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

Michael Shechter, MD, MA, C. Noel Bairey Merz, MD, Maura Paul-Labrador, MPH, Simcha R. Meisel, MD, Robert K. Rude, MD, Mia D. Molloy, MA, James H. Dwyer, PhD, Prediman K. Shah, MD, and Sanjay Kaul, MD

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 mm^2/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.
(Am J Cardiol 1999;84:152–156)

Magnesium is an important intracellular cation and an obligatory co-factor in many enzymes in the human body.[1] Hypomagnesemia is common in hospitalized patients,[2] which is partially due to diuretic therapy.[3] We have previously demonstrated that intravenous magnesium therapy reduces mortality in thrombolysis-ineligible patients with acute myocardial infarction,[4–7] although the mechanisms of benefit remain unknown. Other studies have demonstrated that magnesium can suppress platelet activation.[8,9] We hypothesized that magnesium treatment plays a beneficial role as a regulator of thrombosis, and conducted a study to determine the effects of oral magnesium treatment on potential mediators of acute thrombus formation in patients with coronary artery disease (CAD).

From the Preventive and Rehabilitative Cardiac Center, and the Atherosclerosis Research Center, Cedars-Sinai Burns and Allen Research Institute, Division of Cardiology, Department of Medicine, Cedars-Sinai Medical Center and the UCLA School of Medicine; the Department of Endocrinology, USC Orthopaedic Hospital; and the Institute for Health Promotion and Disease Prevention Research, Department of Preventive Medicine, USC School of Medicine, Los Angeles, California. This study was supported by Blaine Company, Inc., Erlanger, Kentucky, and in part by Nutrition 21, San Diego, California, and the American Physicians Fellowship for Israel, New York, New York. Manuscript received November 25, 1998; revised manuscript received and accepted March 5, 1999.

Address for reprints: Michael Shechter, MD, MA, Preventive & Rehabilitative Cardiac Center, Cedars-Sinai Medical Center, 444 S. San Vicente Blvd., Suite # 901, Los Angeles, CA 90048. E-mail: shechtes@netvision.net.il.

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, hyper/hypothyroidism, type 1 (insulin-dependent) diabetes mellitus, peripheral vascular disease, history of drug or alcohol abuse, 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.

0002-9149/99/$–see front matter
PII S0002-9149(99)00225-8

TABLE I Baseline Characteristics of Study Population

Concomitant Medication			
β-Blocking agents	15 (42%)	Aspirin	36 (100%)
Calcium antagonists	14 (39%)	Long-acting nitrates	4 (11%)
Hypoglycemic agents	2 (6%)	ACE inhibitors	12 (33%)
Lasix	5 (14%)	Lipid-lowering agents	27 (75%)
Digoxin	4 (11%)		
CAD Documentation*		**CAD Risk Factors**	
Prior MI	23 (55%)	Diabetes mellitus	2 (6%)
Prior CABG	26 (62%)	Systemic hypertension	21 (58%)
Prior PTCA	23 (55%)	Current smokers	0
		History of hypercholesterolemia	27 (75%)

*Twenty-five patients had multiple diagnoses.

ACE = angiotensin-converting enzyme; CABG = coronary artery bypass grafting; MI = myocardial infarction; PTCA = percutaneous transluminal coronary angioplasty.

Study medication: MAG-OX 400 (magnesium oxide 400 mg tablets [241.3 mg (19.86 mEq) elemental magnesium], Blaine Co. Inc., Kentucky) or placebo was used 2 to 3 times daily. Compliance was assessed by pill count.

Ex vivo measurement of platelet-dependent thrombosis: After an overnight fast, a 19-gauge butterfly catheter was inserted atraumatically without a tourniquet into an antecubital vein. Flowing unanticoagulated 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 diameter 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 removed from the chambers, fixed in 2% gluteraldehyde in 2 M sodium cacodylate, and processed for morphologic analysis. The stained (hematoxylin-phloxine-safranin) histologic tissue was quantified by computer-assisted morphometry for PDT under light microscopy, 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 morphometric 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 thrombosis experiment, and diluted with an equal volume of isotonic saline. Collagen (2 μg/ml) induced whole blood platelet aggregation was measured by impedance aggregometry.[13] Platelet aggregation was measured as the maximal change in impedance produced 6 minutes after the addition of collagen, and expressed in ohms.

