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Magnesium Reduces Free Radicals in an In Vivo Coronary Occlusion-Reperfusion Model

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Objective. **This study demonstrated that magnesium (Mg) reduces free radicals after a brief coronary occlusion-reperfusion sequence.**

Background. **Magnesium has been shown to reduce infarct size in patients with acute myocardial infarction. We hypothesized that this action of Mg occurs through its action on free radicals.**

Methods. **Eighteen mongrel dogs were studied (nine control, nine receiving Mg). Catheters were placed into the coronary sinus for continuous blood withdrawal. A Varian E-4 electron paramagnetic resonance spectrometer was used to monitor the ascorbate free radical (AFR) signal in the coronary sinus blood; AFR is a measure of total oxidative stress. Occlusion of the left anterior descending coronary artery for 20 min was followed by reperfusion. The study animals received 4 g Mg intravenously starting at 15 min of occlusion (5 min before reperfusion) and continuing during reperfusion.**

Results. **Results are presented as percent change from baseline ± SEM. Magnesium blunted the peak AFR increase: at 4 min of reperfusion there was a 4.7 ± 3.3% increase in AFR signal in the dogs receiving Mg versus an 18.2 ± 3.3% increase in the control animals ($p < 0.05$). Total radical flux was reduced during reperfusion by 53% in the Mg dogs compared with controls ($p < 0.05$).**

Conclusions. **Magnesium attenuates AFR increase after an occlusion-reperfusion sequence. To our knowledge this is the first in vivo real-time demonstration of Mg's impact on free radicals.**

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Rapid restoration of coronary blood flow after coronary artery occlusion provides a significant decrease in infarct size and mortality. However, early reperfusion is associated with reperfusion injury due primarily to calcium overload and oxygen free radicals generated by reintroduction of molecular oxygen into the ischemic myocardium (1,2).

Attenuation of both free radical increase and calcium overload may limit reperfusion injury (3–5). Clinical studies suggest magnesium (Mg) has beneficial effects in patients with acute myocardial infarction by preserving left ventricular ejection fraction, decreasing infarct size and limiting mortality (6–8). This has been postulated to occur through the effect of Mg as a physiologic calcium channel blocker (9). Whether Mg can attenuate free radicals as well is uncertain; there are few data evaluating the effect on free radicals of Mg administered to a whole animal during occlusion-reperfusion sequences. This study sought to evaluate the hypothesis that Mg reduces free radicals during an occlusion-reperfusion sequence in a canine model.

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Methods

This study was approved by the Animal Care and Use Review Board at The University of Iowa and conformed to all regulations for animal use. Eighteen mongrel dogs (nine control/nine receiving Mg), weighing between 23 and 27 kg, were studied.

Experimental preparation. The dogs were anesthetized with a ketamine/rompin (both 100 mg/mL) 3:1 combination at 1 ml/9 kg, intubated and ventilated. Arterial blood gases were monitored and ventilator settings adjusted as necessary. Anesthesia was maintained with intravenous pentobarbital as needed. Sixteen of the 18 dogs underwent a left lateral thoracotomy with exposure of the heart. The left anterior descending (LAD) coronary artery and mid- to distal coronary sinus (CS) were isolated by dissection and snare ligatures were placed. The LAD coronary arterial ligature was placed proximal to the first visible epicardial diagonal branch. A Dacron-woven catheter (7F Gensini) was manually placed into the CS via the right external jugular vein, downstream from the great cardiac vein, to sample the venous drainage from the area of subsequent ischemia-reperfusion (anterior left ventricular myocardium) and secured in position by the snare ligature. We tried to achieve identical catheter placement in all dogs. The remaining 2 dogs had percutaneous instrumentation without a thoracotomy; in these two, the woven Dacron catheter was placed into the CS under fluoroscopic guidance and anchored in position at its exit from the sheath. A 2.5- or 3.5-mm

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Abbreviations and Acronyms

AFR = ascorbate free radical
CS = coronary sinus
EPR = electron paramagnetic resonance
LAD = left anterior descending
Mg = magnesium

angioplasty balloon catheter was positioned in the LAD artery proximal to the diagonal branch for the later coronary occlusion-reperfusion sequence.

