Magnesium and diltiazem relaxes phenylephrine-precontracted rat aortic rings

Mustafa Dogan, Recep O. Peker, Soner Donmez and Osman Gokalp

INTRODUCTION

The superiority of the internal mammary artery (IMA) over the saphenous vein as a conduit for coronary artery bypass grafting (CABG) has prompted the consideration of alternative arterial grafts such as the radial artery (RA) [1]. Several studies on the use of radial artery bypass grafts have documented excellent clinical results and satisfactory short- and mid-term patency rates on control angiography [2]. However, the main disadvantage of the RA is its tendency to spasm, which has been reported to be as high as 5–10% [3]. In its most severe form, the entire RA graft can close off (string sign) with the use of vasopressor therapy intraoperatively and postoperatively [4]. The precise mechanism of radial graft spasm is still unclear and strategies developed to treat it have not succeeded in preventing radial graft spasm completely. During routine surgical practice, vasodilator agents such as papaverine, nitroglycerin and three different types of calcium antagonists (nifedipine, verapamil and diltiazem) are frequently utilized to prevent spasms of the RA graft [5].

Magnesium is a physiological calcium antagonist that has been used for many years in obstetric practice for the treatment of eclampsia and pre-eclampsia whereas diltiazem is a well-known calcium-channel antagonist [6]. During cardiac surgery, magnesium in the prime solution of cardiopulmonary bypass (CPB) circuit prevents tachyarrhythmias and potassium flux. Previous studies have also shown magnesium to be effective in the treatment and prevention of coronary artery vasospasm in variant angina [7]. In addition to vasodilation, magnesium also has potentially analgesic effects, mediated via calcium channels and N-methyl-D-aspartate receptors [8].

In the literature there are few documented vasomotor studies on magnesium. We sought to evaluate the efficacy of magnesium as a vasodilator molecule in an experimental model and designed this aortic ring experiment. Since the effects of diltiazem are well known, we used it as a positive control agent. The aim of the current study was to determine the vasodilator effects of magnesium and diltiazem in vitro on phenylephrine-precontracted rat thoracic aorta and compare their vasorelaxation potencies.

MATERIALS AND METHODS

Animals

The study was approved by the institutional ethics committee and all of the experimental procedures were performed in accordance with European guidelines for the handling of animals.
Ten young adult female Wistar albino rats weighing 230–260 g were used in the experiments. The animals were provided with food and water *ad libitum*. Before sacrifice, rats were not administered any medical treatment or anaesthetic agent.

**Preparation**

The rats were sacrificed by cervical dislocation. After thoracotomy, the descending thoracic aortas distal to arcus were obtained, placed in a Petri dish filled with Krebs-Henseleit solution. The adhering perivascular fat and connective tissue was carefully removed and arterial segments were cut into ≈3-mm rings. Two triangle-shaped parallel steel strings were gently inserted into the lumen to measure tension alterations. One of the steel strings was fixed to the bottom of an *in vitro* chamber and the other was attached to a tension transducer, and tension measurement was performed using isometric transducers (TRI201, Panlab SA, Spain), an amplifier (ML118/D Quad Bridge, AD Instruments), interface PowerLab/4SP (ML750, AD Instruments) and computerized system with ProtoWin v 1.0 software. Special precautions were taken not to damage the endothelial layer or to overdistend the vessel during this procedure [9].

The artery rings were set up in an organ bath containing Krebs-Henseleit solution. The solutions were maintained at 37°C and gassed with 95% O₂ and 5% CO₂. The vessels were superfused with normal Krebs-Henseleit solution (composition in mmol/l; NaCl 119, KCl 4.7, CaCl₂ 2.5, MgSO₄ 1.5, NaHCO₃ 25, KH₂PO₄ 1.2 and glucose 11) under 1.5 g tension for 60 min to allow equilibration, which was found to be optimal for measuring the changes in tension. At the same time, the solution was changed every 15 min for fresh solution.

