite preliminary experimental evidence that magnesium plays a beneficial role as a regulator of thrombosis. This study examines whether oral magnesium treatment inhibits platelet-dependent thrombosis (PDT) in patients with coronary artery disease (CAD). In a randomized prospective, double-blind, crossover, and placebo-controlled study, 42 patients with CAD (37 men, 5 women, mean age 68 ± 9 years) on aspirin received either magnesium oxide tablets (800 to 1,200 mg/day) or placebo for 3 months (phase 1) followed by a 4-week wash-out period, and the crossover treatment for 3 months (phase 2). PDT, platelet aggregation, platelet P-selectin flow cytometry, monocyte tissue factor procoagulant activity (TF-PCA), and adhesion molecule density were assessed before and after each phase. PDT was evaluated by an ex vivo perfusion model using the Badimon chamber. Median PDT was significantly reduced by 35% in patients who received magnesium versus placebo (Δ change from baseline −24 vs 26 mm²/mm; p = 0.02, respectively). There was no significant effect of magnesium treatment on platelet aggregation, P-selectin expression, monocyte TF-PCA, or adhesion molecules. Oral magnesium treatment inhibited PDT in patients with stable CAD. This effect appears to be independent of platelet aggregation or P-selectin expression, and is evident despite aspirin therapy. These findings suggest a potential mechanism whereby magnesium may beneficially alter outcomes in patients with CAD. ©1999 by Excerpta Medica, Inc. (Am J Cardiol 1999;84:152–156)

METHODS

Study design and population: The study was a randomized, prospective, double-blind, crossover, and placebo-controlled trial. Patients were recruited from a supervised cardiac exercise and rehabilitation program at Cedars-Sinai Medical Center. Inclusion criteria included men and women aged >20 years, with documented CAD. Exclusion criteria included unstable angina, congestive heart failure more than New York Heart Association class IV, chronic diarrhea, renal failure (serum creatinine >3 mg/dl), acute myocardial infarction within the preceding 3 months, hypertension, chronic liver disease, or refusal to sign the informed consent. The study was approved by the institutional review board, and all participants gave written informed consent.