Ascorbate-free radical measurements. We measured ascorbate free radical (AFR) signals in the CS or femoral artery as a parameter of total oxidative stress. Our in vivo, real-time method has been previously described in detail (10). Normally, the AFR signal at baseline is below the limit of detection. Therefore, the baseline AFR concentration was enhanced by a continuous vitamin C (ascorbate) infusion at an appropriate rate that produced a steady-state AFR electron paramagnetic resonance (EPR) signal in both the femoral artery and CS. A Varian E4 EPR spectrometer was used to measure the amplitude of the characteristic AFR signal; the amplitude is proportional to the concentration of AFR in the blood. Once a stable signal (≈8 nM) was obtained, the ascorbate infusion was held constant. Sampled blood was continuously drawn from the CS or femoral artery and passed through the spectrometer at a constant rate and then returned to the dog via the femoral vein. The total time for blood to traverse the catheter to the electron paramagnetic spin resonance device was 5 s.

Experimental protocol. Once a stable AFR signal was achieved, occlusion of the LAD coronary artery was accomplished by snare occlusion or balloon inflation; subsequent reperfusion was accomplished by snare release or balloon deflation. The absence of coronary flow on occlusion was documented through loss of coronary Doppler flow signals, measured using an epicardial Doppler flow probe in the dogs undergoing thoracotomy, and by loss of measurable blood pressure from the intracoronary angioplasty catheter in the closed-chest dogs. The coronary occlusion period was 20 min as was the reperfusion period. The study animals received 2 g Mg (as Mg sulfate) IV over 5 min starting at min 15 of occlusion. Without interruption, the study dogs received an additional 2 g Mg over the first 12 minutes of reperfusion during which time the coronary sinus AFR signals were measured (see later). These Mg doses were based on published Mg data (i.e., Intravenous Magnesium Intervention Trial-2) (8) adjusted for the animal weights. The control dogs received a saline infusion.

AFR signals were obtained via continuous CS sampling during the occlusion-reperfusion period, as previously described. During the protocol, AFR signals from the femoral artery were also monitored, and, if necessary, to maintain a stable arterial AFR signal, the ascorbate infusion rate was adjusted before coronary occlusion. The infusion rate was not altered during the coronary occlusion-reperfusion sequence (10). At the end of the protocol, myocardial risk areas were determined through intracoronary Evans blue injection distal to the point of arterial occlusion. The mass of the area stained was divided by the total left ventricular mass to determine percent left ventricular area at risk.

Two dogs receiving Mg had serum Mg levels drawn before Mg infusion was begun, and again 10 min after infusion was begun (i.e., 5 min after reperfusion was initiated)—the time when the peak AFR increase was expected, based on our earlier work (10).

To ascertain whether Mg could have a direct effect on the AFR signal, we performed an in vitro experiment. We added 100 μM of freshly prepared ascorbate and 7 μg/mL of Mg^{2+} (similar to the Mg^{2+} concentration achieved in the animal experiments, see later) to a solution of 50 mM phosphate buffer (pH 7.4, air-saturated). The steady-state AFR EPR signal was measured before and immediately after the addition of Mg^{2+}.

Statistical analysis. The reperfusion data were analyzed using the SAS/STAT procedure MIXED. The repeated-measures analysis was performed involving two factors, treatment (control vs. Mg groups), a between-animal factor, and time, the repeated-measures factor. Comparisons of interest, involving mean changes over time within each group and treatment group comparisons at each time point, were evaluated by estimation of mean contrasts and its corresponding standard error and then tested using the *t*-test statistic. The p values were adjusted using Bonferroni's method to adjust for the number of comparisons performed. All data are presented as mean ± SEM.

Results

There were no differences between the two groups with respect to left ventricular area at risk as assessed by Evans blue dye staining: 30.6 ± 2.6% vs. 33.4 ± 3.0% for Mg and control dogs, respectively (p = NS).

