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The changes in the antioxidant status of heart during experimental hypomagnesemia in balb/c mice

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Abstract

The present experiment was performed to assess if hypomagnesemia can influence antioxidant status in mice heart. The results could explain possibly a free radical theory of heart damage in magnesium deficiency. We used a rodent model of hypomagnesemia. The magnesium sufficient group received a standard diet whereas a magnesium deficient group received the diet containing a trace amount of magnesium. The activities of the most important antioxidant enzymes – catalase, glutathione peroxidase and superoxide dismutase were assessed in mice heart and liver in a time dependent manner, on the 10th and the 20th day of experiment. The level of magnesium in plasma of animals receiving the magnesium deficient diet dropped twice after the 8th day and four times after the 13th day and then reached a plateau value. The activity of catalase in heart in the magnesium deficient group increased gradually and was significantly ($P < 0.05$) elevated by 27% on the 20th day of experiment whereas the superoxide dismutase activity was significantly decreased by 17% on the 20th day. Glutathione peroxidase activity was insignificantly elevated. The alterations of antioxidant enzyme activities in the heart indicate cardiomyocytes's exposure to oxidative stress, which can be responsible for the cardiac lesions observed during hypomagnesemia.

Abbreviations: CAT – catalase; GPX – glutathione peroxidase; HYPO 10, HYPO 20 – study groups received hypomagnesemic diet for 10 and 20 days, respectively; ROS – reactive oxygen species; SOD – superoxide dismutase.

Introduction

Magnesium, the most abundand intracellular ion, activates many enzymes involved in the central pathways of cellular metabolism. Glycolysis, oxidative phosphorylation, nucleotide metabolism, and protein synthesis are magnesium-dependent processes. This element also influences physic-chemical properties of cellular membranes, thus is involved in establishing and maintaining intracellular electrolyte content (Langley *et al.* 1991; Bronzetti *et al.* 1995). The participation of magnesium in so many metabolic processes could explain why hypomagnesemia induces a large variety of pathologies. In animal studies, it has been shown that Mg-deficiency may affect many tissues, including heart-vessels and muscular system (Robeson *et al.* 1980; Bloom *et al.* 1988; Astier *et al.* 1996). According to electron microscope investigations of rat skeletal muscles it was confirmed, that the primary changes of cell ultrastructure occur in cellular membranes (Robeson *et al.* 1980; Astier *et al.* 1996). After 2 weeks of experimental hypomag-

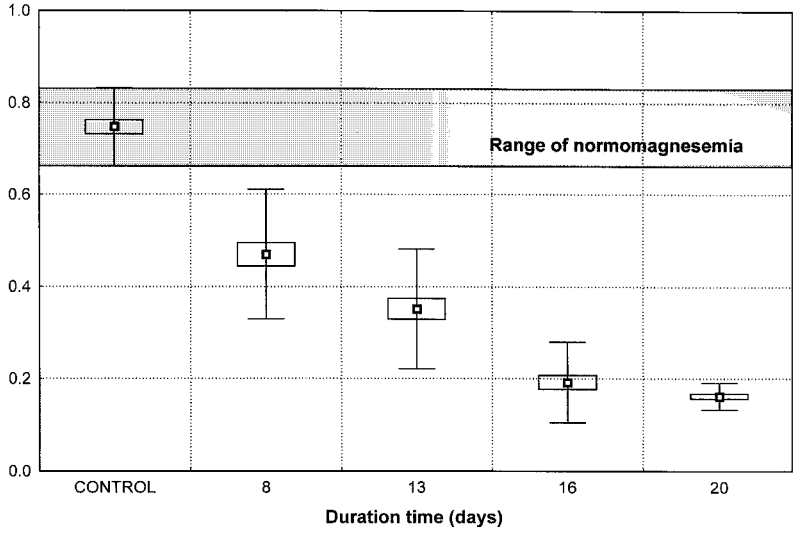

Fig. 1. The level of magnesium during the experiment (mmol/l).

