Intravenous Magnesium in Experimental Stent Thrombosis in Swine

Vladimir Rukshin, Babak Azarbal, Prediman K. Shah, Vivian T. Tsang, Michael Shechter, Ariel Finkelstein, Bojan Cercek, Sanjay Kaul

Abstract—We investigated the effects of magnesium on acute platelet-dependent stent thrombosis in an ex vivo porcine arteriovenous shunt model of high-shear blood flow. Control nitinol stents were expanded to 2 mm in diameter in a tubular perfusion chamber interposed in the shunt and exposed to flowing arterial blood at a shear rate of 2100 s⁻¹ for 20 minutes (n=156 perfusion runs in 10 swine). Animals were treated with intravenous heparin or MgSO₄ alone (2 g bolus over 20 minutes, followed by 2 g/h infusion) and combined heparin plus MgSO₄ in random fashion. Effects on thrombus weight (TW), platelet aggregation, bleeding time, activated clotting time, mean arterial blood pressure, and heart rate were quantified. Data points in the magnesium-treated animals were examined within 20 minutes after bolus (Mg-early) and >40 minutes after bolus (Mg-late). Stent TW (20±3 mg, pretreatment) was reduced by 42±21%, 47±19%, 48±16%, 67±12%, and 86±8% in the groups treated with Mg-early alone, Mg-late alone, heparin alone, heparin+Mg-early, and heparin+Mg-late, respectively (all P<0.001 versus pretreatment, P<0.001 for heparin+Mg-early and Mg-late versus heparin or magnesium alone, and P<0.05 for heparin+Mg-late versus heparin+Mg-early, ANOVA). Magnesium had no significant effect on platelet aggregation, activated clotting time, or bleeding time. There were no significant effects on heart rate or mean arterial blood pressure. The serum magnesium level was inversely correlated with TW (r=-0.70, P=0.002). In conclusion, treatment with intravenous MgSO₄ produced a time-dependent inhibition of acute stent thrombosis under high-shear flow conditions without any hemostatic or significant hemodynamic complications. Thus, magnesium may be an effective agent for preventing stent thrombosis. (Arterioscler Thromb Vasc Biol. 2001;21:1544-1549.)

Keywords: platelets ■ antithrombotic effects ■ animal models ■ experimental thrombosis

Methods

Experimental Model

Animal Surgery
All procedures were approved by the Institutional Animal Care and Use Committee and conformed to the American Heart Association guidelines for animal research. Experiments were performed in 10 swine weighing 25 to 35 kg. After overnight fasting, swine were sedated with phenobarbital (5 mg/kg), and anesthesia was maintained with 1% isoflurane after endotracheal intubation. The right carotid artery and jugular vein were isolated and cannulated with 8F sheaths to establish an extracorporeal circuit as described previously.7 Arterial blood gases and pH were monitored periodically and maintained at normal levels by adjustment of the ventilation rate and tidal volume. Invasive arterial pressure measurement, oxygen saturation, ECG, and rectal temperature were monitored continuously. A thermostatically controlled blanket was used to maintain temperature at 37°C. Venous blood was collected for baseline platelet aggregation, complete blood cell count, and activated clotting time (ACT) measurements. After this, all animals received heparin at a dose of 10 U/kg as a bolus before the study to prevent thrombotic occlusion of the catheters and tubing. Each swine received an average of 200 U
heparin, an amount that produces negligible effects on thrombus formation at high-shear conditions in this model.1 At the conclusion of the experiment, blood was collected for complete blood cell counts, the carotid artery and jugular vein were ligated, and the animals were allowed to recover from anesthesia before being returned to the vivarium.

**Coronary Stents**
The stents tested were 7-mm-long slotted-tube-geometry devices made from the nickel-titanium alloy nitinol (Advanced Coronary Technology). They weighed 24±3 mg and had a strut thickness of 0.006 in. They had a silicon carbide grit–blasted surface finish, which creates a uniform roughened surface known to be highly thrombogenic in this model.2–4 Stents were expanded on a tapered mandrel to an outer diameter of 2.0 mm before being mounted in the perfusion chamber.

