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Intracoronary and Intravenous Magnesium Does Not Reduce Myocardial Infarct Size in a Canine Model of Regional Ischaemia and Reperfusion

S. Grunert¹, D. Ebel¹, W. Schlack², V. Thämer¹

Clinical trials show controversial effects of magnesium infusion in patients with acute myocardial infarction. In LIMIT-2, intravenous magnesium lowered mortality, but in the much larger ISIS-4, intravenous magnesium had no effect. Because of these conflicting results, we tested two hypotheses in a dog model of ischaemia and reperfusion.

A) Intracoronary magnesium infusion given in the early reperfusion period has a protective effect against myocardial reperfusion injury.

B) Systemic magnesium-potassium infusion with doses comparable to those used in clinical studies, may reduce myocardial infarct size.

Anaesthetized open chest dogs underwent 1 h of left anterior descending artery occlusion followed by 6 h of reperfusion.

A) Animals received intracoronary magnesium aspartate (Mg i.c., n=5) or vehicle infusion (Control i.c., n=5) for the first hour of reperfusion to increase regional plasma concentration by 4 mmol l⁻¹.

B) Animals received intravenous magnesium-potassium-aspartate (Mg-K i.v., n=6) or vehicle infusion (Control i.v., n=8) beginning 1 h before occlusion until the end of the 6 h reperfusion period.

Intracoronary magnesium had no influence on infarct size (Mg i.c. 20.6 ± 5.0 % of area at risk, Control i.c. 24.4 ± 8.7 % of area at risk; P = ns) or regional post-ischaemic wallfunction. Application of intravenous magnesium-potassium did not reduce myocardial infarct size (Mg-K i.v. 14.1 ± 14.8 %; Control i.v. 18.1 ± 12.2 % of area at risk; P = ns). The possible beneficial effect of magnesium infusion is probably not due to an early, direct protective effect on ischaemic-reperfused myocardium. J Clin Basic Cardiol 2002; 5: 23–28.

Key words: dog, heart, infarct size, ischaemia, reperfusion, magnesium, myocardial infarction

Several randomised clinical trials, eg the Second Leicester Intravenous Magnesium Intervention Trial (LIMIT-2), have shown beneficial effects of magnesium infusion in patients with acute myocardial infarction [1]. In contrast, the Fourth International Study of Infarct Survival (ISIS-4) failed to show any protective effect of magnesium infusion [2]. There are a number of actions of magnesium on the cardiovascular system that could have influenced these diverging results [3]. Besides its antithrombotic [4, 5] and anti-thrombotic [6, 7] functions, magnesium can protect the ischaemic-reperfused myocardium [8–10]. Reperfusion injury, structural damage following ischaemia and reperfusion, is caused by energy rich metabolites and oxygen reperfusing the energy-depleted myocardium [11, 12]. Magnesium may influence these mechanisms as well as acting as a natural calcium antagonist, preventing ischaemic calcium overload [13]. Experimental studies on both canine and swine models have underlined the importance of magnesium administration prior to the onset of reperfusion [14, 15].

In this study we tested the hypothesis that magnesium can reduce myocardial infarct size in a model of regional ischaemia and reperfusion in the anaesthetized dog after coronary artery occlusion. To examine a direct effect of magnesium on the reperfused myocardium, the animals received intracoronary magnesium for the first hour of reperfusion to increase the regional magnesium plasma concentration by 4 mmol l⁻¹ in the coronary arteries, an elevation that did not alter the electrophysiological excitation of the heart [16, 17]. Since a combination of magnesium and potassium has been shown to reduce reoxygenation arrhythmias [4] in a second study, animals received intravenous magnesium potassium aspartate starting before the occlusion and until the end of reperfusion, in a dose comparable to that which could be achieved clinically.

Materials and Methods

Animal preparation

Twenty-four mongrel dogs of either sex, weighing 18 to 34 kg, were anaesthetized with sodium thiopental, anaesthesia was maintained with enflurane in a mixture of oxygen/nitrous oxide. For further details see [18].

After thoracotomy and pericardiotomy as illustrated in Figure 1, sonographic crystals were implanted into the epicardial and subendocardial myocardium of the anterior wall perfused by the left anterior descending artery (LAD) and the posterior wall perfused by the left circumflex artery (LCX) as a control region. These crystals were used to determine regional wall thickness by sonomicrometry. A suture was placed around the LAD for later occlusion. Left ventricular pressure (LVP) was measured with a catheter tip manometer through the left auricle of the heart. Coronary blood flow of LCX and LAD at the site of occlusion was measured with ultrasonic flow probes. Ischaemic regional myocardial blood flow (RMBF) during the occlusion was measured using coloured microspheres in the group of intravenous treatment. For further details see [19].