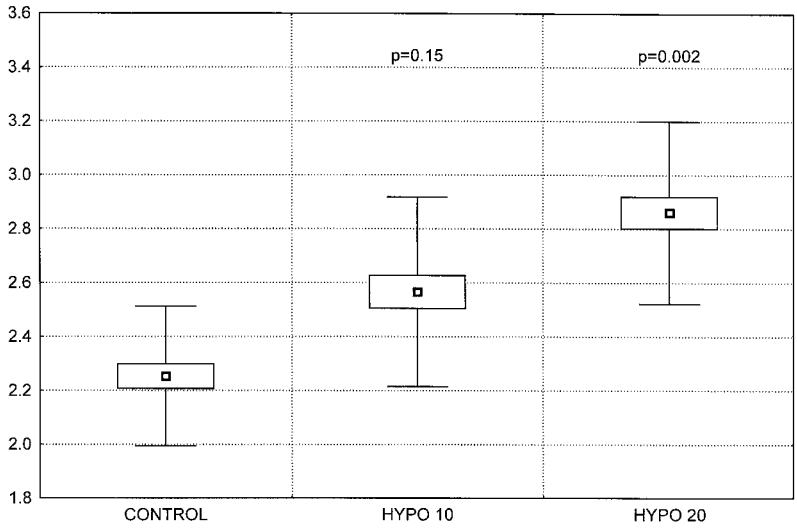

Fig. 2. Catalase activity in mice heart (U/mg).

nesemia there was observed swelling of mitochondria and disorganisation of endoplasmatic reticulum, which was probably caused by decreased activity of Mg-dependent ATPase, and changed permeability of membranes (Astier *et al.* 1996).

Although magnesium is a cofactor for multiple enzymes and its severe deficiency in animals induces oxidative damage, no specific link to antioxidant defence is known. Increased production of ROS *in vitro*, enhanced susceptibility to oxidative stress and changes in antioxidant status during hypomagnesemia were reported (Guenther 1991; Dickens *et al.* 1992; Freedman *et al.* 1992; Kramer *et al.* 1994; Mak *et al.* 1994; Kumar *et al.* 1997; Wiles 1997).

Catalase activity in heart tissue in hypomagnesemic groups increases in a time-dependent manner during hypomagnesemia: after 10 days was higher by 14% and after 20 days by 27% ($p = 0.002$). Similar tendencies have been observed in liver tissue, where the catalase activity was higher by 31% ($p = 0.0003$) after 20 days (Figures 2 and 3).

Observed formation of multifocalis damages (lesions) in heart during hypomagnesemia can be decreased by supplementation with vitamin E and other antioxidants (Freedman *et al.* 1990, 1991a, b;

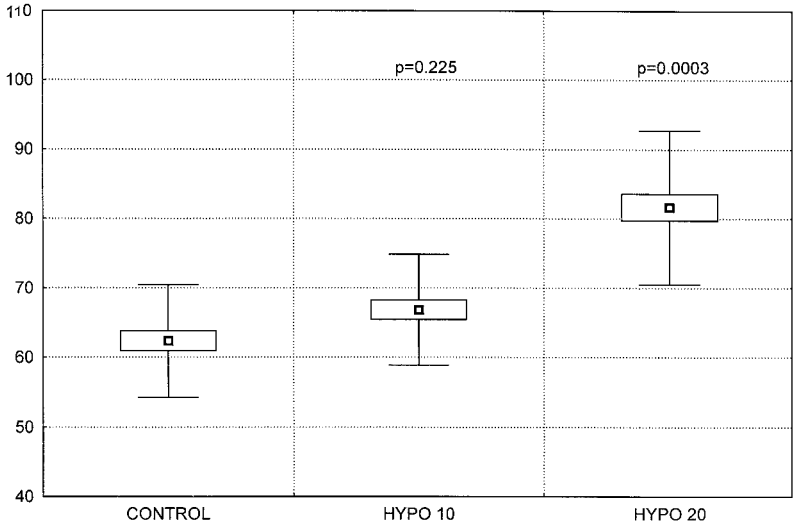

Fig. 3. Catalase activity in mice liver (U/mg).