Study protocol: Forty-two patients were randomized to receive either magnesium oxide tablets (800 to 1,200 mg/day) or placebo for 3 months (phase 1), followed by a 4-week wash-out period, and the alternative treatment for 3 months (phase 2). The patients were instructed to continue taking their other regular medications. Before and after each phase, the patients underwent a physical examination, blood tests for measurement of platelet-dependent thrombosis (PDT), platelet aggregation, flow cytometry, monocyte tissue factor procoagulant activity (TF-PCA), monocyte adhesion molecule density, lipids, blood cell count, electrolytes, and fibrinogen levels.
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labeled platelets deposited on the media and the mor-
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Thrombus size was measured as the average of 6
thermous in square micrometers and normalized to
analyzed sections per tissue, and expressed as the
Study medication: MAG-OX 400 (magnesium ox-
te 400 mg tablets [241.3 mg (19.86 mEq) elemental
Blaine Co., Inc., Kentucky) or placebo
used 2 to 3 times daily. Compliance was assessed by
pill count.
Ex vivo measurement of platelet-dependent throm-
bosis: After an overnight fast, a 19-gauge butterfly
catheter was inserted atraumatically without a tourni-
quet into an antecubital vein. Flowing unanticoagu-
ulated venous blood from the patient was drawn over a
segment of prepared porcine aortic media held in a
tubular superfusion flow chamber by a peristaltic
pump placed distal to the chamber. The chamber was
designed to mimic the cylindrical shape of blood
vessels and contained a window that permitted direct
exposure of the aortic media to the flowing venous
blood.10,11 A perfusion chamber with an internal di-
meter of 1.0 mm was selected to generate shear rates
of 800 s⁻¹, at a flow rate of 5 ml/min.
After perfusion, the aortic media strips were re-
moved from the chambers, fixed in 2% gluteraldehyde
in 2 M sodium cacodylate, and processed for morpho-
logic analysis. The stained (hematoxylin-phloxine-sa-
franin) histologic tissue was quantified by computer-
assisted morphometry for PDT under light micro-
copy, using an image analysis software (Bioscan,
Optimas, Washington). All measurements were made
in a blinded fashion by 1 of the authors (MDM), and
intra-assay observer variability was 5.5 ± 5.6%. Thrombus size was measured as
the average of 6 analyzed sections per tissue, and expressed as the
surface area in square micrometers and normalized to
the cross-sectional diameter of the exposed media (in
millimeters). This morphometric method has been
previously validated and shows a strong correlation
(r = 0.84, p = 0.0001) between the amount of indium-
labeled platelets deposited on the media and the mor-
phometric assessment of thrombus size.12
Platelet aggregation and P-selectin flow cytometry:
Citrated whole blood samples (4.5 ml) were taken
from the patients before starting the ex vivo thrombo-
sis experiment, and diluted with an equal volume of
isotonic saline. Collagen (2 µg/ml)
induced whole blood platelet aggreg-
ation was measured by impedance aggregometry.13 Platelet aggregation
was measured as the maximal change in impedance produced 6
minutes after the addition of colla-
gen, and expressed in ohms.
Platelet α-granule release, de-
tected by expression of P-selectin
(the CD62 antigen), was measured
by whole blood flow cytometry using the
method described by Janes et
al.14 P-selectin expression was mea-
sured in response to adenosine
diphosphate stimulation (5 µmol/L).
All samples were analyzed within 1
hour of collection, in a FACScan
flow cytometer (Becton Dickinson
Immunocytometry systems, San Jose, California). Five thousand platelets were analyzed and the values
were expressed as the percentage of cells positive for
fluorescent antibody binding.
Monocyte isolation and flow cytometry of monocyte
adhesion molecules: Mononuclear cells were isolated
from concentrated citrated human peripheral blood
over Ficoll-Paque (Pharmacia Biotech AB, Uppsala,
Sweden) by density gradient centrifugation.15 Cells
were washed 3 times and resuspended in RPMI-1640
(GIBCO BRL, Grand Island, New York). Cell viability
was assessed by trypan blue exclusion and mono-
cyte origin was determined by nonspecific esterase
stain and flow cytometric analysis for CD14-positive
cells. Suspension cell counts were obtained by Tech-
nicon H-1 or H-3 System (Miles, Tarrytown,
New York). Monocytes were evaluated for surface-mem-
brane expression of the adhesion molecules very late
after activation antigen-4, platelet endothelial cell
adhesion molecule-1, macrophage antigen-1 (CD11b),
and sialylated Lewis-X by direct staining with respec-
tive FITC-conjugated anti-human monoclonal anti-
bodies (Becton Dickinson).
Tissue factor procoagulant activity assay: TF-PCA
of cells was quantitated by a 1-stage recalcification
clotting time assay.16
Lipids and electrolyte: Fasting blood samples were
taken for total cholesterol, low-density lipoprotein
cholesterol, high-density lipoprotein cholesterol, very
low-density lipoprotein cholesterol, triglycerides, glu-
cose, apolipoprotein A-I and B, and fibrinogen levels,
using standardized autoanalyzer techniques. Serum
magnesium was measured by the atomic absorption
spectrophotometry method.
Statistical analysis: Group data are expressed as
mean ± SD. Comparison of treatment groups before
and after treatment as well as the Δ change in those
values after treatment were performed using both
paired and unpaired Student’s t test. Association be-
tween normally distributed variables were determined
determining using Pearson’s correlation coefficient analysis. The Wilcoxon signed-rank test and Spearman correlation

<table>
<thead>
<tr>
<th>TABLE I Baseline Characteristics of Study Population</th>
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<tbody>
<tr>
<td>Concomitant Medication</td>
</tr>
<tr>
<td>β-Blocking agents</td>
</tr>
<tr>
<td>Calcium antagonists</td>
</tr>
<tr>
<td>Hypoglycemic agents</td>
</tr>
<tr>
<td>Losix</td>
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<tr>
<td>Digoxin</td>
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<p>| CAD Documentation* |</p>
<table>
<thead>
<tr>
<th>CAD Risk Factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prior MI</td>
</tr>
<tr>
<td>Prior CABG</td>
</tr>
<tr>
<td>Prior PTCA</td>
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<tr>
<td>History of hypercholesterolemia</td>
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</tbody>
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*Twenty-five patients had multiple diagnoses.
ACE = angiotensin-converting enzyme; CABG = coronary artery bypass grafting; MI = myocardial infarction; PTCA = percutaneous transluminal coronary angioplasty.
coefficients were used to analyze data that were not normally distributed. Log transformations were used to normalize data for regression analysis. Predictors of PDT were determined using log linear regression and multiple stepwise regression analysis. A p value ≤0.05 was required to reject the null hypothesis.

RESULTS
Our study population included 42 coronary patients (37 men and 5 women), with a mean age of 68 ± 9 years (range 48 to 83) (Table I). Six patients were excluded from final analysis: 1 for noncompliance; 1 who did not return for follow-up; 3 who had coronary artery bypass surgery during the trial (2 on magnesium, 1 on placebo); and 1 patient who had a stroke while on placebo tablets; this left 36 subjects with complete data for analysis. Overall compliance with the study medication was 89%. There was no significant change in other medication use over the course of the study. There were no serious adverse effects.