In two dogs receiving Mg infusion, serum Mg levels increased from 1.25 ± 0.55 mg/dl (before Mg) to 7.25 ± 0.85 mg/dl (10 min after the infusion was begun, 5 min after reperfusion). There was a significant but modest decline in systolic and diastolic blood pressure in the dogs receiving Mg (Table 1). No heart block occurred and no intravenous pressor support was administered.

Magnesium blunted the coronary sinus peak free radical rise: at 4 min of reperfusion there was a 4.7 ± 3.3% increase in dogs receiving Mg vs. an 18.2 ± 3.3% increase in control dogs ($p < 0.05$, Fig. 1). Although the peak rise in AFR reflects the flux of radicals present in the blood at that time point, the total integrated area under each curve was proportional to the total free radical flux during the time of reperfusion. With use of a Simpson integration, the control area was 66.5 ± 12.4 arbitrary units, whereas the Mg area was 31.3 ± 10.7 arbitrary units ($p < 0.05$), an overall reduction in total radical flux by 53%).

In the two dogs that were studied with the closed-chest

Table 1. Arterial Pressure Response to Magnesium or Saline (control group) Infusion

Dogs	Systolic Arterial Pressure (mm Hg)		Diastolic Arterial Pressure (mm Hg)	
	Before Infusion	After 10-min Infusion	Before Infusion	After 10-min Infusion
Control dogs	116.7 ± 6.1	120.6 ± 8.2	75.0 ± 3.1	77.2 ± 5.3
Magnesium dogs	109.4 ± 8.6	100.0 ± 4.7*	70.5 ± 8.1	56.1 ± 3.3†

*p = 0.05; †p < 0.05. Data presented are mean value ± SEM.

preparation—one control and one dog receiving Mg—peak AFR increases were 30.2% and 12.1%, respectively.

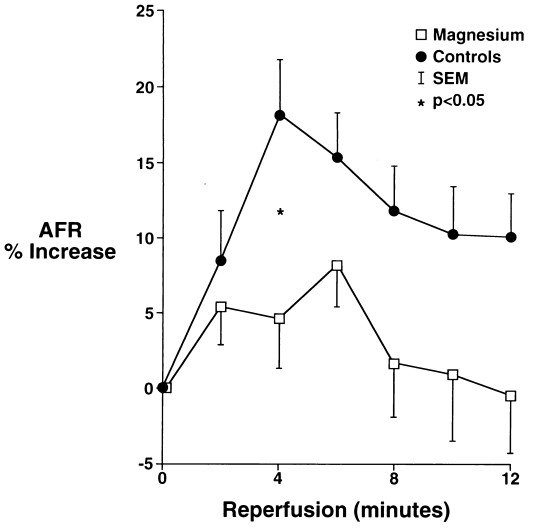
In the in vitro experiment, performed to determine whether Mg^{2+} had any direct effect on the AFR signal, we observed no change in the amplitude of the AFR signal with the addition of Mg^{2+} at a concentration similar to that achieved in the in vivo animal experiments.

Discussion

The major finding of this study is that Mg attenuates AFR increase (total oxidative stress) in an occlusion-reperfusion canine model.

Mechanisms of magnesium's effect. Magnesium may attenuate free radical production in one of two ways: It may directly inhibit free radical production or it may facilitate scavenging of free radicals. A recent study (11) showed that Mg inhibits reduced nicotinamide-adenine dinucleotide phosphate oxidase, an enzyme that produces superoxide radical; the effect of Mg at higher concentrations was comparable to other metals (such as copper) that are usually considered excellent inhibitors of free radical generation by reduced nicotinamide-adenine dinucleotide phosphate oxidase. This same study (11) found that although Mg does facilitate free radical scavenging, it does so at only a minimal level compared with other scavengers such as the transition metal manganese. Thus, the mechanism for Mg's attenuation of free radicals may be through inhibition of free radical production upon reperfusion and not by direct scavenging of radicals already present.

Figure 1. Increase in ascorbate free radical (AFR) after 20 min of coronary occlusion followed by reperfusion. During reperfusion, the nine dogs that received intravenous magnesium had a significantly lower increase in ascorbate free radical than the nine control dogs that did not receive magnesium.