After the end of the reperfusion period, hearts were arrested in diastole with Bretschneider’s cardioplegic solution via the aorta. The area at risk was perfused with Dextran in sodium chloride via the LAD, the rest of the heart was perfused with 0.2 % Evans blue, staining the non-risk-area

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Influence of Mg on Myocardial Infarct Size

blue. After excision of the heart, transversal slices of the left ventricle were stained with triphenyltetrazolium chloride (TTC) to identify necrotic tissue by its red colour [20, 21]. Myocardial infarct size was assessed by planimetry.

Drug preparation

A) Intracoronary magnesium
The solutions used for anoxic pre-reperfusion contained 20 mmol l⁻¹ HEPES (N-2-hydroxyethylpiperazin-N’-2-ethanesulfonic acid) and either 2 mmol l⁻¹ magnesium-aspartate (Mg i.c., n = 5) or 12.5 mmol l⁻¹ sodium-chloride (control i.c., n = 5) adjusted to pH 7.4 and equilibrated with 100 % N₂. Normoxic infusion contained 20 mmol l⁻¹ magnesium-aspartate or 145 mmol l⁻¹ sodium-chloride, each buffered with HEPES and adjusted to pH 7.4.

B) Intravenous magnesium and potassium
The infusion for the intervention group (Mg-K i.v., n = 6) contained 16.6 mmol l⁻¹ magnesium-aspartate and 16.6 mmol l⁻¹ potassium-aspartate and 0.1 mmol l⁻¹ HEPES adjusted to pH 7.35 and sodium chloride 0.9 %. Animals of the placebo group (Control i.v., n= 8) received sodium-chloride 0.9 %.

Experimental protocol

After surgical preparation, sufficient time was allowed for stabilization of the haemodynamic parameters. Baseline values were then measured. The hearts of Group A were paced via the left atrium throughout the experiment.

A) Intracoronary magnesium
The animals underwent 60 min of LAD occlusion with subsequent 360 min of reperfusion. Anoxic intracoronary perfusion of the LAD was administered for the last 5 min of the occlusion with a rate of 10 ml min⁻¹, followed by normoxic intracoronary infusion for the first 60 min of reperfusion. The infusion rate was adapted to LAD-flow to increase regional plasma concentration by approximately 4 mmol l⁻¹ (Fig. 1A).

B) Intravenous magnesium and potassium
Sixty minutes after baseline measurements the animals underwent 60 minutes of LAD occlusion followed by 360 min of reperfusion. Either saline or Mg-K aspartate were administered intravenously using a foreleg vein after completing baseline measurements 60 minutes before occlusion until the end of reperfusion. The infusion rate for both groups was 0.1 ml min⁻¹ [kg body weight]⁻¹, that is 0.1 mmol magnesium potassium aspartate per hour and kg body weight (Fig. 1B). In contrast to the protocol using intracoronary infusion, animals were not paced.

Data analysis and statistics

LVP, its first derivative dP/dt, anterior and posterior wall thickness, coronary blood flow in the LAD and the LCX were continuously recorded on an ink recorder. The signals were stored on videotapes after pulse-code modulation. The data were digitized with an analogue-to-digital recorder at a sampling rate of 500 Hz and processed on a personal computer. Systolic wall thickening (WThSYS) as a parameter for regional myocardial wall function was calculated by the following formula:

$$WThSYS = \frac{(WThES – WThED)}{WThED} \times 100$$

where WThES stands for wall thickness at the end of systole and WThED for wall thickness at the end of diastole.

All data are expressed as means ± standard deviation. Analysis was carried out by an analysis of variance (ANOVA) followed by a Duncan post-hoc test or a t-test for independent samples. Differences with a level of significance P < 0.05 were considered as statistically significant.

Results

Serum magnesium and potassium

A) Intracoronary magnesium
Under baseline conditions systemic serum magnesium concentration was 0.65 ± 0.05 mmol l⁻¹. During intracoronary infusion systemic serum magnesium increased to 0.99 ± 0.05 mmol l⁻¹ at 15 min reperfusion (P < 0.001 vs. Control i.c.) with a maximum of 1.19 ± 0.08 mmol l⁻¹ at the end of intracoronary infusion (P < 0.001) and returned to baseline values by the end of the experiment.

B) Intravenous magnesium and potassium
Infusion as illustrated in Figure 2, induced a significant increase of systemic serum magnesium from 0.71 ± 0.02 mmol l⁻¹ at baseline to 1.42 ± 0.29 mmol l⁻¹ at the end of reperfusion (P < 0.01 vs. baseline and Control i.v.). With the onset of reperfusion serum magnesium was significantly elevated (P < 0.05 vs. control and baseline; Mg-K i.v.: 1.29 ± 0.20 mmol l⁻¹; Control i.v.: 0.81 ± 0.24 mmol l⁻¹)

Serum potassium was not increased in either the control or the Mg-K-groups.

