Captopril Protects Against Myocardial Injury Induced by Magnesium Deficiency

Anthony M. Freedman, Marie M. Cassidy, and William B. Weglicki

We have previously reported that antioxidant drug intervention protects against magnesium deficiency–induced myocardial lesions. In the present study, Golden Syrian male hamsters were fed either a magnesium-deficient diet or a magnesium-supplemented diet. Animals from each group received sulfhydryl-containing angiotensin converting enzyme inhibitors: captopril, epi-captopril (a stereoisomer of captopril), and zofenopril* (arginine blend of zofenopril containing a free SH group); another group of animals received the non-sulfhydryl-containing angiotensin converting enzyme inhibitor enalaprilat. The animals were killed after 14 days, and their hearts were isolated for morphological and morphometric analyses. Hematoxylin and eosin-stained sections were examined by a computer image analysis system for a morphometric determination of the severity of myocardial injury. Captopril reduced both the density of lesions, from 0.32 to 0.08 lesions/(mm²) (p<0.01), and the area fraction of lesions, from 7.42×10⁻⁴ to 2.03×10⁻⁴ lesion area/(mm²) (p<0.01), as well as the degree of inflammatory infiltration around the blood vessels. Epi-captopril and zofenopril* were virtually equipotent to captopril, but enalaprilat afforded only slight (nonsignificant) protection. These results indicate that a significant component of the protective effect of captopril in this model was attributable to its sulphydryl moiety, rather than solely due to the inhibition of the angiotensin converting enzyme. These data further support our previous findings of possible free radical participation in cardiomyopathy due to magnesium deficiency. (Hypertension 1991;18:142-147)

In recent years, the correlation between magnesium deficiency and various cardiovascular diseases, such as coronary artery disease, cardiac arrhythmias, and ischemic heart disease,1-3 has become well established. In addition, magnesium deficiency has been associated with an increased severity of myocardial infarction,4 coronary and cerebral vasospasm,5,6 and hypertension.7,8 Neuromuscular disturbances, such as increased excitability and convulsions, have also been correlated with magnesium deficiency.9-11 Epidemiological studies have documented that a substantial segment of the population in the Western world is magnesium deficient12 and also that populations which dwell in hard water regions or consume a magnesium-rich diet are less prone to cardiovascular diseases and sudden death.13-15 In this country, magnesium deficiency has also been reported in various groups such as alcoholics and those receiving diuretics.16 Despite the importance of magnesium deficiency, mechanisms of its pathobiology remain unclear.

We have previously shown that drugs such as probucol and certain β-blockers provided protection against magnesium-deficiency–induced cardiomyopathy.17,18 These preliminary findings led to the suggestion of a possible role for free radical participation in this model. We proposed that magnesium deficiency may reduce the threshold antioxidant capacity of the cardiovascular system leading to an enhanced susceptibility to free radical injury. We tested this hypothesis with the natural antioxidant vitamin E,19 which substantially attenuated the development of magnesium deficiency–induced myocardial lesions. In the present study, we demonstrated the protective effects of a sulphydryl (SH)-containing angiotensin converting enzyme (ACE) inhibitor, captopril, on magnesium deficiency–induced cardiomyopathy. In addition, we tested epi-captopril, a stereoisomer of captopril, zofenopril* (arginine blend of zofenopril containing a free SH group), and enalaprilat (a non-SH-containing ACE inhibitor).

Methods

Captopril, its stereoisomer epi-captopril, and the arginine salt of zofenopril were obtained from the
Enalaprilat was kindly provided by Merck Sharp & Dohme Research Laboratories, Rahway, N.J. Three-week slow release pellets containing the above drugs were made by Innovative Research of America, Toledo, Ohio.

Golden Syrian male hamsters (80–90 g), purchased from Harlan Sprague Dawley, Inc., Haslett, Mich., were kept under a 12-hour light/dark cycle with food and deionized water ad libitum. The magnesium-deficient diet, containing less than 1 mmol/kg magnesium, was supplied by Teklad, Inc., Madison, Wis. Control animals received the identical diet supplemented with 100 mmol/kg MgCl₂. Animals from each group received captopril, 2.2 mg 3-week slow release pellets, as subcutaneous implants; a dose regimen within the clinical range of 1 mg/kg/day. Other animals from both groups received the same dose of epi-captopril and zofenopril*. For comparison, the non-SH-containing ACE inhibitor enalaprilat was administered at two doses (1.1 mg and 2.2 mg pellets). The recommended clinical range for enalaprilat is half that of captopril. On this basis, a direct comparison between captopril and enalaprilat effectiveness can be made with the lower dose of enalaprilat (1.1 mg). However, it has been demonstrated that the potency of enalaprilat is at least an order of magnitude greater than captopril.²⁰ The K₅ of the inhibitor-enzyme complex for captopril is 1.7×10⁻⁹ M, whereas enalaprilat has a value of 2×10⁻¹⁰ M. Experimentally, enzyme activity has been assayed for the effect of angiotensin I or of bradykinin, giving IC₅₀ values of 0.025 M and 0.001 M for captopril and enalaprilat, respectively. These studies were performed in similar rodents to the hamster: rats and guinea pigs, as well as other species such as rabbits, dogs, and primates. Thus, the ACE-inhibiting potency of enalaprilat at the above doses exceeded that of captopril. The choice of drug administration was made so that the animals would receive a constant dose throughout the time period of the experiment; also, it was simple to administer, and oral administration by adding the drugs to the animals’ feed may have resulted in substantial variations in dose, dependent on the feeding habits of the animals. At day 14 the animals were killed. The hamsters were anesthetized with ketamine (100 mg/kg) after ether induction. The chests were opened and the hearts were excised and rinsed in saline.

