Magnesium attenuates isoproterenol-induced acute cardiac dysfunction and β-adrenergic desensitization

Yin-Tie Jin, Naoyuki Hasebe, Tomoyuki Matsusaka, Shunsuke Natori, Takaumi Ohta, Shiro Tsuji, and Kenjiro Kikuchi

Department of Internal Medicine, Division of Cardiology, Asahikawa Medical College, Asahikawa, Hokkaido, Japan

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Magnesium is known as a cardioprotective factor in the treatment of hypertension, ischemic heart disease, and chronic heart failure (2, 21, 38, 43). In experiments on cardiac ischemia-reperfusion, magnesium has been reported to reduce myocardial calcium overload (44), free radical generation (11), and myocardial infarct size (26). High magnesium levels have been reported to suppress β-adrenergic desensitization (9). However, the beneficial effects of magnesium in acute heart failure remain to be elucidated.

The goal of the present study was to investigate whether magnesium prevents the development of cardiac dysfunction and β-adrenergic desensitization in acute heart failure, in which excess catecholamine plays a particularly crucial role. To achieve this goal, we used an ISO-induced cardiac dysfunction and β-adrenergic desensitization dog model (31).

METHODS

Experimental Animals and Preparation

Sixteen adult mongrel dogs of either sex, weighing 11.2 ± 2.4 kg, were anesthetized with pentobarbital sodium (30 mg/kg iv) and ventilated with oxygen enriched air using a volume-limited ventilator (model 613; Harvard, South Natick, MA). Arterial blood gas was maintained within the physiological range by adjusting the ventilator. An incision was made in the fifth left intercostal space. A polyethylene tube occluder was placed around the descending aorta to regulate aortic blood pressure. A bipolar pacing electrode was attached to the left atrial appendage through a small pericardial incision and connected to a cardiac stimulator (SEC-2102; Nihon Kohden, Tokyo, Japan). A polyethylene catheter was inserted through the left carotid artery into the ascending aorta and connected to strain-gauge manometers (TP-101T; Nihon Kohden) to monitor arterial pressure. Catheters were inserted through the femoral artery to take blood samples and through the femoral vein to infuse drugs. LV pressure, the rate of LV pressure change (LV dP/dt), and the time constant of LV isovolumic relaxation (τ) were measured by a catheter-tipped transducer (PC-350; Millar, Houston, TX) inserted from the right carotid artery into the LV. A 5-MHz single-plane transducer for transesophageal echocardiography (SSD-830; Aloka, Tokyo, Japan) was placed just behind the LV to monitor changes in LV dimensions.

The present study was reviewed and approved by the Ethics Committee for Animal Experiments at Asahikawa Medical College and according to The Law (no. 105) andNotifications (no. 6) of the Japanese Government.

Experimental Protocol

The ISO loading dose was determined from both a preliminary study and previous study using the same experimental model (31). As the maximal dose, one which did not induce crucial hemodynamic

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deterioration or fatal arrhythmias during more than 4 h of experiments, 1 µg·kg⁻¹·min⁻¹ of ISO was selected. For the maximal dose of magnesium, 1 mg·kg⁻¹·min⁻¹ of continuous magnesium sulfate (MgSO₄) infusion was selected as it did not provoke any significant hemodynamic changes during the experiment.

Dogs were randomly assigned into two groups (Fig. 1). One group received 1 µg·kg⁻¹·min⁻¹ of ISO infusion (ISO group). The other group received 1 mg·kg⁻¹·min⁻¹ of magnesium infusion started 10 min before the ISO infusion and continued throughout the experiment (ISO + Mg group). Every 1 h, ISO infusion was stopped for 15 min, when the hemodynamics and dose response to low doses of ISO (0.025–0.2 µg·kg⁻¹·min⁻¹) were compared with baseline hemodynamics and the dose response to low doses of ISO. The 1 h of ISO loading infusion and 15-min ISO-free period followed by the dose-response test protocol was repeated three times. During continuous hemodynamic measurements and dose response to 0.025–0.2 µg·kg⁻¹·min⁻¹ of ISO, aortic pressure and heart rate were maintained at baseline levels using an aortic occluder and atrial pacing.

