

ANTIHYPERTENSIVE EFFECT OF MAGNESIUM SULFATE (CORMAGNESIN®) AND ITS COMBINATION WITH FUROSEMIDE ON CONSCIOUS SPONTANEOUSLY HYPERTENSIVE RATS**Katerina D. Simeonova,
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Received: June 19, 2015**Revision received:** September 28, 2015**Accepted:** December 01, 2015**Summary**

The present study demonstrates the antihypertensive effect of magnesium sulfate (Cormagnesin®) and its combination with Furosemide on conscious spontaneously hypertensive rats (SHR) after intravenous infusion. Experiments were carried out on six groups of conscious male SHR (n=6). Under short anesthesia the rats were chronically instrumented for intravenous (i.v.) drug administration. The arterial blood pressure (AP) was measured by indirect tail method. Cormagnesin® was applied by i.v. infusion in doses of 5, 20 and 40 mg/kg; and furosemide (10 mg/kg) was applied intraperitoneally. Experimental results showed significant decrease of AP after i.v. infusion of 20 mg/kg Cormagnesin® as well as after application of the Cormagnesin® and furosemide combination. The hypotensive effects of 40 mg/kg Cormagnesin® and of furosemide were not significant. There was no significant difference between the antihypertensive effects of Cormagnesin® and its combination with furosemide but the combination showed much better hypotensive effect than Furosemide (p<0.05). Our study demonstrated the antihypertensive effect of magnesium sulfate on conscious SHR after i.v. application. Our results suggest that the antihypertensive effect of magnesium sulfate in the doses applied is not dose-dependent. Magnesium sulfate potentiates the antihypertensive effect of furosemide in SHR.

Key words: magnesium sulfas, antihypertensive effect, SHR

Introduction

Arterial hypertension is one of the most common diseases and is a major cause of death worldwide. It is a risk factor for the development of many cardiovascular complications and other diseases [1, 2]. Peripheral vascular resistance is one of the most important factors determining arterial blood pressure. Many experimental and clinical studies demonstrate that vascular magnesium levels are reduced in hypertension, and the vascular changes occurring lead to increase in peripheral vascular resistance and blood pressure [3-5]. Obviously, magnesium deficit is part of the pathogenesis of hypertension.

It is well known that loop diuretics, particularly

after prolonged use, lead to reduction of serum magnesium concentration, which in turn further aggravates cardiovascular diseases. This requires maintenance of its serum level to improve life quality.

Adequate experimental models have been developed for the experimental study of the antihypertensive effect of different substances. Spontaneously developing hypertension in rats is a suitable model. The Okamoto-Aoki SHR we used in our experiments were developed from Prof. Kozo Okamoto and D-r Kyuzo Aoki in Kyoto University, Japan in 1963 [6].

There are only few data in the literature concerning the influence of magnesium preparations on arterial blood pressure in rats with experimental hypertension and the concomitant use of magnesium preparations with other antihypertensive drugs in the treatment of hypertension.

The aim of this work was to study the antihypertensive effect of Magnesium sulfate (Cormagnesin[®]) and its combination with Furosemide on conscious spontaneously hypertensive rats (SHR) after intravenous infusion.

Materials and Methods

Experiments were carried out on 6 groups of conscious male SHR (n=6) employing procedures approved by the local ethic committee. The SHRs used were 14-18 weeks of age, with body weight 240-280 g. The animals were kept under standart conditions with 12 h light/darkness cycle with access to food and water ad libitum. To avoid stress, the rats were habituated to the experimental procedure by being handled for a few minutes on each of several days before the actual experiment.

Under short pentobarbital sodium (1 mg/100 g i.p.) and ketamin (5 mg/100 g i.p.) anesthesia rats were chronically instrumented for intravenous (i.v.) drug administration by inserting of a saline-filled (heparinized saline, 15 IU/ml) catheter (Portex polythene tubing; ID 0.28 mm; OD 0.61 mm) into the right iliac vein via the right femoral vein. The catheter was tunneled under the skin to exit at the back of the neck and the end was heat sealed. At the end of the operation rats received i.m. injection of gentamicin (8 mg/kg). Experiments were performed after 24-hour recovery period. During the i.v. infusion rats were kept in a Bollman- type

restrainer (KN-326, type III, Natsume, Japan) [7]. The arterial blood pressure (AP) was measured through the indirect tail-cuff method using UGO BASILE blood pressure recorder 8005. The cuff containing a highly sensitive piezoelectric crystal transducer embracing all three caudal arteries enabled this measurement. Before each measurement, rats were held at 37°C in a "hot plate"-heat-controlled box for 5-10 min in order to increase the regional blood flow in the tail artery, which allowed accurate AP measurement by the indirect method [8].