The severity of myocardial injury was determined by light microscopy. The hearts were sliced into four segments, and fixed in 4% formalin. Hematoxylin and eosin-stained sections, an average of 30 per heart (sections at different levels, not serial sections), were prepared for morphological and morphometric analyses. The morphometric analysis was made via the Bioquant (R & M Biometrics, Nashville, Tenn.), image analysis system. The Bioquant, an IBM interactive image analysis system, provides fast direct morphometric measurements from digitized images obtained via the (VCMTE) automated video counting and microdensitometry accessory program. Once a lesion was identified microscopically, the image was viewed on the monitor screen and its area was traced manually. Although the morphometric analysis was not truly blinded, since the operator was aware of the sample identification, he was not directly involved in the study and was not informed of the details of the experiments. In addition, since the magnesium-deficient animals yielded 40–60 individual lesions per animal, we believe that when repeated in five or more animals, sufficiently representative data are obtained. The values obtained for magnesium-deficient animals were consistent with our previous studies. In the present study, a minimum of seven animals was used for each set, with controls and deficient animals in each group. In particular, the magnesium-deficient and captopril group contained larger numbers of animals. Thus, we are confident that the data presented are a true representation of the relative myocardial injury occurring in each treatment group and any unconscious bias on the part of the operator was negligible. The results obtained are quoted as the density of lesions (the number of lesions per mm² of tissue) and the area fraction (the total lesion area per mm² of tissue). The numbers of animals used in this study were as follows: magnesium-deficient group, 22; magnesium-deficient+captopril group, 19; magnesium-deficient+epi-captopril group, 11; magnesium-deficient+zofenopril* group, 7; magnesium-deficient+enalaprilat (1.1 mg) group, 7; and magnesium-deficient+enalaprilat (2.2 mg) group, 7. Control groups of animals were used for each of the drug treatments.

Statistical analysis was determined by an analysis of variance of several means and the Tukey test was used for all paired comparison of means. Significance was considered at p<0.05. All values are expressed as mean±SEM.

Results

The animals subjected to the magnesium-deficient diet suffered a small nonsignificant weight reduction compared with the control animals. After 14 days on the diet, the control animals weighed an average of 105.7 g compared with 96.4 g, the deficient group weight average. We have previously established in this model that the serum magnesium levels were reduced almost a third, from 4.8±1.0 to 1.8±0.5 meq/l (p<0.05), over the 14-day period due to the magnesium-deficient diet.¹⁹ The administration of drugs, although protective to the cardiovascular system, did not result in any measurable changes in either weight or magnesium levels from those of the untreated magnesium-deficient animals.

Morphologically the magnesium-deficiency-induced myocardial lesions are characterized by focal necrosis and calcification, as previously described.²¹ Figure 1 shows light micrographs of representative sections from (panel A) a control animal and (panel B) a magnesium-deficient animal with several typical myocardial lesions clearly apparent, as indicated by
the arrows. Inflammatory infiltration and tissue injury around the blood vessel were also characteristic of this model; however, due to their irregularity, only the lesions were quantified.

Quantitative assessment of the lesions, determined by light microscopy, is shown in Figures 2 and 3. Figure 2 shows a comparison of the relative effectiveness of the two clinically used ACE inhibitors captopril and enalaprilat. Figure 2A shows that captopril reduced the density of lesions by 75% ($p<0.01$), whereas enalapril afforded no significant protection (9% and 31% protection for 1.1 mg and 2.2 mg doses, respectively). Similarly, captopril reduced the area fraction by 73% ($p<0.01$). Although nonsignificant, enalaprilat had a tendency to reduce the size of the lesions, as indicated by the reduction in area.
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FIGURE 2. Bar graphs represent a comparison between the relative protection against magnesium deficiency-induced myocardial lesions afforded by captopril (CAPT, 2.2 mg) and enalaprilat (ENAL 1, 1.1 mg and ENAL 2, 2.2 mg, respectively). Panel A: Density of lesions (no. of lesions/mm² tissue). Panel B: Area fraction of lesions (lesion area/mm² tissue).

*Magnesium deficiency vs. captopril (p<0.01) and captopril vs. enalaprilat (1.1 mg) (p<0.05).

fraction (25% and 45% for 1.1 mg and 2.2 mg doses, respectively) with only slight decrease in the density of lesions, particularly at the higher dose. Significance was obtained between captopril and enalaprilat (1.1 mg) for both the density and area fraction of lesions. These data suggest that only for higher doses of enalaprilat, there may be some protection due to ACE inhibition alone. However, this is relatively small compared with captopril, which exhibits substantial protection.