Blood Sampling

Aortic blood samples were periodically collected through the catheter in the femoral artery. Aortic blood gas was monitored with a blood-gas analyzer (Bayer 850; Sudbury, UK). Serum-ionized magnesium (s-Mg²⁺) was measured with a free magnesium analyzer (NOVA CRT8; MC Medical, Waltham, MA). Serum-ionized calcium (s-Ca²⁺) was measured with a free calcium analyzer (ICA2; Radiometer, Copenhagen, Denmark). Serum lipid peroxide (LPO; hemoglobin methylene blue method), the MB fraction of creatine phosphokinase (CK-MB; chemiluminescent immunoassay method), lactic dehydrogenase (LDH; Wroblewski La Due method), troponin T (TnT; enzyme immunoassay method), and plasma atrial natriuretic peptide (ANP; immuno-radiometric assay method) were also measured.

Data Analysis and Statistics

All hemodynamic data and the lead II ECG were continuously monitored on a direct-writing oscillograph (RM 6200: Nihon Kohden), digitized, and then recorded on a personal computer by a physiological data-acquisition system (AD Instruments, Taunton, Australia, New South Wales). LV end diastolic pressure was defined as the point immediately before the onset of LV contraction, indicated by the initial increase in LV dP/dt. LV end systole was defined as the point of maximum negative LV dP/dt (17). The τ was derived from a digitized plot of LV pressure wave against time (42). LV end-diastolic pressure (LVEDP) and LV end-systolic pressure (LVESD) were measured by M-mode tranesophageal echocardiography. LV percent fractional shortening was calculated as (LVEDD – LVESD/LVEDD) × 100. LV ejection fraction was calculated as (LVEDD² – LVESD²/LVEDD²) × 100 (16). The data were stored and analyzed with a personal computer; all values are expressed as means ± SE. Differences between baseline hemodynamic and subsequent values were assessed by repeated-measures ANOVA. If an overall difference was found, comparisons were performed by a two-tailed Student’s t-test for unpaired data. A P value of 0.05 or less was considered to be statistically significant.

RESULTS

Baseline Hemodynamic Parameters

Baseline hemodynamic parameters are summarized in Table 1. There were no significant differences between baseline parameters in the ISO and ISO + Mg groups obtained after a 10-min pretreatment of magnesium infusion.

Time Course Hemodynamic Changes

Effects of magnesium on maximum LV dP/dt after ISO infusion. The maximum LV dP/dt was time dependently decreased in ISO groups. However, it was significantly smaller in the ISO + Mg group, where the maximum LV dP/dt was decreased from 4,179 ± 233 to 2,920 ± 200 mmHg/s in the

Table 1. Baseline hemodynamic parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ISO Group (n = 8)</th>
<th>ISO + Mg Group (n = 8)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR, beats/min</td>
<td>179 ± 11</td>
<td>170 ± 9</td>
<td>NS</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>102 ± 12</td>
<td>107 ± 5</td>
<td>NS</td>
</tr>
<tr>
<td>Max LV dP/dt, mmHg/s</td>
<td>4,179 ± 232</td>
<td>4,132 ± 325</td>
<td>NS</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>26 ± 2</td>
<td>24 ± 1</td>
<td>NS</td>
</tr>
<tr>
<td>LVF, %</td>
<td>61 ± 3</td>
<td>67 ± 4</td>
<td>NS</td>
</tr>
<tr>
<td>LVFS, %</td>
<td>28 ± 2</td>
<td>34 ± 3</td>
<td>NS</td>
</tr>
<tr>
<td>τ, ms</td>
<td>21 ± 2</td>
<td>25 ± 2</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means ± SE. HR, heart rate; MAP, mean aortic pressure; LV dP/dt, rate of change of left ventricular pressure; LVEDD, left ventricular end-diastolic dimension; LVF, left ventricular fractioning; LVFS, left ventricular fractional shortening; τ, time constant of isovolumic left ventricular pressure decay; NS, not significantly different. There were no significant differences between the baseline hemodynamic parameters in the isoproterenol (ISO) and ISO + Mg groups.
ISO group ($P < 0.05$) but from 4,132 ± 325 to 3,477 ± 206 mmHg/s in the ISO + Mg group (not significant) at 3 h after ISO infusion. Changes in maximum LV dP/dt from baseline were time dependently decreased by ISO in both groups; however, they were significantly smaller in the ISO + Mg group ($P < 0.05$) (Fig. 2, left). *

**Effects of magnesium on the time constant of LV pressure decay.** The $\tau$ increased significantly in a time-dependent manner from 21.1 ± 2.4 to 36.1 ± 5.7 ms at 3 h in the ISO group. It also increased from 25.5 ± 2.6 to 34.1 ± 3.4 ms at 3 h but was significantly attenuated in the ISO + Mg group. The changes in $\tau$ from baseline were time dependently increased by ISO in both groups; however, they were significantly smaller in the ISO + Mg group ($P < 0.05$) (Fig. 2, right).