Systolic AP (SAP) [mmHg] and heart rate (HR) [beats per minute] were determined 30, 60 and 120 minutes after the i.v. infusion of the drugs. Changes (- %) of these parameters to the administered drugs were calculated. Data were statistically evaluated using one-way analysis of variance (ANOVA), followed by Tukey-Kramer multiple comparisons test. Results were expressed as mean S.E.M. and p-values less than 0.05 were considered statistically significant: ** p0.01; * p0.05. GraphPad InStat (GraphPad Software, Inc., USA) was used for the statistical analysis.

Groups and treatments

The animals were divided into groups as follows: group 1 (control group) received 0.9 % NaCl; group 2 – Cormagnesin[®] 5 mg/kg; group 3 – Cormagnesin[®] 20 mg/kg; group 4 – Cormagnesin[®] 40 mg/kg; group 5 – Furosemide 10 mg/kg; group 6 – Cormagnesin[®] 20 mg/kg and Furosemide 10 mg/kg.

The administered doses of Magnesium sulfate were 2.5%, 10% and 20 % from LD₅₀ based on toxicological studies by Mochizuki M., 1998 [9]. Cormagnesin[®] and NaCl were applied as 30-minute i.v. infusion – 0.4 ml/100 g; Furosemide was injected intraperitoneally (i.p. 0.1 ml/100 g). The rats from the group 5 received i.v. NaCl infusion (0.4 ml/100 g).

Drugs: Cormagnesin[®] 200 Inject (Magnesium sulfas) – Woerwag Pharma GmbH; Furosemide Sopharma[®] – Sopharma AG; Gentamicin Sandoz[®] (40 mg/ml 2 ml) – Sandoz GmbH; Heparin natrium 25000 IU/5 ml and Natrium Chloride Braun[®] (B Braun Melsungen AG). For anesthesia: Calypsol (Ketamine) – Gedeon Richter Ltd. Hungary and Pentobarbitalnatrium – Apoteksbolaget, Sweden.

Results

The SHR_s used showed basal levels of SAP from 170 to 200 mmHg. After the i.v. infusion of Cormagnesin[®] (5, 20 and 40 mg/kg) we obtained a hypotensive effect, which was maximal after the application of 20 mg/kg. The hypotensive effect of 20 mg/kg Cormagnesin[®] was significant after 60 and 120 minutes after its i.v. infusion ($p < 0.05$) and was significantly more pronounced in comparison to the hypotensive effect obtained 60 min after the application of Cormagnesin[®] in a dose of 40 mg/kg ($p < 0.05$) (Fig. 1 and Fig. 2).

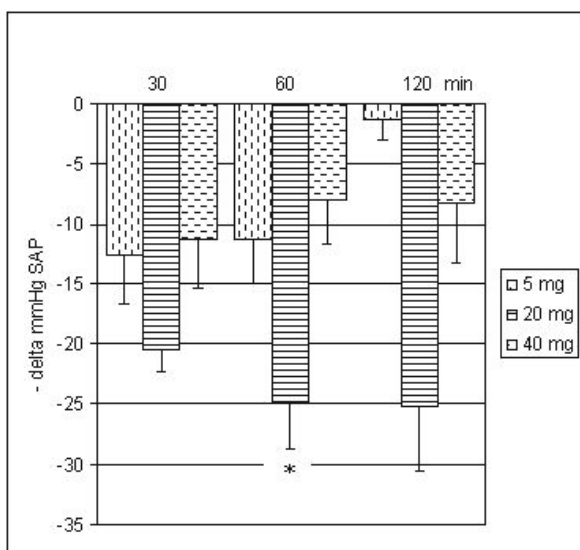


Fig. 1. Maximal changes [-mmHg] of systolic arterial pressure (SAP) after i.v. infusion of Magnesium sulfas (Cormagnesin[®] - 5, 20 and 40 mg/kg) on conscious male SHR_s (n = 6). Each column represents the mean \pm S.E.M. Asterisk represents the significant difference between the hypotensive effects after application of 20 and 40 mg/kg Cormagnesin[®] (* $p < 0.05$)

