Intravenous Magnesium Reduces Infarct Size After Ischemia/Reperfusion Injury Combined with a Thrombogenic Lesion in the Left Anterior Descending Artery

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Abstract—Experimental studies have demonstrated that intravenous magnesium (Mg) can protect the ischemic myocardium and has an antithrombotic effect. In patients with myocardial infarction, the reperfusion injury is complicated by the presence of a thrombogenic area in the affected coronary artery that may cause repetitive thrombus formation and embolization. We investigated the effect of Mg on infarct size in a randomized study in pigs. Myocardial infarction was induced by a 50-minute mechanical occlusion of the left anterior descending artery combined with an arterial injury, which stimulated a dynamic thrombus formation with emboli shedding on reperfusion. Magnesium sulfate (6 mmol/20 min plus 3 mmol/h) or saline was started at 30 minutes after coronary occlusion. Real-time ventricular pressure–volume loops were generated from the left ventricle by using a microtip pressure manometer and a conductance catheter. Platelet accumulation in the myocardium was evaluated by using $^{111}$In-labeled platelets. After 4 hours of reperfusion, the infarct size/area at risk ratio in the placebo group was $46 \pm 0.06\%$ (n=8) compared with $22 \pm 0.07\%$ (n=6) in the Mg-treated animals ($P=0.03$). Ejection fraction decreased significantly in the control group but not in the Mg-treated animals ($P=0.03$). Platelet accumulation in the myocardium did not change significantly between the Mg- and placebo-treated animals (placebo group, 191 ± 19%; Mg group, 177 ± 29%; NS). The present study demonstrates that intravenous Mg infusion is able to reduce infarct size by >50% and preserve the ejection fraction in this model where ischemia/reperfusion injury was evaluated in the presence of a thrombogenic area in the nutrient artery. (Arterioscler Thromb Vasc Biol. 1999;19:569-574.)

Key Words: magnesium n animals n reperfusion injury n thrombosis n platelets

Experimental data indicate that intravenous magnesium (Mg) may be a promising agent in the treatment of acute infarction not only during reperfusion of the ischemic myocardium,1,2 but also as an antithrombotic drug.3,4 Acute myocardial infarction (MI) is caused, in most cases, by the formation of an occlusive thrombus in the coronary artery.5 The therapeutic aim in MI patients is to obtain recanalization of the artery, to salvage the ischemic myocardium. However, after thrombolysis or angioplasty, intermittent occlusion of the artery often occurs because of recurrent thrombus formation at the thrombogenic plaque. Thrombus formation and embolization is likely to occur repetitively, until the disrupted plaque is sealed off as part of a healing process. This may lead to recurrent episodes of ischemia/reperfusion as well as accumulation of microaggregates from the thrombus in the myocardium downstream to the lesion. In animal models of reperfusion injury, a mechanical and standardized occlusion of the nutrient artery has generally been used. However, the clinical setting differs with respect to the experimental setup, because reestablishment of coronary blood flow is not always well defined in patients. In the present model, reperfusion injury was combined with a thrombogenic lesion in the left anterior descending artery (LAD), thereby increasing the risk of occlusive episodes after reperfusion. This was done to evaluate the effect of Mg in an experimental model, which has closer resemblance to the pathophysiology in MI patients.

We have previously shown that intravenous Mg is able to reduce not only thrombus formation, but also emboli frequency.4 We were therefore encouraged to evaluate if intravenous Mg was able to reduce not only infarct size but also the embolic burden in the myocardium downstream to the thrombogenic lesion in the LAD.

Methods

Animals and Study Design

Twenty-one 3-month-old, 45-kg (40 to 51 kg) Danish Landrace pigs (Institute of Experimental and Clinical Research, Aarhus, Denmark)

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were included in the study. The animals were randomly assigned to 2 groups, ie, Mg and control. The Mg group received 6 mmol of intravenous magnesium sulfate for 20 minutes starting 30 minutes after occlusion of the LAD and then continued with 3 mmol of magnesium sulfate per hour. The control group received intravenous isotonic saline in volumes equal to the Mg-treated pigs, starting 30 minutes after occlusion of the LAD.

The animals were treated according to the principles stated in the Danish law on animal experiments.