**Effects of magnesium on transesophageal echocardiographic parameters.** Echocardiographic LV systolic functional indexes of LV ejection fraction and LV fractional shortening were significantly decreased in a time-dependent fashion by the ISO infusion. These changes were significantly attenuated in the ISO + Mg group (Fig. 3).

**Effects of Magnesium on the Dose Response to ISO**

The dose responses of LV dP/dt to low doses of ISO challenge infusion were not significantly affected by magnesium pretreatment (Fig. 4A). They were gradually attenuated in a time-dependent fashion by ISO loading; however, they were significantly preserved in the ISO + Mg group (Fig. 4, B–D).

$s$-Mg$^{2+}$ and s-Ca$^{2+}$ Levels

The time course of changes in s-Mg$^{2+}$ and s-Ca$^{2+}$ levels is summarized in Fig. 5. As expected, the s-Mg$^{2+}$ level significantly increased (7-fold) in the ISO + Mg group. In contrast, it decreased, but not significantly, in the ISO group (Fig. 5, left). The s-Ca$^{2+}$ level gradually but significantly decreased in the ISO group. In contrast, it was not significantly affected in the ISO + Mg group (Fig. 5, right).

**Changes in Serum LPO and Serological Markers of Myocardial Damage**

The serum LPO level, a radical product of polyunsaturated fatty acid, significantly increased from 0.9 ± 0.1 to 4.6 ± 0.3 nmol/ml at 3 h after ISO loading (Fig. 6). In the ISO + Mg group, the LPO level increased from 0.9 ± 0.2 to 2.8 ± 0.4 nmol/ml; however, it was significantly smaller than in the ISO group ($P < 0.01$) (Fig. 6).

Myocardial leakages of CK-MB, LDH, and TnT and the release of ANP were significantly increased by ISO in both
groups. However, these were significantly attenuated in the ISO + Mg compared with the ISO group at 3 h after ISO loading: CK-MB = 44.1 ± 4.5 vs. 25.4 ± 2.3 ng/ml (P < 0.05) (Fig. 7A), LDH = 627.1 ± 98.5 vs. 427.7 ± 78.2 IU/ml (P < 0.05) (Fig. 7B), TnT = 1.7 ± 0.6 vs. 0.5 ± 0.1 ng/ml (P < 0.01) (Fig. 7C), ANP = 21.7 ± 4.1 vs. 14.4 ± 3.5 ng/ml (P < 0.05) (Fig. 7D).

**DISCUSSION**

We demonstrated for the first time that magnesium attenuated acute cardiac dysfunction and β-adrenergic desensitization induced by a large dose of ISO infusion in dogs. Chronic infusion of ISO has been reported to induce LV systolic and diastolic dysfunction and LV hypertrophy, accompanied by myocardial necrosis, apoptosis, and interstitial fibrosis (14, 24, 36). In the present study, using a dog model, we demonstrated that a large dose of ISO infusion provoked acute LV dysfunction, accompanied by evidence of myocardial damage, i.e., leakage of myocardial enzymes and contractile proteins, within 2 h of administration, as well as the release of ANP.

The mechanism of cardiac dysfunction induced by excess ISO is not yet fully understood. The most plausible explanation of ISO cardiotoxicity is intracellular calcium overload through β-adrenergic receptor stimulation (6). Cardiomyocytes are injured by the excess calcium influx (20), leading to irreversible cell injury, apoptosis, and necrosis. Therefore, ISO-induced cardiac dysfunction is prevented by calcium channel blockades as well as by β-adrenergic receptor blockades (6, 20).

Magnesium inhibits extracellular calcium influx via a voltage-sensitive calcium channel (L-type calcium channel) and calcium release from the sarcoplasmic reticulum (SR), i.e.,...

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Fig. 4. Dose responses to low doses of challenge infusion of ISO, compared in the ISO (○) and ISO + Mg (■) groups at baseline (A) and at 1 (B), 2 (C), and 3 h (D) after ISO loading infusion. The dose response of LV dP/dt to ISO was significantly attenuated in a time-dependent fashion. In contrast, magnesium supplementation significantly preserved it. Data are means ± SE. *P < 0.05 vs. ISO + Mg.