Global haemodynamics

A) Intracoronary magnesium
Peak systolic left ventricular pressure (LVPMAX), maximal and minimal left ventricular dP/dt (dP/dtMAX and dP/dtMIN) were not different within the groups. LVPMAX was stable dur-
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ing the experiment (Fig. 3A), in both groups \( \frac{dP}{dt_{\text{MIN}}} \) was decreased with onset of the occlusion and remained depressed during reperfusion (Tab. 1).

B) Intravenous magnesium and potassium

Treatment with Mg-K decreased heart rate at the beginning of occlusion (P < 0.05 vs. baseline). While animals of the control group showed an increase in HR during the experiment, Mg-K induced an increasing bradycardia from 114.1 ± 7.1 min\(^{-1}\) to 100.2 ± 16.7 min\(^{-1}\) (Fig. 4). LVP\(_{\text{MAX}}\) was similar in both groups (Fig. 3B).

There was no difference in the maximal \( \frac{dP}{dt} \) representing left ventricular inotropy (Tab. 1).

Regional haemodynamics

A) Intracoronary magnesium

Systolic wall thickening as a parameter of regional function is shown in Figure 5A. WThSYS of the test region was similar in both groups, it decreased with LAD occlusion to negative values, the depression remaining even during the reperfusion period. Ischaemic myocardium lost its ability to contract, so the systolic myocardial wall thickness is not greater than diastolic values. The myocardial wall was even thinned by surrounding non-ischaemic regions so that the ratio shows negative values.

Regional wall function of the control region was similar in both placebo and magnesium groups during the experiment.

B) Intravenous magnesium and potassium

Depression of WThSYS of the ischaemic region occurred in both groups commencing with the start of LAD occlusion, similar to the i.c. study (Fig. 5B).

Regional wall function of the control region was better in the Mg-K group (P < 0.001). Improvement of WThSYS was not only limited to the ischaemic period, the Control i.v.-group also showed a mild improvement of WThSYS.

Infarct size

A) Intracoronary magnesium

Application of magnesium did not reduce the infarct size as illustrated in Figure 6 (P = 0.41; Mg i.c. 20.6 ± 5.0 % of area

...
at risk; Control i.c. 24.4 ± 8.7 %). Area at risk was equal (Mg i.c. 28.0 ± 7.4 % of the left ventricle; Control i.c. 29.6 ± 8.6 % of the left ventricle).

B) Intravenous magnesium and potassium
Myocardial infarct size was not reduced by Mg-K as shown in Figure 6 (P = 0.61). The mean infarct size was 18.1 ± 14.7 % of area at risk for the control group, the mean infarct size in the Mg-K-group was 14.1 ± 12.2 % of the area at risk. Size of the area at risk was not different (Control i.v.: 35.6 ± 8.2 % of the left ventricle; Mg-K i.v.: 36.7 ± 11.3 % of the left ventricle).

Regional myocardial blood flow
Ischaemic collateral blood flow did not differ in the animals of the intravenous treatment-study. No differences in epicardial, endocardial or transmural areas were observed.

Discussion
The main result of the intracoronary magnesium study is that there is no protective effect of magnesium against myocardial reperfusion injury; myocardial infarct size and post-ischaemic wall function were not improved by magnesium infusion. Magnesium has been shown to protect against reperfusion injury, eg prevention of mitochondrial calcium overload [9] or preservation of ATP [8], in isolated rodent hearts [22]. However, the comparison to in vivo-studies is difficult, because of the differences in the concentrations used. Concentrations of in vitro-experiments cannot be achieved with either intravenous or intracoronary administration in in vivo-studies due to the systemic toxicity of high magnesium concentrations [23]. Because of this we chose magnesium concentrations comparable to LIMIT-2 or ISIS-4 to avoid the supratherapeutic effects of magnesium.

After failing to see a protective effect with an elevation of regional serum magnesium by 4 mmol l−1 in the intracoronary treatment, we examined in a second study the effect of systemic magnesium-potassium administered in a dose comparable to clinical trials. This study of intravenous magnesium-potassium-administration also failed to show an infarct-size reduction. The regional wall function of post-ischaemic myocardium was also not improved.

After intravenous infusion, serum magnesium was nearly doubled, while potassium was not different to baseline value. Increased renal excretion of potassium was not observed. A possible explanation for this difference is a rapid cellular uptake of potassium but not for magnesium.