**Extracorporeal Shunt and Perfusion Protocol**
The extracorporeal shunt system used in the present study has been extensively characterized and described previously.5–7 After a 60-minute stabilization period, stents were mounted in the tubular chamber and perfused with normal saline for 60 seconds at 37°C. Care was taken to ensure that the stents were well apposed to the chamber wall. With a switch valve used to prevent stasis, blood was circulated through the system, and flow was regulated at 70 mL/min for 20 minutes by using a peristaltic pump (Masterflex, Cole-Palmer Instrument Co) placed in the circuit distal to the perfusion chamber. This flow rate generates a wall shear rate of 1486 s⁻¹ at the chamber surface and 2100 s⁻¹ at the stent surface. The shear rates were calculated according to the formula for laminar flow of homogeneous newtonian fluid in a cylindrical tube: shear rate = 4 Q / πR², where Q is volume flow, and R is radius.8 At high shear rates, as used in the present study, blood is considered to be essentially a newtonian fluid. At the end of the perfusion period, saline was circulated through the chamber and ex vivo system for several minutes at 40 mL/min to clear any visible blood before another stent was mounted. At the completion of each perfusion period, the stents (weighed before perfusion) were removed from the chamber, dried, and weighed again. Thrombus weight was calculated as a difference between preperfusion and postperfusion stent weights. The top of each stent was covered with heterologous porcine aortic strip (Pel Freeze) from which the intimal layer was removed to simulate the thrombogenic conditions induced by vascular injury associated with stent implantation. The number of stent perfusion runs examined was ≈10 during each experiment. Of the 10 animals studied, 5 underwent 2 experiments each at a minimum interval of 2 weeks, and 5 underwent 1 experiment only. Thus, a total of 15 experiments were performed in 10 animals with the use of a total of 44 stents for 156 perfusion runs. Stents were cleaned meticulously at the end of each experiment with BTS-450 solution (Beckman instruments, Inc). Stents were subsequently sterilized with 2.4% glutaraldehyde solution and suspended in sterile 0.9% saline solution for at least 24 hours before being used in the next experiment. Each stent can be used and cleaned up to 30 to 40 times without any demonstrable effect on the thrombogenic properties of the stent. Digital images of the stents were obtained with a Nikon 950 digital camera, downloaded into a PC, and processed with image analysis software (PhotoShop Adobe 5.0).

The perfusion protocol is illustrated in Figure 1. The stents were perfused before and after treatment with heparin or magnesium alone or combined treatment with heparin and magnesium in a random fashion. Heparin was administered as a 50 U/kg IV bolus, followed by 25 to 50 U · kg⁻¹ · h⁻¹ IV to maintain ACT at >150 seconds. MgSO₄ was administered as a 2 g bolus IV over 20 minutes, followed by a 2 g/h maintenance infusion. This was determined to be the optimal dose on the basis of the initial dose-response experiments. To assess a potential time-dependent antithrombotic effect of magnesium, data points were examined within 20 minutes after the bolus (Mg-early) and >40 minutes after the bolus (Mg-late). The time-dependent effects of magnesium were also examined in a random fashion. The administration of magnesium or heparin plus magnesium was stopped for 2 to 3 hours before obtaining new control thrombus weights. ACT was measured to ensure no residual effects of heparin. Effects on thrombus weight, whole-blood platelet aggregation, bleeding time, ACT, serum magnesium level, and complete blood count were quantified at various time points as shown in the protocol schematic. Mean arterial blood pressure (MABP) and heart rate were monitored and recorded throughout the protocol.

**Platelet Aggregation assay**
Thirty minutes after administration of the drug, 3 mL venous blood was collected in a siliconized test tube containing 0.3 mL of 0.129 molar sodium citrate or sodium heparin (Becton Dickinson Vacutainer System). Whole blood aggregometry (Chronolog Corp) was used to measure collagen (2.5 and 5 μg/mL)–induced and ADP (2.5 μmol/L)–induced platelet aggregation. Aggregation was expressed as maximal increase in electrical impedance measured in ohms at 6 minutes after the addition of agonist.

**Bleeding Time, ACT, and Serum Magnesium Assay**
Bleeding time was measured from an incision on the ventral surface of the thigh with a No. 11 surgical knife. The time between incision and cessation of bleeding was recorded as bleeding time. ACT was performed with a Hemochron 400 (International Technidyne Corp) machine in standard fashion.8,9 Serum magnesium was measured spectrophotometrically by using the magon dye method.11

**Statistical Analysis**
Data are presented as mean±SD. The statistical difference between means was determined by 1-way ANOVA. If means were shown to be significantly different, multiple comparisons by pairs were performed by the Bonferroni test (Graphpad Prism, version 3.0). Spearman correlation analysis was performed to explore the relationship between serum magnesium and other variables and between thrombus size and other variables. A value of P<0.05 was considered significant.