Surgery
Animals were anesthetized with fentanyl 0.3 mg and propofol 150 mg intubated and connected to mechanical ventilation at 4.5 L/min with a mixture of atmospheric air and O₂. Anesthesia was maintained with an infusion of fentanyl 0.3 mg/h, propofol 8 to 10 mg·kg⁻¹·hr⁻¹, and pancuronium 3 mg/h. Serial blood gas measurements were performed hourly to maintain a physiological level of oxygenation and ventilation. All animals received amiodarone 150 mg prophylactically to reduce the rate of arrhythmias during reperfusion. Temperature was kept between 36.5°C and 38.0°C by using a heating blanket.

The right and left carotid arteries and right internal jugular vein were cannulated. Access to the heart was obtained through a median sternotomy, and the heart was suspended in a pericardial cradle. A sternotomy, and the heart was suspended in a pericardial cradle. A

The pulmonary artery was carefully dissected free for placement of a transit-time flow-probe (Medi-Stim AS, Cardiomed).

Thrombogenic Lesion
A medial injury resulting in exposure of adventitial tissue into the vessel lumen was created by external application of 2 dented forceps, followed by a twisting of the artery by moving 1 clamp clockwise and the other clamp counterclockwise. Before the experiment, a series of pilot studies (n = 4) was performed in which in vivo thrombus formation was visualized by transilluminating the LAD from beneath, as described previously. A growing thrombus shedding emboli could be seen inside the vessel lumen after the thrombogenic injury. In this pilot series, the vessels were cut out 30 minutes after reperfusion, and a combined red and white thrombus was found within the lumen. However, because of the movement of the heart, a major concern was whether the transilluminator would intermittently cause obstruction of the blood flow in the artery, and the transilluminator was therefore not applied during the final experiment.

To evaluate the nature of the thrombogenic lesion, the isolated part of the LAD was cut out at the end of the experiment and placed in formaldehyde/sodium phosphate (4%, wt/vol). Vessel specimens were cut out in longitudinal sections and prepared for light microscopy examination by using trichrome staining.

Ischemia/Reperfusion Injury
A vessel clamp was applied on the LAD just proximal to the thrombogenic lesion immediately after the injury was created. Occlusion was maintained for 50 minutes, and afterward a 4-hour reperfusion period ensued. At 4 hours a suture was placed around the injured part of the LAD and the corresponding vein to occlude the LAD. Immediately after occlusion, the heart was perfusion stained with intra-atrial injection of sodium fluorescein to determine the area at risk. The heart was fibrillated with a 9-V battery and excised 10 to 15 seconds after injection.

Infarct Size/Area at Risk
The heart was cut into 5-mm slices, perpendicular to the septum from the apex to the base. All slices were weighed. The area at risk was marked with a glow needle on each slice under a Woods lamp. Viable myocardium was stained bright red by using a charge-coupled device camera (JAI Protec 2040, JAI) and a video machine (Sony SVO 9500 MMP) and stored on S-VHS videotapes for later analysis. For each slice area at risk, area not at risk and infarct size were assessed by computer planimetry, on an IBM personal computer, and the mass-ratio of the area at risk to the left ventricular (LV) mass, and the infarct size to the area at risk, were calculated.

Hemodynamics
Real-time ventricular pressure–volume loops were generated by using pigtail conductance catheters (7F, NuMed) and microtip pressure catheters (2.5F, Millar Houston). The conductance catheter was inserted retrogradely into the LV via the right carotid artery under fluoroscopic guidance. The pressure catheter was inserted through the LV apex. All volume measurements were corrected for blood resistivity and parallel conductance and calibrated from a transit time flow probe placed around the pulmonary artery. Parallel conductance was determined by the hypertonic saline method, using an injection of 7 mL of 10% saline into the inferior vena cava. Preload was varied by transient snaring of the inferior vena cava. Volume and pressure data were fed into a dedicated microcomputer, where they were integrated and analyzed offline in custom-designed software.

Calculation of Ejection Fraction
Raw volume data were corrected for parallel conductance and the gain constant α offline. The gain constant α was derived as the ratio of conductance-derived stroke volume to transit time flow averaged over 5 consecutive cardiac cycles.

Absolute LV volume = (raw volume – parallel conductance)/α

The ejection fraction (EF) was then derived in the normal way as (EDV – ESV)/EDV, where EDV is end-diastolic volume and ESV is end-systolic volume.