In vivo-studies have shown a protective effect of magnesium, but concentrations used are difficult to reproduce in a physiological or clinical setting [4, 10, 24]. When magnesium is given in supratherapeutic doses, negative inotropy occurs. The protective effect of negative inotropic substances in myocardial infarction is well known eg β-blockers, Ca²⁺-receptor antagonists or volatile narcotics [25–27]. In this study we used concentrations of magnesium that showed no change in maximal dP/dt, a marker for negative inotropy. The improved myocardial wall function of the posterior wall, that is seen in the i.v. treatment group, is due to a Frank-Starling-mechanism. Bradycardia prolonged diastolic filling time and elevated LVPEd, so end-diastolic wall-thickness becomes thinner, leading to relative improvement of WThsys. In the Mg i.c. experiments, wall thickness or WThsys did not change, because the hearts were paced, thus eliminating frequency-related effects.

The decrease in myocardial wall function of the test region with onset of occlusion is due to loss of myocytes and contractility reserves, that is followed by myocardial stunning in the reperfusion period [28]. Magnesium did not change these pathophysiologic phenomena, wall function remained depressed, although magnesium has been shown to improve stunned myocardium [29]. The potency of bradycardic agents to reduce myocardial infarct size [30] is well established and the protection is not dependent on the negative inotropic effects [31]. A trend towards smaller infarct size was seen but was not significant. A power analysis was done before the study, the number of dogs was sufficient to detect a reduction in infarct size compared with prior studies of our working group investigating reperfusion injury [18].

The findings of this study that there is no change in infarct size after either intracoronary or intravenous magnesium infusion are in contrast to those of Christensen et al. [14], Herzog et al. [15; reviewed in 32] and the recent experiments of Ravn et al. [33].

In contrast to our study, in those three studies the levels of magnesium reached on infusion were not measured. We measured the concentra-

Table 1. Global haemodynamics

<table>
<thead>
<tr>
<th></th>
<th>LV dP/dtMAX (mmHg s⁻¹)</th>
<th>LV dP/dtMIN (mmHg s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A) Intracoronary magnesium</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intervention</td>
<td>Control i.c.</td>
<td>Mg-K i.c.</td>
</tr>
<tr>
<td>Baseline</td>
<td>1566 ± 121</td>
<td>1583 ± 167</td>
</tr>
<tr>
<td>Occlusion</td>
<td>1363 ± 229</td>
<td>1380 ± 165</td>
</tr>
<tr>
<td>Reperefusion</td>
<td>1202 ± 246</td>
<td>1413 ± 134</td>
</tr>
<tr>
<td>1 h</td>
<td>1472 ± 190</td>
<td>1577 ± 234</td>
</tr>
<tr>
<td>2 h</td>
<td>1547 ± 196</td>
<td>1530 ± 211</td>
</tr>
<tr>
<td>3 h</td>
<td>1482 ± 182</td>
<td>1520 ± 226</td>
</tr>
<tr>
<td>4 h</td>
<td>1507 ± 209</td>
<td>1451 ± 164</td>
</tr>
<tr>
<td>5 h</td>
<td>1454 ± 222</td>
<td>1343 ± 119</td>
</tr>
<tr>
<td>6 h</td>
<td>1486 ± 253</td>
<td>1262 ± 51</td>
</tr>
<tr>
<td><strong>B) Intravenous magnesium and potassium</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intervention</td>
<td>Control i.v.</td>
<td>Mg-K i.v.</td>
</tr>
<tr>
<td>Baseline</td>
<td>1582 ± 295</td>
<td>1517 ± 249</td>
</tr>
<tr>
<td>Mg²⁺-K⁺</td>
<td>1552 ± 254</td>
<td>1552 ± 254</td>
</tr>
</tbody>
</table>

Values are means ± SD; LV dP/dtMAX = maximum of first derivative of left ventricular pressure; LV dP/dtMIN = minimum of first derivative of left ventricular pressure. * P < 0.05 vs baseline
ton of total magnesium, but the concentration of ionised magnesium, which is responsible for the biological effects was not measured. Aspartate could bind Mg$^{2+}$ and thus reduce the ionised concentration, but recent measurements of such binding with Mg$^{2+}$-microelectrodes showed that aspartate did not significantly bind Mg$^{2+}$ and reduced the ionised Mg$^{2+}$ concentration [34]. Different protocols, with or without initial bolus of magnesium were also used which could also have influenced the magnesium concentrations. The lack of the possibility to compare serum concentrations of magnesium during occlusion and at the onset of reperfusion makes it difficult to explain the different results concerning infarct size reduction. It is thus essential in future studies, to measure both total and ionised magnesium concentrations to allow comparison amongst different studies.

In conclusion, our results on anaesthetized animals do not support a beneficial effect of magnesium infusion for patients with acute myocardial infarction. Indeed, magnesium infusion could even be detrimental, as the Second National Registry of Myocardial Infarction [35] concluded that there is tendency to an increased mortality in those patients who received an infusion of magnesium.

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This study was approved by the Bioethics Committee of the District of Düsseldorf, Germany.

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