**Results**

**Stent Thrombosis**
Dose-response experiments were performed initially in the first 3 animals to determine the optimal bolus and maintenance dose regimen. The first set of experiments evaluated...
the optimal maintenance dose after a bolus dose of 2 g over 20 minutes, a dose found to be hemodynamically well tolerated in previous studies. The results shown in Table 1 demonstrate a dose-dependent reduction in stent thrombus weight with an optimal maintenance dose of 2 g/h. At higher doses, a slightly greater antithrombotic effect was observed. However, this was associated with a significant drop in heart rate and blood pressure. Next, we evaluated different bolus doses, with the maintenance dose fixed at 2 g/h. Heparin plus a 2 g bolus plus 2 g/h maintenance dose produced a greater reduction in stent thrombus weight compared with heparin plus a 1 g bolus plus 2 g/h maintenance dose (84 ± 6% versus 43 ± 14%, respectively; P < 0.005; n = 6 to 8 stents). The 4 g bolus produced a profound effect on heart rate (from 206 to 62 bpm) and blood pressure (MABP dropped from 79 to 60 mm Hg), thereby precluding evaluation of the antithrombotic effects at this dose. On the basis of these initial results, a bolus dose of 2 g over 20 minutes followed by maintenance infusion of 2 g/h was used in the present study.

Representative examples of stents perfused during pretreatment conditions and after treatment with magnesium alone, heparin alone, and combined magnesium + heparin are shown in Figure 2, and the data are quantified in Figure 3. Stent thrombus weight was reduced by 42 ± 25% (from 21 ± 4 to 13 ± 6 mg), 47 ± 19% (from 21 ± 3 to 11 ± 3 mg), 48 ± 16% (from 20 ± 3 to 10 ± 3 mg), 67 ± 12% (from 19 ± 3 to 6 ± 3 mg), and 86 ± 8% (from 20 ± 3 to 2 ± 2 mg) in the groups treated with Mg-early alone, Mg-late alone, heparin alone, heparin + Mg-early, and heparin + Mg-late, respectively (all P < 0.001 versus pretreatment). The antithrombotic effects were significantly more pronounced with a combined treatment with heparin and magnesium compared with treatment with magnesium or heparin alone (P < 0.001, ANOVA). Heparin + Mg-late produced a significantly greater reduction in thrombus weight compared with heparin + Mg-early (P < 0.05, ANOVA).

To exclude the possibility that prolonged anesthesia and repetitive instrumentation may have effects on stent thrombosis, control stents were perfused 2 to 3 hours after stopping the administration of treatment agents to ensure the return of stent thrombus weights toward baseline pretreatment values. The stent thrombus weight averaged 20 ± 3 mg at the beginning of the experiment (n = 16 stents in 5 animals) and 19 ± 4 mg at the end of the experiment (n = 12 stents in 5 animals). One typical example is shown in Figure 4A. In addition, heparin and heparin plus magnesium were given randomly at equivalent times after establishment of the extracorporeal shunt. The results, as shown in Figure 4B, indicate no differences in the antithrombotic effects during the first 3 hours of treatment.

**Table 1. Dose-Response Effects on TW, Heart Rate, and MABP**

<table>
<thead>
<tr>
<th>Intervention</th>
<th>% TW Reduction (n=5–8)</th>
<th>% Δ Heart Rate (n=3–5)</th>
<th>% Δ MABP (n=3–5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heparin alone</td>
<td>49 ± 11*</td>
<td>3 ± 8</td>
<td>5 ± 6</td>
</tr>
<tr>
<td>Hep + 2 g bolus + 1 g/h M</td>
<td>51 ± 22*</td>
<td>6 ± 7</td>
<td>5 ± 5</td>
</tr>
<tr>
<td>Hep + 2 g bolus + 2 g/h M</td>
<td>84 ± 6†</td>
<td>5 ± 9</td>
<td>2 ± 8</td>
</tr>
<tr>
<td>Hep + 2 g bolus + 3 g/h M</td>
<td>87 ± 8†</td>
<td>22 ± 11†</td>
<td>13 ± 4*</td>
</tr>
</tbody>
</table>

TW indicates thrombus weight, %Δ, percent change from baseline; Hep, heparin; and M, maintenance infusion rate. Values are mean ± SD.

*P < 0.01 vs baseline; †P < 0.01 vs Hep alone or Hep + 2 g bolus + 1 g/h M.
hours compared with the next 3 hours after establishment of the extracorporeal shunt.