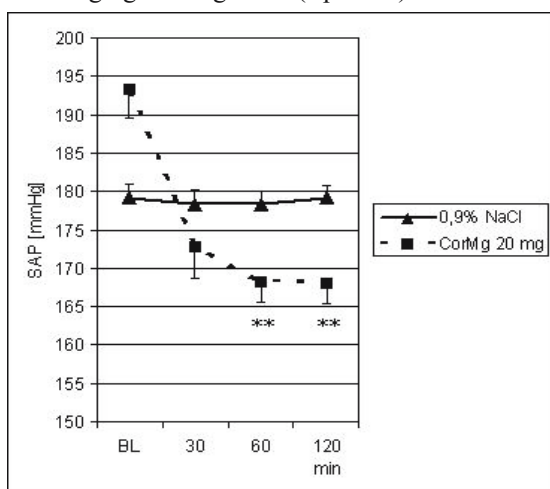


Fig. 2. Hypotensive effect of Magnesium sulfas - Cormagnesin[®] (CorMg: 20 mg/kg) after its i.v.

infusion on conscious male SHR_s (n = 6). The control group SHR received i.v. infusion of 0.9% NaCl. Systolic arterial pressure (SAP) is represented in mmHg. Each data-point represents the mean \pm S.E.M. Asterisks represent the significant difference of the SAP in comparison to its basal level (** $p < 0.01$)

In our experiments, Furosemide (10 mg/kg) showed hypotensive effect which was not significant but in combination with Cormagnesin[®] (20 mg/kg) the hypotensive effect was significant at the 60th minute of the AP measurement ($p < 0.05$) (Fig. 3). The decrease of the SAP after 20 mg/kg Cormagnesin[®] was significantly more pronounced in comparison to the hypotensive effect of Furosemide at the 30th ($p < 0.05$) and the 60th ($p < 0.01$) minute of the AP measurement (Fig. 3).

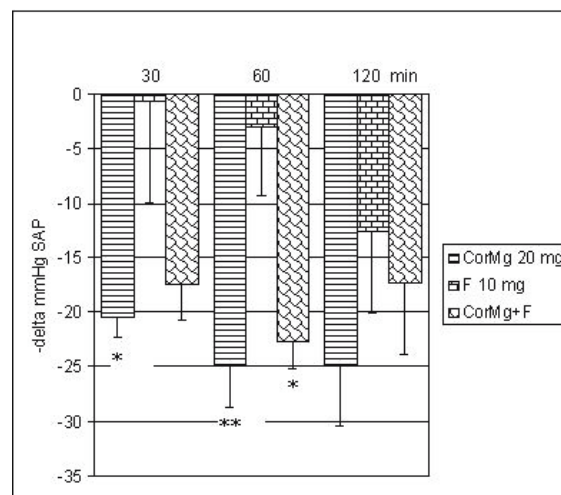


Fig. 3. Maximal changes [-mmHg] of systolic arterial pressure (SAP) after i.v. infusion of Magnesium sulfas (Cormagnesin[®] - CorMg: 20 mg/kg), Furosemide (F: 10 mg/kg i.p.) and their combination (CorMg+F) on conscious male SHR_s (n = 6). Each column represents the mean \pm S.E.M. Asterisks represent the significant difference of the hypotensive effects of Cormagnesin[®] and of its combination with Furosemide in comparison to the hypotensive effect of Furosemide at the 30th and the 60th minute (* $p < 0.05$; ** $p < 0.01$).

During our experiments we did not observe bradycardic effect after the i.v. infusion of Cormagnesin[®] and its combination with Furosemide. The values obtained for the HR were not statistically different for each group during the experiment.

Discussion

Many years ago it was demonstrated that the extracellular magnesium concentration influences blood flow, vascular reactivity and blood pressure in mammals. Hypomagnesemia leads to vasoconstriction and elevated blood pressure, whereas increased serum Mg^{2+} produces vasodilation and hypotension [10]. The role of magnesium in the processes determining peripheral vascular resistance was studied by many authors. Elevated levels of Mg^{2+} compete with influx of Ca^{2+} and stimulate Ca^{2+} -ATPase-mediated Ca^{2+} efflux, resulting in decreased intracellular free Ca^{2+} concentration and consequent reduced vascular contractility [4]. It

has been established that increased concentrations of extracellular magnesium also cause attenuation of agonist-induced vasoconstriction, increased responsiveness to vasodilators, increased production of endothelial-derived relaxant factors and decreased oxidative stress, whereas decreased concentrations cause increased vascular tone and vascular contraction, increased endothelial permeability, decreased endothelium-dependent vasodilation, enhanced vascular reactivity to vasoconstrictor agents, increased production of vasoactive agents and cytokines (e.g. ET-1, catecholamines), decreased production of endothelial-derived vasodilators (e.g. prostacyclin), and increased oxidative stress (Table 1).