Platelets
Indium-labeled platelets were produced from 100 mL of blood withdrawn from a central venous line into acid citrate dextrose anticoagulant and gently rotated. The blood was centrifuged at 1000 rpm for 10 minutes to isolate platelets in plasma. Platelet-rich plasma was centrifuged at 2200 rpm for 10 minutes and the platelet-poor plasma (PPP) removed for later resuspension. The platelet pellet was gently resuspended in 1.5 mL of plasma and incubated with a mixture of 111 In-Tropolone for 10 minutes. After incubation, 5 mL of PPP was added and the sample centrifuged at 2200 rpm for 10 minutes to remove unbound indium. After the second centrifugation, the platelets were resuspended in 5 mL of PPP. The labeling efficiency was 98±0.02%. The radioactive solution was injected into the pig 30 minutes before harvesting of the heart.

Blood samples were withdrawn into heparin-anticoagulated (0.1 mg/mL) tubes at baseline and after 15 minutes, 1 hour, and 3 hours of reperfusion. Platelet-rich plasma (PRP) was prepared by centrifugation at 180g for 10 minutes at room temperature. PPP was obtained by centrifugation at 2500g for 10 minutes. Platelet aggregation was measured in a single-channel aggregometer (Model 560 versus Chronolog, Havertown), using the turbimetric method described by Born. Platelet aggregation was induced with fixed concentrations of collagen (0.2 mg/mL) (Sigma) and the aggregation response was recorded during the next 5 minutes.

Platelet Accumulation
After the LV had been stained with 2,3,5-triphenyltetrazolium chloride, the slices were divided into 3 parts based on staining characteristics. The area at risk was cut out according to the glow needle marks, and within this area the necrotic tissue (white-yellowish) was separated from the viable tissue (red stained). Representative samples of the central part of the right ventricle were obtained. The tissue was placed in preweighed scintillation vials in according to the following 4 separate groups: (1) area at risk, necrotic; (2) area at risk, viable; (3) area not at risk, LV; and (4) right ventricle. Two persons, who were unaware of the treatment given, performed all procedures (H.B.R. and L.B.-S.). Quantitative counting of the tissue radioactivity was performed on a Packard γ-counter. The window was set at 350 to 500 keV for indium 111, and counts were corrected for background. Counts per gram of tissue was
calculated and the radioactivity was expressed as the percentage of the counts per gram of tissue in the area not at risk in the LV.

Ischemic Markers

Blood samples were withdrawn into heparinized Venoject tubes (Terumo Europe) at baseline and after 15 minutes, 1 hour, and 3 hours of reperfusion. Samples were immediately centrifuged at 3000 rpm, at 4°C for 15 minutes, and plasma was stored at −20°C for later analysis. Plasma levels of creatine kinase (CK) were measured by the use of Enzymun-Test Troponin-T-1556428 (Boehringer Mannheim GmbH). Ischemic markers, ie, CK and TNT. CK increased significantly over time (Table 2) but was not significantly different between the treatment groups during clamping of the LAD and 1, 2, and 3 hours after reperfusion of the artery. Data are shown as mean±SEM values. Two-way ANOVA showed a significant difference between the Mg and the placebo group (P=0.03) but no significant difference between the Mg-treated animals (0.15±0.02). The ratio of infarct size to area at risk was 0.46±0.06 in the control group and 0.22±0.07 in the Mg group, showing that the mean infarct size was reduced by >50% in animals receiving intravenous Mg (P=0.03).

Statistics

All data are mean±SEM values. Comparison between 2 groups was performed by Student’s t test or the rank sum test when appropriate. With more than 2 groups, data were analyzed by using 2-way ANOVA with time and treatment being the 2 factors explored. The relation between infarct size and ischemic markers was evaluated by using Spearman’s correlation coefficient. P<0.05 was considered significant.

Results

Exclusion of Animals

Of the 21 pigs studied, 7 animals were excluded because of irreversible ventricular fibrillation during occlusion or early reperfusion. The incidence of irreversible ventricular fibrillation did not differ between the Mg-treated (4 of 10 animals) and placebo-treated (3 of 11) animals. The remaining 14 pigs (6 Mg-treated and 8 placebo-treated animals) were included in the final analysis.

Hemodynamic Variables

Table 1 gives a summary of these data. Heart rate increased significantly in both groups over time, but no differences were observed between the Mg- and placebo-treated animals (two-way ANOVA: P<0.001 [time]; NS [treatment]). There were no significant differences in end-diastolic volume, cardiac output, LV systolic pressure, or LV diastolic pressure between the control and the Mg-treated group at baseline or during the first 3 hours. The mean EF was calculated in each group and no significant difference was observed at baseline (control, 47±3%; Mg, 41±3%). EF decreased to 38±4% at 2 hours of reperfusion in the control animals, whereas the EF decreased <1% during the observation period in the Mg group (Figure 1). The decrease in EF was significantly larger in the controls than the Mg group (2-way ANOVA: NS [time]; P=0.03 [treatment]).