**Experimental protocols**

After the equilibration period, aortic rings were contracted with the alpha-1 adrenoceptor agonist, phenylephrine, (0.001 mmol/l). After stability of maximal contraction was observed in ≈10 min, the two groups of aortic rings were treated with consecutively increasing doses of either diltiazem (10⁻⁶, 10⁻⁵, 10⁻⁴, 0.0001, 0.001, 0.01, 0.1 mmol/l) or magnesium (0.1, 1, 2, 4, 10 mmol/l) with 5-min intervals. The relevant responses of aortic segments to diltiazem and magnesium were recorded.

**Drugs**

Magnesium sulphate 15% 10 ml was obtained from Biofarma (Istanbul, Turkey). Diltiazem hydrochloride 25 mg/5 ml was obtained from Mustafa Nevzat (Istanbul, Turkey). Phenylephrine and the salts for the Krebs-Henseleit solution were purchased from Sigma Chemical (St. Louis, MO, USA).

**Data and statistical analysis**

The concentration of the agonist that elicited a 50% maximal response (Eₘₐₓ) was designated as the EC₅₀, which was calculated by linear regression. The sensitivity of an agent was expressed as pD₂ (−log EC₅₀). The statistical calculations were performed with Graphpad Prism 4 software. All values were expressed as the mean ± SEM for each group. Determination of the significant differences between the groups was performed with an analysis of variance (ANOVA). When a significant difference was detected with ANOVA, Tukey’s multiple comparison test was used to reveal the significant differences. Differences with a P-value <0.05 were considered significant. Relaxation responses to magnesium sulphate or diltiazem were expressed as percentages of the phenylephrine-induced contraction.

**RESULTS**

After aortic rings were precontracted with phenylephrine (0.001 mmol/l), a mean peak contraction was recorded at 800 ± 200 mg. Both diltiazem and magnesium caused concentration-dependent relaxation. The mean relaxation with a maximum dose of diltiazem (0.1 mmol/l) was nearly total and robust.
The calculated EC50 values for magnesium and diltiazem were 4.064 mmol/l and 0.01035 mmol/l, respectively (P < 0.05) (Fig. 3). Also in terms of phenylephrine contraction, the sensitivity (−log EC50) of diltiazem (4.985) was significantly higher than that of magnesium (2.391) on contracted aortic rings (P < 0.05).

FIGURE 3: Relative log concentration and mean relaxation response graphs of diltiazem (n = 9) and magnesium (n = 9).

DISCUSSION

During cardiac surgery, vasospasm is a challenging problem for the patient and should be managed precisely. Calcium antagonists in combination with nitroglycerin are currently used for the prevention of graft spasm but they may cause systemic hypotension. Magnesium, a vasorelaxant and antiarrhythmic agent is used in CPB solutions and may have a role in the treatment of perioperative atrial fibrillation. However, the vasorelaxant potency of magnesium in an organ bath has not been studied in detail. We aimed to compare the vasorelaxant effects of magnesium and diltiazem on precontracted rat aortic rings.

In the current study, relaxation with magnesium was significantly limited compared with diltiazem. Nevertheless, in 1991, Janis and Triggle [10] demonstrated the complete inhibition of coronary artery rings contraction with magnesium. Differences between the results may be attributed to the changing material and methods or the vessel type used, the strain and the age of animals, the calculation methods for contractile responses and the integrity of endothelium.

The EC50 value for diltiazem was ~400 times lower than that of magnesium in the current study and the sensitivity expressed as −log EC50 was higher for diltiazem compared with magnesium. Therefore, diltiazem was found to be a more sensitive and more potent molecule than magnesium on precontracted rat aortic rings.

The maximum relaxation with magnesium was achieved at 10 mmol/l concentration. This value is much higher than normal blood magnesium levels in humans (1.1–1.2 mmol/l) and in rats (1.2 mmol/l) [11, 12]. Therefore, the magnesium concentration required for adequate vasorelaxation may be toxic to humans. However, Bryne et al. compared magnesium with verapamil in a clinical study and demonstrated that, during radial catheterization, magnesium is a more effective vasodilator with a reduced haemodynamic effect, and is equally effective at preventing radial artery spasm [8].