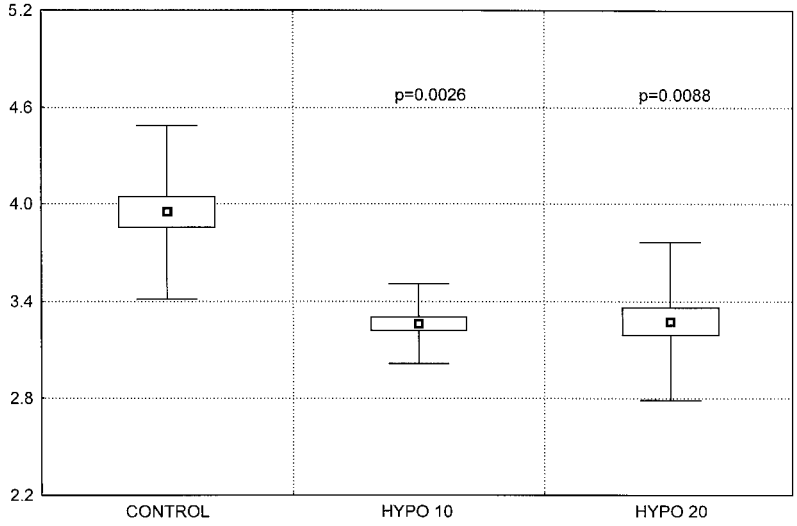

Fig. 4. SOD activity in mice heart (U/mg).

Weglicki *et al.* 1992a, 1993; Mak *et al.* 1994). Besides, some authors have observed increased level of lipid peroxidation products in animals suffering from magnesium deficiency (Guenther *et al.* 1992a, b; Calviello *et al.* 1994; Gueux 1995).

Magnesium is required for the glutathione synthesis and non-specifically in the processes of transcription/translation of the enzymes concerned with antioxidant defence (Whank *et al.* 1994; Rahman *et al.* 1999). It activates ATPase enzymes involved in homeostasis of intracellular electrolyte content. The calcium pump, that preserves intracellular calcium concentration is believed to be regulated by magnesium (Rude 1993). Excessive of intracellular free Ca^{2+} accumulation under oxidant stress has been implicated as a primary event in cell necrosis and injury (Rude 1993; Altura *et al.* 1996; Chakraborti *et al.* 1998).

The aim of our study was to investigate the mechanism by which magnesium deficiency promotes heart muscle damages. We examined the activity of the three main antioxidant enzymes: catalase (CAT, E.C. 1.11.1.6), superoxide dismutase (SOD, E.C. 1.15.1.1) and glutathione peroxidase (GPX, E.C. 1.11.1.9). In order to state if the observed alterations are specific for

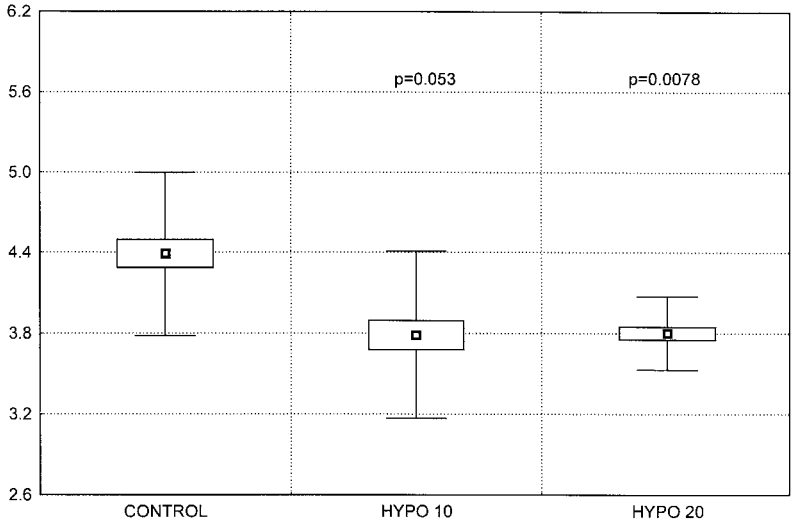

Fig. 5. SOD activity in mice liver (U/mg).

heart muscle, we also measured the same parameters in liver.

Materials and methods

In our experiments we used 8 weeks old male balb/c mice. The animals were divided into 3 groups (10 animals per group): a control group, which received normomagnesemic diet (Altromin, Germany) and deionized water ad libitum and the next two study groups received hypomagnesemic diet (Altromin, Germany) and deionized water ad libitum for 10 and 20 days. Animals were kept under standard laboratory conditions with 12:12 h light:night cycle. After the experiments animals were sacrificed by cervical dislocation. For further assessments heart and liver tissue were taken and stored at −75 °C.