Platelet α-granule release, detected 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 measured 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 monocyte origin was determined by nonspecific esterase stain and flow cytometric analysis for CD14-positive cells. Suspension cell counts were obtained by Technicon H-1 or H-3 System (Miles, Tarrytown, New York). Monocytes were evaluated for surface-membrane 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 respective FITC-conjugated anti-human monoclonal antibodies (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, glucose, 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 between normally distributed variables were determined using Pearson's correlation coefficient analysis. The Wilcoxon signed-rank test and Spearman correlation

TABLE II Effects of Oral Magnesium on Platelet and Monocyte Function and Lipids

	Baseline	Magnesium	p Value
Platelet function			
PDT (μm^2/mm)	91	59	<0.05
PLT aggregation (max ohms)	9 ± 6	8 ± 4	0.40
P-selectin (% gated)*	33 ± 20	35 ± 23	0.69
Monocyte function			
MAC-1†	95 ± 44	76 ± 29	0.03
VLA-4†	55 ± 22	49 ± 17	0.27
PECAM-1†	198 ± 114	164 ± 68	0.14
Sialyl Le^{x}†	71 ± 46	55 ± 29	0.18
TF-PCA ($\mu g/10^6$ cells)	124 ± 127	239 ± 248	0.09
Lipids			
Total cholesterol (mg/dl)	173 ± 28	180 ± 12	0.10
Triglycerides (mg/dl)	148 ± 93	148 ± 100	0.99
HDL-C (mg/dl)	40 ± 10	41 ± 9	0.26
LDL-C (mg/dl)	104 ± 26	110 ± 27	0.22
Fibrinogen (mg/dl)	307 ± 66	309 ± 51	0.83

Values are expressed as mean ± SD except PDT which is median.
*P-selectin expression stimulated by adenosine diphosphate (5 μM).
†Intensity of adhesion molecules is expressed in mean channels.
HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; MAC-1 = macrophage antigen-1 (CD11b); PECAM-1 = platelet endothelial cell adhesion molecule-1; PLT = platelets; Sialyl Le^{x} = sialylated Lewis-X; VLA-4 = very late after activation antigen-4.

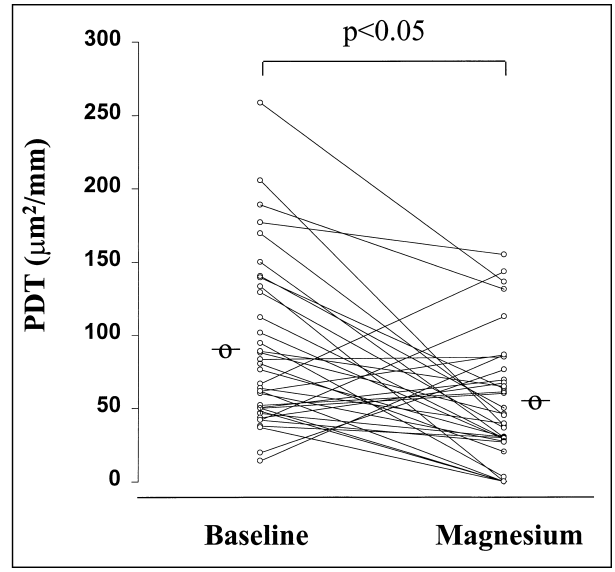

FIGURE 1. Changes in PDT with oral magnesium. *Open circles,* mean values; *0,* median values.

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 ($r = -24$, $p = 0.16$), 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

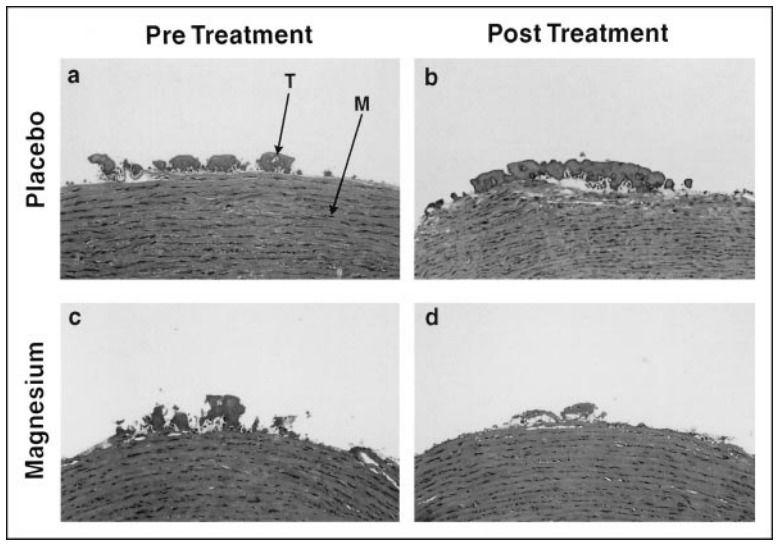

FIGURE 2. A representative histologic section (hematoxylin-phloxine-safranin stain) showing the PDT deposited (T) on porcine aortic media (M) taken from a patient before *(a)* and after *(b)* receiving placebo, and before *(c)* and after *(d)* receiving magnesium supplementation for 3 months.

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 triphospate 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.

Acknowledgment: The authors are indebted to Edwin R. Alexander, MD, Tony Stephen, Aalok Agarwala, and Care Felix for their technical assistance.