Infarct Size

Figure 2 shows the area at risk to LV mass-ratio in the control group (0.13±0.01), which was not significantly different from the Mg-treated animals (0.15±0.02). The ratio of infarct size to area at risk was 0.46±0.06 in the control group and 0.22±0.07 in the Mg group, showing that the mean infarct size was reduced by >50% in animals receiving intravenous Mg (P=0.03).

Ischemic Markers

The cellular damage was evaluated by using ischemic myocardial markers, ie, CK and TNT. CK increased significantly over time in both groups (Table 2) but was not significantly different between the treatment groups (2-way ANOVA: P=0.02 [time]; NS [treatment]). In a similar manner, TNT increased significantly over time (Table 2), but although the difference increased between the 2 treatments, numbers did not differ significantly between the Mg- and placebo-treated animals (two-way ANOVA: NS [time]; P=0.03 [treatment]).
Intravenous administration of Mg resulted in a >50% reduction in infarct size in the Mg-treated group compared with controls. This is in accordance with previous experimental studies showing a significant reduction in infarct size after an isolated ischemia/reperfusion injury.\(^1,2,8\) In patients with acute MI, repetitive occlusion of the artery often occurs because of recurrent thrombus formation at the culprit lesion. We wanted to evaluate the effect of Mg in a model where both reperfusion injury and thrombosis would occur. This was done to observe whether the reduction in infarct size after Mg therapy would attenuate with several pathophysiological events being present simultaneously. This could perhaps explain the discrepancy between previous experimental studies and the most recent clinical trial (ISIS-4), where Mg did not affect early mortality. However, data from the present model, which has a closer resemblance to the clinical setting, indicate that Mg has a highly protective effect on the ischemic myocardium and is able to reduce infarct size and preserve myocardial contractility.

Mg was administered before reperfusion of the artery, as this has been shown to be of utmost importance, both in terms of reducing reperfusion injury\(^1,2\) and in obtaining an anti-thrombotic effect.\(^4\) It has previously been shown that infarct size can be reduced with bradycardic agents\(^9\) and bradycardia is a known side effect of intravenous Mg, but the occurrence of bradycardia is more dependent on the dose rate than the actual dose given. We performed 4 preliminary studies with different infusion rates and observed that by extending the bolus infusion to 20 minutes the bradycardic effects of Mg could be avoided (unpublished data). A regimen with continuous Mg infusion was chosen, as stuttering cycles of occlusion and recanalization were anticipated at least during the early phase of reperfusion.

The reduction in infarct size could not be ascribed to any changes in hemodynamics as heart rate and LV systolic and diastolic pressures were not significantly different between the Mg-treated animals and the controls. Furthermore, the difference could not be explained by a reduction in the occurrence of ventricular fibrillation observed during or after coronary occlusion. Earlier trials indicated a reduction in the frequency of various types of arrhythmias with Mg in suspected MI,\(^10\) but this finding could not be substantiated either in the LIMIT-2 or the ISIS-4 study.\(^11,12\)

The results from the LIMIT-2 study showed a significant reduction in mortality and reduced incidence of LV failure in the Mg-treated patients.\(^11\) Subgroup analysis in LIMIT-2

### Discussion

| TABLE 2. Ischemic Markers and Platelet Aggregation Measured at Baseline and at 15 Minutes and 1, 2, and 3 Hours After Reperfusion |
|------------------|------------------|------------------|------------------|------------------|
|                   | t=0              | t=15 Min         | t=1 h            | t=3 h            |
| CK (U/L)          | Control 802±79   | 848±106          | 1295±229         | 1732±372         |
|                   | Mg 1010±146      | 1097±180         | 1494±474         | 1826±571         |
| TNT (µg/L)        | Control 0.03±0.00| 0.17±0.007       | 0.91±0.36        | 2.21±0.72        |
|                   | Mg 0.03±0.00     | 0.16±0.09        | 1.30±0.98        | 1.38±0.94        |
| Platelet aggregation | Control 32±5     | 21±4             | 25±2             | 28±6             |
|                   | Mg 38±8          | 17±3             | 32±11            | 35±9             |

Two-way ANOVA: CK: \(P=0.02\) (time), NS (treatment); TNT: \(P=0.006\) (time), NS (treatment); platelet aggregation: NS (time and treatment).
showed a transient increase in cardiac output, probably as a consequence of reduced afterload. However, the increase in cardiac output lasted only 15 minutes after bolus injection, indicating that the hemodynamic effects did not contribute to the reduction in LV failure. Accordingly, we observed a preservation of the EF in the Mg-treated group without any sustained increase in cardiac output, indicating that the effect is more likely to be derived from a protection of the myocardium during ischemia/reperfusion injury.