Tissue samples were crushed in a temperature of liquid nitrogen and homogenized in phosphate buffer, pH 7.0. Homogenized samples were transferred to Eppendorf tubes and centrifuged at $10\,000 \times g$ for 10 min. The resulting supernatant was used for enzyme activity measurements and stored at −75 °C for further assessment.

SOD activity was measured using a spectrophotometric method (McCord *et al.* 1969; Flohe *et al.* 1984). In this method the inhibition of the cytochrome c reduction rate is monitored at 550 nm at 25 °C, utilising the xanthine/xanthine oxidase system as the source of O_2^-. SOD competes for superoxide and decreases the reduction rate of cytochrome c. One unit of SOD activity is defined as the amount of enzyme that inhibits by 50% the rate of cytochrome c reduction.

GPX activity was measured by following the rate of oxidation of the reduced form of glutathione, which was similar to the method previously reported (Paglia *et al.* 1967). The formation of oxidised glutathione was monitored by a decrease in the concentration of NADPH, measured at 340 nm and 37 °C, caused by the addition of glutathione reductase to the reaction mixture.

CAT activity was measured using a method reported by Aebi (Aebi 1984). This method uses the change in absorbance at 240 nm and 25 °C of a solution of 10 mM H_2O_2 in phosphate buffer, pH 7.0. The decrease in absorbance per unit time is a measure of the catalase activity.

The plasma level of magnesium was assessed by colorimetric method (Randox kit). Protein concentration was assessed by Lowry method (Lowry *et al.* 1951).

All enzyme activities were reported as unit per mg of protein.

Data are expressed as the means ± SE and ± SD. Differences between groups were analysed by Student's *t*-test and by ANOVA. A P value <0.05 was considered to be significant.

Results

The level of magnesium in blood of the balb/c mice, which received the diet with a trace amount of magne-

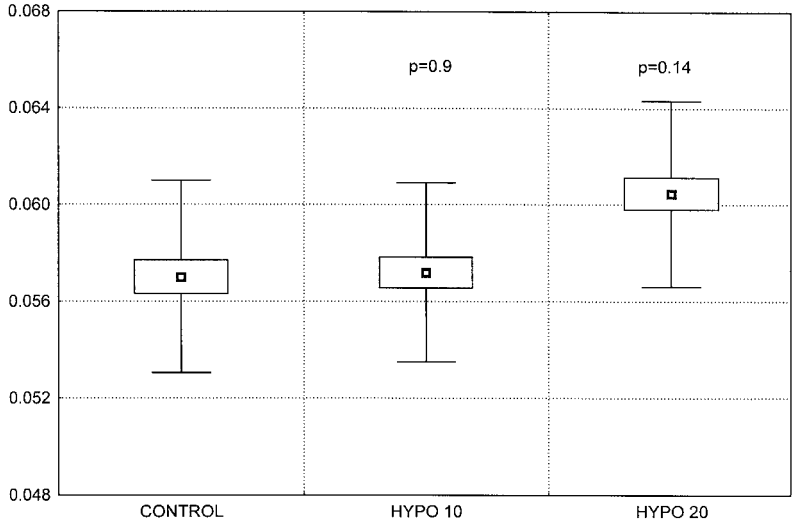

Fig. 6. GPX activity in mice heart (U/mg).

sium decreased systematically. After 8 days the level of magnesium was reduced by 37% and after 2 weeks by 75% (Figure 1).

Superoxide dismutase activity decreases in both examined tissues by 17% (heart, $p = 0.0088$) and 13% (liver, $p = 0.0078$) (Figures 4 and 5).

Different tendencies have been observed in the changes of GPX activity. In the heart tissue an increase in GPX activity was not significant whereas in the liver tissue GPX activity decreased by 29% ($p = 0.000044$) (Figures 6 and 7).