Fig. 5. Effects of ISO on serum magnesium (s-Mg²⁺) and serum calcium (s-Ca²⁺) levels with or without magnesium supplementation. The s-Mg²⁺ level was significantly increased in the ISO + Mg group (solid bars), but it was not significantly affected in the ISO group (open bars). The s-Ca²⁺ level was gradually and significantly decreased in the ISO group; in contrast, it was not significantly affected in the ISO + Mg group. Data are means ± SE. *P < 0.05 and **P < 0.01 vs. ISO; †P < 0.05 and ‡P < 0.01 vs. baseline.
calcium-induced calcium release, and facilitates the sequestration of calcium released by the SR in myocardial cells (28, 45, 46). Calcium channel-blocking action of magnesium is more potent than that of other cations, such as cadmium and nickel. These findings suggest that magnesium supplementation potentially suppresses calcium overload in the myocardium. The cardiac and hemodynamic changes that we observed, i.e., contractile dysfunction paralleled with diastolic dysfunction accompanied by increased end diastolic dimension, were consistent with physiological changes induced by a calcium overload (1). A large dose of ISO significantly decreased the s-Ca²⁺ level, but this was almost totally prevented by the magnesium infusion. The decrease in the s-Ca²⁺ level by ISO may represent a shift of extracellular calcium into the cytosol, i.e., intracellular calcium loading. Thus prevention of the decrease in the s-Ca²⁺ level by magnesium may indirectly indicate evidence of suppression of intracellular calcium overload.

Prolonged exposure of hearts to β-adrenergic agonist results in attenuated inotropic responsiveness to agonists, i.e., β-adrenergic desensitization, which may contribute to the progression of heart failure. The process of β-adrenergic desensitization involves decreases in β-adrenergic receptor density, which is triggered by the phosphorylation of receptors, a rapid uncoupling of receptors from Gs protein, and decreases in basal adenylate cyclase activity and calcium channel density (3, 5, 25, 39, 40). Although the mechanism of ISO-induced β-adrenergic receptor desensitization has not been entirely clarified, a time-dependent decrease in the inotropic response to ISO was observed even after 2 h of ISO loading, and it was significantly prevented by magnesium supplementation in the present study. Magnesium is known to modulate receptor-G protein-catalytic interactions at several sites (12). Magnesium acts as a cofactor...
with ATP at the catalytic site and is required for GTPase activity and G activation as well as GTP binding (22). Feldman (9) reported that β-adrenergic desensitization is obscured by high magnesium concentrations via maintenance of adenylate cyclase and cAMP-dependent protein kinase activities. These findings suggest that magnesium supplementation may inhibit β-adrenergic desensitization by regulation of the receptor-G protein-catalytic interactions.

Another plausible mechanism of magnesium efficacy on cardiac dysfunction and β-adrenergic desensitization is related to oxidative stress. Excess ISO generates oxygen free radicals, suppresses the activity of anti-oxidative enzymes, and consequently augments oxidative stress (35). Excess catecholamine can also be oxidized and generate oxidative products (10), which may be responsible for impaired inotropic responses to adrenergic stimulation and myocardial necrosis and contractile failure (33). Magnesium has been reported to reduce free radical production in ischemia-reperfusion of the myocardium (11). In the present study, the time-dependent increase in LPO, radical product of polyunsaturated fatty acid, was significantly diminished by magnesium supplementation. A chain reaction of lipid peroxidation by reactive oxygen species impairs the myocardial cell membrane and consequently causes cardiac dysfunction. The antioxidative effects of magnesium may contribute to the prevention of cardiac dysfunction and β-adrenergic desensitization induced by excess ISO.

Magnesium deficiency is a risk factor of various cardiovascular diseases. Magnesium supplement therapy reduces coronary and systemic vascular resistance, which consequently increases coronary artery blood flow and improves cardiac index (2). When the external magnesium is depleted, the activity of the calcium pump on the myocardial cell membrane and SR is reduced, thereby resulting in impaired calcium handling (41). Excess ISO has been reported to transiently increase and subsequently decrease myocardial intracellular magnesium, suggesting that prolonged β-adrenergic stimulation itself facilitates the magnesium-extruding mechanism (30). Whether excess β-adrenergic stimulation affects intracellular and s-Mg2+ levels remains controversial (4, 15, 23, 47). Magnesium supplementation may break the vicious cycle of calcium handling and prevent cardiac dysfunction as well as β-adrenergic desensitization.

In conclusion, magnesium supplementation attenuated LV systolic and diastolic dysfunction induced by excess ISO and preserved responses to ISO in dogs. The mechanism may be facilitated, at least in part, by the inhibition of calcium overload and the suppression of free radical generation in myocardial cells by magnesium. Magnesium supplementation is an important addition to the treatment of patients with acute cardiac dysfunction, particularly in those with a highly activated sympathetic nervous system.

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