**Platelet and Hematologic Studies**

The effects of study drugs on 5 μg/mL collagen–induced platelet aggregation, bleeding time, and ACTs are shown in Table 2. Magnesium had no significant effects on platelet aggregation. To exclude the possibility that the lack of a magnesium effect on platelet aggregation may be related to the citrate anticoagulant used (which may influence ionic concentrations because of calcium chelation in the samples), we performed aggregation in heparinized as well as citrated concentrations because of calcium chelation in the samples), the citrate anticoagulant used (which may influence ionic magnesium effect on platelet aggregation may be related to aggregation. To exclude the possibility that the lack of a magnesium effect on platelet aggregation may be related to the citrate anticoagulant used (which may influence ionic concentrations because of calcium chelation in the samples), we performed aggregation in heparinized as well as citrated blood in 3 pigs. Magnesium produced no significant inhibitory effect on platelet aggregation in heparin-stabilized samples (26±2 before magnesium and 27±2 after magnesium) compared with citrate-stabilized samples (25±3 before magnesium and 22±3 after magnesium). There was no effect of magnesium on platelet aggregation in response to either a lower concentration of collagen, 2.5 μg/mL, or to another platelet agonist, ADP at 2.5 μmol/L (data not shown).

Heparin produced a slight, but statistically significant, prolongation of bleeding time and also significantly increased ACT. Magnesium had no significant effects on either bleeding time or ACT beyond the heparin effect. There were no episodes of significant bleeding in any of the animals studied. Treatment with heparin or magnesium had no significant effects on either platelet or white blood cell counts or hematocrit (data not shown).

**Table 2. Effects on Platelet Function, ACT, and Serum Magnesium Level**

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Platelet Aggregation, Vmax</th>
<th>Bleeding Time, min</th>
<th>ACT, s</th>
<th>Serum Magnesium, mEq/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>25±3</td>
<td>4.0±0.5</td>
<td>109±8</td>
<td>1.7±0.2</td>
</tr>
<tr>
<td>Mg-late alone</td>
<td>22±3</td>
<td>4.5±0.5</td>
<td>104±5</td>
<td>3.7±0.6*</td>
</tr>
<tr>
<td>Heparin alone</td>
<td>24±6</td>
<td>5.3±0.6*</td>
<td>185±36*</td>
<td>...</td>
</tr>
<tr>
<td>Heparin+ Mg-early</td>
<td>23±4</td>
<td>5.8±0.8*</td>
<td>153±17*</td>
<td>3.7±0.3*</td>
</tr>
<tr>
<td>Heparin+ Mg-late</td>
<td>21±5</td>
<td>6.0±1*</td>
<td>162±24*</td>
<td>3.8±0.4*</td>
</tr>
</tbody>
</table>

Values are mean±SD (n=8 or 9 for each variable).

*M<0.01 vs baseline (ANOVA).

**Discussion**

In the present study, we have demonstrated for the first time that intravenous MgSO4 produces a significant inhibition of acute platelet-dependent stent thrombosis under high-shear flow conditions. The antithrombotic effect of magnesium was directly correlated with serum magnesium levels and was evident without any hemostatic or significant hemodynamic complications. The timing of magnesium administration appeared to be important, inasmuch as the maximum antithrombotic effect of magnesium was evident when platelets were treated before exposure to thrombogenic stimuli. Thus, magnesium may be an effective agent for preventing stent thrombosis.
Mechanism of Effect
The exact mechanism by which magnesium influences thrombus formation is not clear. Potential mechanisms include effects on platelets, coagulation, and fibrinolysis. Consistent with the dominant role of platelets in this ex vivo model of high shear-mediated acute thrombogenesis, antiplatelet effects of magnesium are likely to contribute significantly to its antithrombotic properties. Previous studies have demonstrated that platelet activation, adhesion, and aggregation are all effectively inhibited by intravenous magnesium supplementation. In the present study, a significant inhibition of platelet-thrombus formation was demonstrated with magnesium treatment without any effect on platelet aggregation or bleeding time. This observation of the discrepancy between profound effect of the magnesium on platelet-thrombus formation and lack of a significant effect on platelet aggregation or bleeding time is not inconsistent with previous findings reported in different experimental models. For example, in a rat thrombosis model, Ravn et al showed that the antithrombotic effect of magnesium was not accompanied by a significant increase in bleeding tendency. In a porcine model of ischemia/reperfusion injury, Ravn et al also showed that platelet aggregation responses were not significantly altered despite substantial reduction in infarct size. It is possible that the lack of an inhibitory effect on platelet aggregation may relate to the fact that whole blood platelet aggregation was performed in citrate-stabilized blood, which may influence the ionic concentrations due to calcium chelation in the samples. However, this possibility is not supported by our findings, in which magnesium had no significant effect on platelet aggregation regardless of the anticoagulant used to stabilize blood, i.e., citrate or heparin.