Dickens et al. (12) showed that Mg deficiency within endothelial cells increased cytotoxicity to oxyradicals beginning at a relatively short 15 min of exposure to the free radicals, compared with Mg-rich cells. Thus, Mg may also protect the endothelial cell from oxyradical injury.

Kramer et al. (13) demonstrated that following a sequence of global ischemia and reperfusion, isolated working hearts from rats fed an Mg-deficient diet displayed greater levels of radical adduct production in EPR spin-trapping experiments and less recovery of cardiac function than hearts from rats fed an Mg-sufficient diet. This prooxidant influence of Mg deficiency is consonant with our study's demonstration of an apparent antioxidant effect of acute Mg administration before an occlusion-reperfusion sequence.

Magnesium is a divalent cation and is a known cofactor in over 300 enzymes. It is essential for all adenosine triphosphatase activity including movement of calcium across and within cell membranes for cardiovascular tissue. Magnesium may also confer benefit during acute occlusion-reperfusion sequences by its action as a physiologic calcium channel blocker (6,9,14). Magnesium inhibits calcium overload during initial phases of reperfusion through inhibition of calcium transport across most calcium channels (9).

Magnesium in our study had a modest but significant arterial pressure-lowering effect. This may have contributed to the attenuation of free radicals by reducing ischemia during coronary occlusion.

The use of AFR to evaluate the effect of Mg on free radicals assumes that there is no direct interaction between AFR and Mg. We demonstrated this in an in vitro experiment.

The dose of Mg used for the current study was based on previous clinical studies of myocardial infarction (Intravenous Magnesium Intervention Trial-2) (8). We only evaluated one Mg dose; a dose-response study would be an appropriate future study.

Ascorbate free radicals as a parameter of free radical generation. Our AFR method has some advantages over other free radical measurement methods, especially spin-trapping

methods: no chemicals are added, no tissue biopsies are necessary, and the method is real time. However, a disadvantage is that we do not know which specific radical(s) are suppressed, because the AFR method only allows determination of total oxidative stress, and does not identify individual radicals (10). Kramer et al. (13) noted strong correlations between post ischemic free radical production—which our AFR technique measures—and markers of tissue injury, such as lactate dehydrogenase release and percent recovery of cardiac work.

To facilitate blood transit from the CS to the EPR spectrometer, we used larger bore tubing, which resulted in a longer transit time from the CS to the EPR measuring device in this study than in our previous studies (10). We attribute the differences in this study's control group peak AFR increase of 18% compared with control AFR increases of >30% in our previous study (10) to the longer transit time. We have recently performed additional experiments with shorter transit times showing peak AFR increases comparable to our earlier studies.

Relevance of this study to clinical effects of magnesium. The use of open-chest preparations is inevitably unphysiologic to some degree. We studied two dogs percutaneously, without a thoracotomy. In the control (saline infusion) dog, the peak coronary occlusion increase in the AFR signal was greater than the increase in AFR in the open-chest animals. The closed-chest dog that received Mg had a lower AFR peak increase than the open-chest dogs. No conclusions can be drawn from only two experiments. These closed-chest experiments do show the feasibility of studying Mg's effect in a more physiologic experimental preparation. Future studies, using such a clinically relevant model, are necessary to confirm the beneficial effect of Mg in sequences of myocardial ischemia and reperfusion.

Direct free radical suppression by antioxidants such as superoxide dismutase and catalase has been shown to improve ventricular function after an occlusion-reperfusion sequence in animals (3,4). Clinical studies using doses of Mg similar to ours have also shown cardioprotective effects, with reductions in infarct size, preservation of ejection fraction and reduced mortality. These clinical benefits are consonant with attenuation of free radical(s) by Mg as our study found.

In summary, magnesium reduces AFR production in an occlusion-reperfusion canine model. Our study is the first, to our knowledge, to demonstrate suppression of free radicals in an occlusion-reperfusion sequence by Mg in a real-time, whole animal model. Magnesium warrants further evaluation as a cardioprotective agent in the setting of myocardial ischemia and reperfusion.

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