In Figures 3A and 3B, we present, separately for clarity, the data for the other SH-ACE inhibitors epi-captopril and zofenopril*. Both these drugs were found to significantly reduce both the density and area fraction of the injury almost as effectively as captopril. Epi-captopril reduced the density and area fraction by 69% and 66% (p<0.01), respectively, and zofenopril* demonstrated similar (62% and 53%; p<0.01) reductions. There were no significant differences discernible between the groups receiving captopril and either epi-captopril or zofenopril*.

FIGURE 3. Bar graph data demonstrate almost equal protection against magnesium deficiency-induced injury to captopril by both epi-captopril (EPI-CAPT) (2.2 mg) and zofenopril* (ZOFENOPRIL*) (2.2 mg). Panel A: Density of lesions (no. of lesions/mm² tissue). Panel B: Area fraction of lesions (lesion area/mm² tissue). *Magnesium deficiency vs. epi-captopril and zofenopril* (p<0.01).

Discussion

In humans, magnesium ranks as the fourth most abundant cation; however, in the intracellular compartment, only potassium is more plentiful. The free intracellular magnesium concentration is low and varies with time and subcellular structure. In the heart, the magnesium levels are relatively high, with the ventricular content greater than that in the atria. However, the myocardium is still highly vulnerable to magnesium deficiency, leading to cell degeneration, fibrosis, necrosis, and calcification. It is known that magnesium is essential in the regulation of enzyme activity and in the preservation of structure and function of many enzymes. In particular, Na⁺,K⁺-ATPase dysfunction results in a decreased intracellular potassium concentration, which can further cause a reduction in the membrane potential, as has been documented in both rats and hamsters following the induction of magnesium deficiency. This impairment will subsequently increase the intracellular sodium, stimulating the Na⁺-Ca²⁺ exchange and leading to a calcium overload and cellular necrosis. Alterations to the mitochondrial
enzyme activities may cause an uncoupling of oxidative phosphorylation, while the MgATP complex is a substrate for numerous enzymatic reactions. It has also been reported that in magnesium-deficient rats, blood glutathione levels are low, indicating a reduction in GSH biosynthesis. Recently, evidence has been reported of increased membrane fluidity during magnesium deficiency in erythrocytes. We have also recently demonstrated that magnesium-deficient erythrocytes are more susceptible to an applied free radical stress than magnesium-supplemented erythrocytes in hamsters.

In the present study, we demonstrated the protective effect of the SH-containing ACE inhibitor captopril. The chemical structure of captopril consists of a carboxyl group on proline with the carbonyl and thiol groups being essential for the activity. It has been shown that the inhibitory potency of this drug is enhanced by the presence of the SH moiety. In related studies, we have demonstrated that SH-containing ACE inhibitors possess antioxidant properties by their protection of endothelial cells in vitro against exogenously generated free radicals. We found that all the SH-containing ACE inhibitors possessed potent hydroxyl (·OH) radical scavenging ability in addition to moderate antiperoxidative properties. In comparison, two non-SH-containing ACE inhibitors, enalaprilat and lisinopril, were found to be ineffective. Thus, we concluded that the antiradical effects appeared to be primarily related to the free SH moiety. Other studies have also reported the ability of captopril to scavenge ·OH radicals, as well as exhibit general free radical scavenging activity.

In our present study, we also found that in contrast to captopril, the non-SH-containing ACE inhibitor enalaprilat exhibited only slight (nonsignificant) protection, indicating the ACE inhibition may not be the only mechanism of protection by captopril.

In addition to the relative protection afforded by the two clinical drugs captopril and enalaprilat, our hypothesis is further supported by the protection obtained with zofenopril* and epi-captopril. Zofenopril*, a sulfur-containing pro-drug, is a fivefold more potent ACE inhibitor than captopril when bioconverted to its active free sulfhydryl-containing form. In this model we found zofenopril* exhibited almost equal, but not enhanced, protection of the myocardium, suggesting that ACE inhibition is not the only mechanism of cardioprotection. This conclusion is further underscored by the equal protection afforded by the stereoisomer compound epi-captopril, a compound containing an SH moiety but essentially devoid of any ACE inhibitory activity.

In conclusion, our data clearly demonstrate the protective properties of captopril against magnesium deficiency-induced myocardial lesions. We have shown that the administration of captopril reduces the development of the cardiac lesions, both in their density and area fraction. However, the drug intervention had little effect on the animals' loss of weight or, as in previous studies, on the serum magnesium levels. We suggest that the action of captopril is more likely to be attributable to antioxidant supplementation, rather than a more direct amelioration of earlier events occurring in the development of magnesium deficiency. For instance, as blood glutathione levels are reduced during magnesium deficiency, these compounds, containing a free SH moiety, may act in part as a replacement therapy as well as ACE inhibitors. Thus, it appears that the protective effects of captopril in this model are related to both its antioxidant properties and to its ACE inhibition. These data further support our previous findings, suggesting that a free radical mechanism may be a significant etiologic factor in magnesium deficiency-induced myocardial injury.

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