Different vasoconstrictor molecules such as serotonin, noradrenaline, prostaglandin F2α, barium chloride or potassium chloride may yield different EC50 values [13]. Because of its selective alpha-1 adrenoceptor agonist effect, we preferred to use phenylephrine for precontraction. The reported EC50 value for diltiazem was 0.56 ± 0.26 μmol/l by Gómez-Alvis et al. [14] on female rat aortic rings precontracted with 80 mM KCl. The nearly 20-fold difference in the EC50 value compared with that in our study might be related with different precontraction agents and techniques used in these two studies.

Magnesium not only changes cellular transmembrane potentials to decrease signal transduction, but also acts as a cofactor for Na-K ATPase to regulate cellular K+ levels and stabilize intracellular calcium. Low magnesium levels after CPB are a frequent finding.

Haemodilution, hormonal changes, stress-related epinephrine release, diabetes mellitus, postoperative use of digoxin, β-blockers and diuretics are thought to be contributing factors to the development of postoperative hypomagnesemia [11]. Both magnesium and diltiazem have various clinical applications. Diltiazem is widely accepted for the prevention of radial artery graft vasospasm. We suggested a potential role of magnesium in this clinical situation as an adjunct to diltiazem rather than being an alternative. For future work, we propose that magnesium supplementation into the radial artery preservation solution to prevent vasospasm may be a subject for investigation.

The relaxant effect of magnesium was clearly observed on different arteries, uterus, bronchus and trachea. The mechanism of this effect is by inhibition of calcium entry from the extracellular space to the intracellular space [15]; thus, magnesium has similar characteristics to calcium-channel blockers (diltiazem, verapamil etc.) that are used to inhibit vasospasm on arterial grafts during CABG. Magnesium is also used to inhibit vasoconstriction during the treatment of pre-eclampsia–eclampsia.

The cause of perioperative spasm remains unclear and is most likely due to a combination of various factors. It can be induced by several different mechanisms such as mechanical trauma, hypothermia, low cardiac output or vasoactive agents. We thought that magnesium could be a good physiological candidate together with diltiazem, a calcium-channel blocker to inhibit vasospasm on arterial grafts during CABG. Further studies on human radial artery and mammary artery rings using magnesium as an adjunct for vasospasm prevention and also measuring oxidative stress may be helpful to clarify the role of magnesium.

For the purpose of vasodilation, the use of magnesium can be more physiological than diltiazem. Since magnesium is an ion that is already present in the body, it may achieve more physiologically smooth muscle dilation. It would be better to use magnesium after measuring its benefit on humans in vitro studies.

Satisfactorily documented clinical results obtained by magnesium may encourage its clinical use similar to diltiazem for the management of vasospasm even though it showed limited benefit relative to diltiazem on phenylephrine-precontracted rat aortic rings.

Vena saphena magna, IMA and radial arteries are still in use during coronary surgery with different levels of sensitivity for vasospasm. Even native vessels of the patient may be subjected to vasospasm.
There are several limitations in this study. First of all, the use of female rats was a major limiting factor, because of cyclic variations of endocrine profile. However, during the study, female rats were the only gender available in our laboratory. We used the thoracic aorta which was a big conductive vessel because we did not have experience with a peripheral vessel. Therefore, the use of a peripheral vessel would better reflect a radial artery spasm model. The study is clinically applicable, but the laboratory results should be transferred very precisely to radial artery grafts performed in surgical practice. Also cardiac surgery induced oxidative stress is a matter of fact and should be taken into consideration here.

In conclusion, diltiazem is more effective in relieving vasoconstriction on rat aortic rings, however; magnesium could be a more physiological and promising candidate than diltiazem to inhibit vasospasm.

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REFERENCES