1. Wacker WEL. Magnesium metabolism. *N Engl J Med* 1968;278:712–717.
2. Chernow B, Bamberger S, Stocko M, Vadnais M, Mills S, Hoellerich V, Warshaw AL. Hypomagnesemia in patients in postoperative intensive care. *Chest* 1989;95:391–397.
3. Dyckner T, Wester PO. Potassium/magnesium depletion in patients with cardiovascular disease. *Am J Med* 1987;82 (suppl 3A):11–17.
4. Shechter M, Hod H, Marks N, Behar S, Kaplinsky E, Rabinowitz B. Beneficial effect of magnesium in acute myocardial infarction. *Am J Cardiol* 1990;66:271–274.
5. Shechter M, Hod H, Chouraqui P, Kaplinsky E, Rabonowitz B. Magnesium in acute myocardial infarction when patients are not candidates for thrombolytic therapy. *Am J Cardiol* 1995;75:321–323.
6. Shechter M, Hod H, Kaplinsky E, Rabinowitz B. The rationale of magnesium as alternative therapy for patients with acute myocardial infarction without thrombolytic therapy. *Am Heart J* 1996;132:483–486.
7. Shechter M, Kaplinsky E, Rabinowitz B. Review of clinical evidence—is there a role for supplemental magnesium in acute myocardial infarction in high-risk populations (patients ineligible for thrombolysis and the elderly)? *Coron Artery Dis* 1996;7:352–358.
8. Nadler JL, Goodson S, Rude RK. Evidence that prostacyclin mediates the vascular action of magnesium in humans. *Hypertension* 1987;9:379–383.
9. Frandsen NJ, Winther K, Pedersen F, Christiansen I, McNair P. Magnesium and platelet function: in vivo influence on aggregation and alpha-granule release in healthy volunteers. *Magnesium Bull* 1995;17:37–40.
10. Badimon L, Badimon JJ, Galvez A, Chesebro JH, Fuster V. Influence of arterial damage and wall shear rate on platelet formation: ex vivo study in a swine model. *Arteriosclerosis* 1986;6:312–320.
11. Lam JYT, Badimon JJ, Ellefson RD, Fuster V, Chesebro JH. Cod-liver oil alters platelet-arterial wall response to injury in pigs. *Circ Res* 1992;71:769–775.
12. Lacoste L, Lam JYT, Hung J, Waters D. Oral verapamil inhibits platelet thrombus formation in humans. *Circulation* 1994;89:630–634.
13. Cardinal DC, Flower RJ. The electronic aggregometer: a novel device for assessing platelet behavior in blood. *J Pharmacol Methods* 1980;3:135–158.
14. Janes SL, Wilson DJ, Chronos N, Goodall AH. Evaluation of whole blood flow cytometric detecting of platelet bound fibrinogen in normal subjects and patients with activated platelets. *Thromb Haemostasis* 1993;70:659–666.

15. Bøyum A. Isolation of mononuclear cells and granulocytes from human blood. *Scand J Clin Lab Invest* 1968;21(Suppl 97):77–89.
16. Morrissey JH, Fair DS, Edgington TS. Monoclonal antibody analysis of purified and cell-associated tissue factor. *Thromb Res* 1989;52:247–261.
17. Hwang DL, Yen CF, Nadler JL. Effect of extracellular magnesium on platelet activation and intracellular calcium mobilization. *Am J Hypertens* 1992;5:700–706.
18. Ravn HB, Vissinger H, Kristensen SD, Husted SE. Magnesium inhibits platelet activity—an in vitro study. *Thromb Haemost* 1996;76:88–93.
19. Chronos NAF, Wilson DJ, Janes SL, Hutton RA, Buller NP, Goodall AH. Aspirin does not affect the flow cytometric detection of fibrinogen binding to, or release of a-granules or lysosomes from, human platelets. *Clin Science* 1994;87: 575–580.
20. Gawaz M, Ott I, Mehringer S, Neumann FJ. Effects of magnesium on platelet aggregation and adhesion. Magnesium modulates surface expression of glycoproteins on platelets in vitro and ex vivo. *Thromb Haemost* 1994;72:912–18.
21. Ravn HB, Kristensen SD, Hjortdal VE, Thygesen K, Husted SE. Early administration of intravenous magnesium inhibits arterial thrombus formation. *Arterioscler Thromb Vasc Biol* 1997;17:3620–3625.
22. Ryzen E, Elkayam U, Rude RK. Low blood mononuclear cell magnesium in intensive cardiac care unit patients. *Am Heart J* 1986;111:475–480.
23. Reinhart RA. Magnesium metabolism. *Arch Intern Med* 1988;148:2415–2420.
24. Elin RJ. Status of the determination of magnesium in mononuclear blood cells in humans. *Magnesium* 1988;7:300–305.