Baseline values in the treatment groups before each treatment period were compared, and no significant differences were found. To assess the possibility of a carryover effect from the initial treatment phase and the next treatment phase, we compared the baseline values before the first treatment period with those before the second treatment period. No significant differences were found.

Platelet-dependent thrombosis: Baseline analysis before initiation of treatment revealed significant positive correlations between PDT and fasting blood sugar (r = 0.44), systolic blood pressure at rest (r = 0.37), apolipoprotein-B (r = 0.39), and total cholesterol level (r = 0.42) (all p <0.05). There was no significant correlation between PDT and TF-PCA, either at baseline or following magnesium treatment.

After 3 months of oral magnesium treatment, the median PDT decreased by 35% (p <0.05) (Table II). As depicted in Figure 1, 75% of the patients (27 of 36) demonstrated a decrease in PDT with magnesium treatment (p <0.05). There was no association between the presence of risk factors (hypertension, diabetes mellitus, smoking) and the magnitude of change in PDT in response to oral magnesium. A representative histologic section of PDT on magnesium and placebo is shown in Figure 2.

Other variables: There was no significant effect of magnesium treatment on platelet aggregation, P-selectin expression, monocyte-derived TF-PCA, serum lipids, fibrinogen, or apolipoprotein A-I and B (Table II). Among the monocyte adhesion molecules, MAC-1 was significantly reduced (p <0.03) during oral magnesium treatment (Table II).

Serum magnesium concentrations Only 1 patient had a subnormal serum magnesium level (1.6 mg/dl) at study entry. There were no significant differences in serum magnesium levels after 3 months of oral magnesium treatment (2.08 ± 0.16 vs 2.11 ± 0.14 mg/dl, p = 0.22), and no correlation between baseline serum magnesium levels and PDT (r = −0.24, p = 0.16), nor changes in serum magnesium levels and in PDT (r = −0.29, p = 0.09). Baseline serum magnesium levels correlated negatively with changes in serum magnesium during magnesium treatment (r = −0.53, p = 0.0008), such that the increase in magnesium level following treatment was greater when baseline levels were low.

DISCUSSION
Our study demonstrates for the first time that an ex vivo measure of acute PDT was significantly reduced...
in stable CAD patients treated with oral magnesium therapy. An antithrombotic effect of magnesium treatment was seen despite 100% utilization of aspirin therapy. Of note, magnesium supplementation did not inhibit either platelet aggregation or P-selectin expression (a measure of platelet α-granule release reaction).

Previous experimental work has shown magnesium levels to be inversely related to platelet aggregation and adenosine triphosphate release.\(^{17,18}\) In contrast, we did not observe any effect on collagen-induced platelet aggregation at an oral dose that caused significant inhibition of platelet adhesion and thrombus formation in the current study. Several factors may have contributed to this observed difference. First, all of the study patients were on aspirin therapy, which is known to suppress platelet aggregation but not platelet adhesion.\(^{19}\) Second, the route of magnesium supplementation (oral in the present study compared with intravenous in prior studies)\(^{5,6,17–21}\) might also have contributed to the disparate results. High magnesium concentration (as achieved via intravenous continuous infusion route or boluses) inhibits platelet aggregation only at high doses.\(^{21}\) Thus, the antiplatelet adhesion effects of oral magnesium may account for its significant effects on PDT formation in the absence of effects on in vitro platelet aggregation.

Serum magnesium, like serum potassium, is often normal despite depletion of total body magnesium.\(^{22}\) Intracellular levels of magnesium are more accurate measurements of total body status,\(^{23}\) and the intracellular mononuclear cell magnesium level is often used to reflect the true magnesium levels in the body.\(^{24}\) In our study, we did not observe significant changes in the serum magnesium levels after 3 months of oral magnesium treatment. We were also unable to demonstrate any relation between the change in serum magnesium levels and change in PDT. Compliance assessment during the study was 89% with the study medication, suggesting serum magnesium levels did not accurately reflect the study intervention.

**Study limitations:** We studied a relatively small number of patients with a low-risk of CAD who were on aspirin. It is possible that potential beneficial effects related to oral magnesium treatment were less likely to be evident in our study due to this low-risk population. Further studies with larger numbers of patients who are at higher risk and received larger doses of magnesium are indicated, given these results.

An additional study limitation is our choice of thrombus measurement tool. Although the clinical relevance of thrombus formation in this experimental model has not been defined, the reproducibility and simplicity of this ex vivo system makes it attractive to study the interaction of blood elements with thrombogenic surfaces and possibly make inferences about the efficacy of therapeutic interventions.

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