The discrepant effect on platelet adhesion/thrombus formation and platelet aggregation may also be likely related to the fact that the antiadhesive effect of magnesium may occur at a lower concentration (<4 mEq/L) compared with the antiaggregatory effect (>5 mEq/L). The serum magnesium levels achieved in the present study were within a range in which the antiadhesive effects would be more evident than the antiaggregatory effects. Magnesium exerts its platelet-inhibitory effect by reducing calcium mobilization in platelets and may also suppress fibrinogen interaction with platelets via competitive inhibition of calcium at the calcium-binding sites of the glycoprotein IIb/IIIa complex.

Hemodynamic Effects
No statistically significant changes in heart rate and blood pressure were observed in the present study. This lack of an effect of magnesium on hemodynamic parameters is not surprising. In the study of Ravn et al, hemodynamic measurements, including blood pressure, heart rate, and cardiac output, were not significantly affected in the magnesium-treated swine. In contrast, in human volunteers, blood pressure and heart rate decreased transiently only during the first 15 minutes, with no difference after 30 minutes of infusion. It is possible that the 20-minute bolus duration used in the present study compared with the 15-minute bolus duration used in the human study may account for the differences, because (according to Ravn et al) by extending the bolus infusion to 20 minutes, the hemodynamic effects of magnesium could be potentially avoided.

Time Dependence of Antithrombotic Effect of Magnesium
The antithrombotic effects were significantly more pronounced when magnesium was given 40 minutes before the initiation of stent perfusion. The difference in the antithrombotic effect in the Mg-early and Mg-late groups cannot be solely explained by a dose-dependent effect, because serum magnesium levels were not significantly different. It is possible that the intracellular levels of magnesium may be higher in the Mg-late group compared with the Mg-early group despite similar serum magnesium levels. It is well known that serum magnesium does not accurately reflect intracellular levels. Thus, platelet-thrombus formation is more easily inhibited when platelets are treated before exposure to thrombogenic stimuli.

The present study confirms the previously observed time dependence of the effect of magnesium in experimental models of ischemia/reperfusion myocardial injury in dogs and thrombosis in rats. The importance of the timing of magnesium administration may also potentially explain, in part, the negative results in ISIS-4 (in which magnesium infusion was delayed) compared with the positive results in LIMIT-2 (in which treatment was initiated earlier). Knowledge of this time dependence may be important and should be implemented in the study design of future clinical trials.

Limitations of the Study
The ex vivo model used in the present study, which primarily examines shear-mediated platelet-dependent thrombus formation, does not allow us to explore the effect of magnesium on coagulation and profibrinolytic and antifibrinolytic factors that may modulate thrombogenesis in vivo conditions. Nonetheless, this validated model is useful for studying the interaction of blood elements with stents and thrombogenic surfaces under controlled and well-defined conditions. The reproducibility and simplicity of this ex vivo system makes it a sensitive tool for assessing the preclinical efficacy of antithrombotic therapeutic interventions. We have used the shunt model to demonstrate superior efficacy of potent antiplatelet agents, such as abciximab and clopidogrel, for the prevention of stent thrombosis well before there was any clinical evidence for this indication. The swine model precludes the assessment of comparative antithrombotic efficacy of the glycoprotein IIb/IIIa inhibitor with magnesium, a subject that we are currently evaluating in a canine model.

It is well known that phenobarbital can potentially affect platelet function. However, it is unlikely that the antithrombotic effects of magnesium were critically modulated by phenobarbital, which was used only for sedation in the present study. All perfusion runs, including pretreatment and posttreatment runs, were performed after phenobarbital administration. Thus, even if phenobarbital were to potentially affect stent thrombosis, all interventions would be likely to be influenced similarly. Moreover, pretreatment thrombus weight did not vary from perfusion to perfusion, thrombus weights were not dependent on the time from the start of the experiments, and, last, thrombus weights returned to baseline after stopping all medications. These data indicate a low likelihood of a significant effect of phenobarbital on stent thrombosis. Isoflurane, which was used for the maintenance...
of anesthesia in the present study, has no significant inhibitory effect on platelet function.\textsuperscript{15}

**Conclusions and Implications**

In summary, intravenous treatment with magnesium produced a time-dependent inhibition of acute stent thrombosis under high-shear flow conditions without any hemostatic or significant hemodynamic complications. The potent anti-thrombotic effects of magnesium together with its safety, ease of administration, and low cost make it a promising adjuvant treatment during percutaneous coronary intervention. We have recently completed a pilot study in humans to evaluate the safety and efficacy of intravenous administration of MgSO\textsubscript{4} as an adjunct to nonacute percutaneous coronary intervention with stent implantation; the results have been favorable.

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**References**

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