Discussion

The observed changes in the antioxidant status during hypomagnesemia indicate that both heart and liver tissue were exposed to oxidative stress. As the parameters of these changes, we used the activities of three main antioxidant enzymes. Oxidant stress occurs in two kinds of situations: with the increased production of free radicals, (not examined by us) and with the decreased level of antioxidants. In our experiment we observed a decreased activity of SOD and GPX in liver and a decreased activity of SOD in heart in comparison to the control group. At the current scientific knowledge and the stage of presented research it is impossible to explain the mechanisms of the reported changes. They can result from the influence of a decreased magnesium level on synthesis and activity of enzymes. Magnesium is required for each step concerned with the replication, transcription and translation of genetic information of mentioned enzymes. Also the activity of antioxidant enzymes can be dependent on this ion. Increased level of peroxidised products observed in oxidative stress during hypomagnesemia may provide potential explanation for the structural and functional alteration of examined enzymes (Rude 1993; Girotti 1998).

On the other site a decreased magnesium level can cause other changes, i.e. increasing production of proinflammatory cytokines, which then can stimulate production of free radicals and changes of antioxidant status (Guenther *et al.* 1991; Freedman *et al.* 1992; Weglicki *et al.* 1992b, c). The results from the other laboratories state that the blood level of substance P increases rapidly after the first week of hypomagnesemia. This leads to the reactions of excitation of macrophages, mast and endothelium cells (Weglicki *et al.* 1996). Activated cells secret then proinflammatory cytokines, IL-1, IL-6, TNF-alfa, histamin, which induce production of free radicals (She *et al.* 1989; Wozniak *et al.* 1989). The role of neuropeptide P is certainly important in pathogenesis of cardiac lesions. Administration of substance P inhibitor results in a reduction of these damages (Weglicki *et al.* 1994, 1996; Walsh *et al.* 1996; Kramer *et al.* 1997). Similar effects are caused by supplementation with vitamin E and other antioxidants, which can prove the presence of elevated production of free radicals during hypomagnesemia.

A decreasing activity of SOD in heart and liver tissue in our experiment could indicate that not all of the superoxide radicals were removed. The superoxide

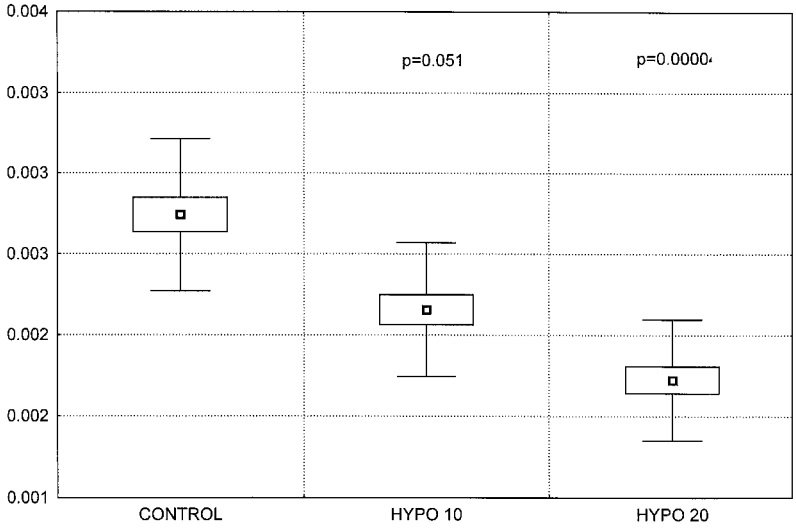

Fig. 7. GPX activity in mice liver (U/mg).

radicals can be then transformed in the presence of transition metals (i.e. Fe, Cu) to very toxic hydroxy radicals, which cause the destruction of DNA, proteins and lipids, and lastly induce cellular death through apoptosis and necrosis (Halliwell *et al.* 1998).

Elevated CAT activity in cells indicates a large increase in the amount of hydrogen peroxide, which is decomposed by two enzymes – CAT and GPX. However we can not state in what degree the processes of removal of hydrogen peroxide are efficient. There could exist a situation, where these two enzymes are not able to remove the excess of this radical, in spite of their increased activities.

An increased activity of CAT indicates that, besides water, a large amount of oxygen arises which can be incorporated to further radical transformations (Halliwell *et al.* 1998).

Results obtained by Kumar *et al.* (1997) demonstrated a decreased level of antioxidant defence regarding CAT and SOD in rat hearts. In our research CAT activity was increased, which indicates that different species respond differently to decreased magnesium level concerning the activity of this enzyme. The activity changes of GPX and SOD were similar in both species (Zhu *et al.* 1993; Kumar *et al.* 1997). The mechanisms that lead to the stated alterations should be explained in the further experiments.

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