To detect myocardial cell damage, CK and TNT levels were analyzed. CK has a low cardiospecificity, as it is also released from skeletal muscle, brain, and the intestinal tract. It has been suggested that TNT release represents irreversible cell damage, whereas CK is also likely to be released after reversible ischemia. Thus, the almost similar increase in CK in both treatment groups may indicate that the animals have been exposed to an equal ischemic insult, which is supported by the finding that the mass-ratio of the area at risk to the LV mass was not significantly different between the Mg-treated animals and the controls. Another explanation may be that the surgical intervention with damage of skeletal muscle tissue constitutes a major part of the measured CK activity, and therefore the signal from the myocardial contribution cannot be properly assessed. The irreversible damage to the myocytes, as shown by the increase in the TNT levels, tended to be most pronounced in the placebo group. Although there was no significant difference between the 2 treatment groups, we cannot rule out the possibility that this is because of the limited number of animals in each group.

Evidence from several platelet aggregation studies has shown that Mg has the ability to inhibit platelet reactivity in vitro. In the present study, we were not able to demonstrate any effect of Mg on collagen-induced ex vivo platelet aggregation. In contrast, we have previously shown reduced ex vivo platelet reactivity after intravenous Mg treatment of healthy volunteers. Herzog et al have demonstrated reduced ex vivo platelet aggregation after intravenous infusion of Mg in swine. The reason for this discrepancy remains unclear. It can be speculated that platelets collected after reperfusion may be more inhomogeneous as a consequence of consumption of more reactive platelets in the thrombogenic area in the LAD. We have previously shown in an in vivo model that Mg reduces not only thrombus formation but also the emboli frequency during ongoing thrombosis. Emboli can theoretically obstruct smaller vessels downstream to the lesion and in this way contribute to the developing infarct. The mean platelet accumulation was reduced in the Mg group, but not significantly, which is probably because of a very large interanimal variation.

Other possible mechanisms may contribute to the observed reduction in infarct size after Mg therapy, including reduction in generation and release of free oxygen radicals. It has previously been shown that Mg blocks free radical formation both in cell cultures and in vivo. We did not try to explore the release of free oxygen radicals in the present study and we can only speculate that this mechanism may be important not only in preserving myocytes, but also in influencing platelet accumulation within the ischemic myocardium. In a study by Leo et al, platelet activation was observed after reoxygenation of anoxic platelets, and the platelet activation was significantly reduced in the presence of free oxygen radical scavengers.

The clinical need for adjunctive therapies to reduce infarct size in patients is still a major issue. Preservation of the ischemic myocardium is extremely important, as LV dysfunction is a strong predictor of poor outcome. The present study shows that intravenous Mg has the ability to reduce infarct size and preserve EF in an experimental model where ischemia/reperfusion injury was evaluated in the presence of a thrombogenic area in the nutrient artery.

### Acknowledgments
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**TABLE 3. Platelet Accumulation in the Myocardium**

<table>
<thead>
<tr>
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<th>AAR-LV</th>
<th>AAR-Vital</th>
<th>AAR-Necro</th>
<th>RV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (% of ANR)</td>
<td>191±19</td>
<td>155±12</td>
<td>*277±30</td>
<td>136±12</td>
</tr>
<tr>
<td>Mg (% of ANR)</td>
<td>177±29</td>
<td>167±29</td>
<td>*188±32</td>
<td>132±15</td>
</tr>
</tbody>
</table>

AAR-LV indicates total area at risk in the left ventricle; AAR-Vital, vital part of area at risk; AAR-Necro, necrotic part of area at risk; RV, right ventricle; ANR, area not at risk in the left ventricle.

Two-way ANOVA showed significant difference in location (P=0.01), NS (treatment). Significantly more platelets were accumulated in AAR-Necro compared with RV, *P<0.05 (Tukey test).
References


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