Table 1. Influence of magnesium on vascular reactivity

Vascular effects of low magnesium concentrations	
• Increased endothelial permeability	[4]
• Decreased endothelium-dependent vasodilation	[11]
• Enhanced vascular reactivity to vasoconstrictor agents	[12]
• Increased vascular tone	[13-15]
• Increased production of vasoactive agents and cytokines (e.g. ET-1, catecholamines)	[16, 17]
• Decreased production of endothelial-derived vasodilators (e.g. prostacyclin)	[18]
• Increased oxidative stress	[11, 14, 19, 20]
Vascular effects of high magnesium concentrations	
• Increased responsiveness to vasodilators	[21]
• Decreased reactivity to vasoconstrictor agents, inhibition of vasoconstrictor agents production (e.g. ET-1)	[22-25]
• Increased vasorelaxation (e.g. prostacyclin) and increased production of endothelium-derived relaxing factors	[4, 13, 26-28]
• Decreased vascular contractility	[29]

Hypertension is due primarily to increased peripheral vascular resistance that is associated with functional, structural and mechanical alterations in peripheral vasculature. Magnesium plays an important role in these processes [4]. Indeed, many studies have demonstrated that vascular magnesium levels are reduced in hypertension [30]. Magnesium probably influences intracellular Ca^{2+} concentration, which is a major determinant of vascular smooth muscle contraction. The influence of magnesium on vascular tone and reactivity could also be due

to effects on Na^+/K^+ ATPase activity, which regulates Na^+ and K^+ transport, and to formation of cyclic AMP and cyclic GMP, which are important factors modulating vasodilation. Since intracellular Mg^{2+} influences many enzymes in signal transduction pathways involved in vascular contraction, low intracellular Mg^{2+} level could have important effects in vascular smooth muscle cell function in hypertension [4].

There are only few data in the literature about the influence of magnesium preparations on arterial blood pressure in rats with experimental

hypertension and about the concomitant use of magnesium preparations with other antihypertensive drugs. Most of the researchers use oral magnesium supplementation. Experiments on mineralocorticoid-salt hypertensive rats [31] show that dietary magnesium supplementation modifies blood pressure and cardiovascular function. Jin K et al. [32] reported that magnesium supplementation enhanced blood pressure reduction induced by angiotensin II receptor blocker losartan in rats with hypomagnesemic hypertension. Findings of Berthon N et al., [16, 22] have shown that magnesium supplementation in DOCA-salt hypertensive rats prevents blood pressure elevation, attenuates peripheral vascular resistance and inhibits endothelin-1 production. But the data about the preventive and therapeutic role of magnesium supplementation for hypertension remains controversial. There are also studies, which did not confirm these findings [33].

Our study demonstrated the antihypertensive effect of magnesium sulfate (Cormagnésin® 5, 20 and 40 mg/kg) on conscious spontaneously hypertensive rats after i.v. infusion. Our results showed a significant decrease of SAP after i.v. infusion of 20 mg/kg Cormagnésin®. The hypotensive effect, however, was not dose-dependent. The maximal effect we obtained was that after applying 20 mg/kg, and this effect was significantly more pronounced in comparison to the hypotensive effect received 60 min after the application of Cormagnésin® in a dose of 40 mg/kg. Our experiments also showed that the hypotensive effect of the loop diuretic Furosemide increased after its concomitant use with magnesium sulfate. There are several reports, which demonstrate that there is a reduction of the magnesium level in hypertension. It is also well known that loop diuretics lead to depletion of magnesium ions. Therefore, this combination can be recommended for antihypertensive treatment. There are also clinical studies, which report further blood pressure-lowering effect of magnesium treatment in patients already receiving diuretics [34].

Conclusions

Our study demonstrates the antihypertensive effect of magnesium sulfate (Cormagnésin®) on conscious SHR after i.v. infusion. Our

results suggest that the antihypertensive effect of magnesium sulfate in the applied doses is not dose-dependent. The maximal effective dose of magnesium sulfate in our experiments was 20 mg/kg i.v. The concomitant administration of Furosemide and Cormagnésin® potentiates the antihypertensive effect of Furosemide on SHR.

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