Prophylaxis of Magnesium Deficiency by High Skeletal Magnesium Content in Adult Rats

Electrolyte Studies

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Zusammenfassung

An 30 männlichen Sprague Dawley Ratten (ca. 1 Jahr, 436 g Körpergewicht) wurde der Einfluß einer 14tägigen Vorphase mit entweder marginal dosiertem (270 ppm) oder hochdosiertem (8880 ppm) Magnesium-Gehalt (Mg-Gehalt) auf klinische und biochemische Parameter während einer 64tägigen Mg-Mangelphase (75 ppm) untersucht. Mg wurde in Form von Monomagnesium-L-aspartat hydrochlorid (MAH, Fa. Verla Pharm, Tutzing) verfüttert. Proben wurden nach der Vorphase (Tag 0) und an den Tagen 32 und 64 entnommen. 5 Tiere, die zu Beginn der Vorphase abgetötet wurden, dienten als Kontrollen. Die 50%-Häufigkeit der Erythembildung konnte durch das Mg-Loading bei den supplementierten Gruppen um im Mittel 9 Tage verzögert werden. [Mittelwert (Vertrauensbereich): 12. (10.–15.) Tag gegenüber 21. (19.–24.) Tag]. Plasma-Mg nahm in Abhängigkeit vom Angebot im Futter zu bzw. ab, erreichte aber bereits nach 32 Tagen ein Minimum. Es ergaben sich in den untersuchten Knochen (Femur, Becken, Schädel, Rippe) für Mg und Calcium (Ca) unterschiedliche Speicher- und Mobilisierungskapazitäten bis zu Versuchsende. Im Myokardgewebe konnten die für eine Hypomagnesiämie typischen zellulären Electrolytverschiebungen (Abnahme von Mg und Kalium, Zunahme von Ca und Natrium) nachgewiesen werden.
Ein Mg-Loading ist auch bei älteren Tieren möglich und erscheint insbesondere vor Phasen unzureichender Bedarfsdeckung oder gesteigerten Verbrauchs bzw. Verlusts angezeigt.

Summary

30 male Sprague Dawley rats rats (ca. 1 year old, 436 g b.w.) were pretreated for 14 days (d) with either marginal (270 ppm) or high doses of dietary Magnesium (Mg). Clinical and biochemical parameters over a 64 d period were investigated. Mg was fed as Monomag-nesium-L-aspartate hydrochloride (MAH, Verla Pharm). Samples of both groups were taken after prephase (d 0), and on d 32 and 64. Five animals sacrificed before prephase served as controls. 50%-frequency of erythema appearance could be postponed for about 9 d [mean (confidence interval): 12. (10.–15.) d vs. 21. (19.–24) d] in Mg supplemented groups. Plasma-Mg depended on dietary Mg content, but lowest concentration was already measured after 32 d. The bones investigated (femur, pelvis, cranum, costa) showed different storage and mobilization capacities for Mg and Calcium (Ca) till termination of experiment. In the myocardial tissue typical electrolyte shifts were present (decrease of Mg and potassium, increase of Ca and sodium). Results suggest the presence of Mg-loading in older rats. It is recommended particulary before periods of decreased supply or increased consumption, resp. loss.

Résumé

Trente rats mâles Sprague Dawley (âgés d'environ 1 an et pesant 436 g) ont reçu, à titre de prétraitement, une dose marginale (270 ppm) ou élevée (8880 ppm) de magnésium dans leur alimentation pendant 14 jours. Les paramètres cliniques et biochimiques ont été étudiés au cours d'une phase d'administration faible (75 ppm) de 64 jours. Le magnésium a été administré sous forme de chlorhydrate de L-asparte de monomagnésium (MAH, Verla Pharm, Tutzing, Allemagne). Des prélèvements sanguins ont été effectués après la phase de prétraitement (J O) et aux jours 32 et 64. Cinq animaux, sacrifiés avant le prétraitement, ont servi de témoins. Dans 50 % des cas, l'apparition des érythèmes a pu être ralentie en moyenne de 9 jours (moyenne (intervalle de confiance): 12 (10-15) jours contre 21 (19-24) jours) dans les groupes recevant la supplémentation. La magnésémie a été dose-dépendante et a atteint un minimum dès le 32ème jour. Jusqu'à la fin de l'étude, différentes capacités à capter et à stocker le magnésium et le calcium ont été observées dans les os étudiés (fémur, os du bassin, du crâne et côtes). Le tissu myocardique a, quant à lui, présenté des modifications électrolytiques typiques (diminution du magnésium et du potassium, augmentation du calcium et du sodium). Ces résultats indiquent la nécessité d'une supplémentation en magnésium chez le rat âgé, en particulier avant une période de carence ou d'augmentation des besoins résultant en une perte magnésique.

Introduction

In contrast to chronic administration of high doses of oral Magnesium (Mg), short-term loading of stores – especially before periods of increased Mg requirements or losses seem to be commonly accepted as Mg-therapy. Studies in young rapidly-growing animals have shown that filling of Mg stores in the skeleton depend directly on the dietary supply (*Blanc* and *Classen*, 1980; *Classen* et al., 1988). These animals were able to store up to twice as much Mg, if dietary Mg supply was excessive. The model of *Anast* and *Gardner* (1986) suggests stores in bones which keep Mg non-exchangeable and others which keep Mg exchangeable. Mobilization during Mg deficiency is possible as long as exchangeable or additional stored Mg exists. Thereby symptoms of acute

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(erythema, stress and noise sensitivity) and chronic deficiency appear significantly later, if stores were optimally filled. A chronic Mg deficiency induces pathological calcifications in animals. Most frequently affected are the kidneys (*Leder* et al. 1981, *Grimm*, 1990), but electrolyte disturbances are also found in other soft tissues (*Günther*, 1981; *Stein*, 1988).

Only little information is available about the lability of bone Mg in the adult resp. older rats. Intention of the following study was to investigate the extent of exchangeable Mg stores – loaded and unloaded – in older rats. Thereby Mg and Calcium (Ca) storage and mobilization capacities of four different bones were analysed. Further, secondary electrolyte shifts in soft tissues (heart, aorta, liver, and kidneys) during Mg deficiency were registered.

Methods

35 male SD rats (Interfauna, Tuttlingen) were randomly distributed to 7 groups of equal size. The animals weighed ca. 436 g (+/-18 g) and were about one year old. After two days of acclimati-zation samples of 5 animals (group I) were taken for baseline values. During the following prephase (14 d) groups II, III, and IV were treated with a diet containing only 270 ppm Mg, while the diet of groups V, VI, and VII was supplemented to 8880 ppm Mg. For preparation of the diet a Mg deficient pulverized diet containing 75 ppm Mg (Altromin C1035; Lage) was enriched with monomagnesium-L-aspartate hydrochloride (MAH, Verla Pharm, Tutzing). After prephase all animals were fed Mg deficient diet for 64 days (d). Fig. 1 depicts the experimental design. Diets and deionized water were available ad libitum. All experiments were performed under standard conditions. Samples were taken on d 0 (groups II and V), d 32 (groups III and VI), and d 64 (IV and VII): Plasma, four different bones (femur, pelvis, cranum, costa) and soft tissues (heart, aorta (5 cm), liver (lobus quadratus), kidneys). In addition appearance of ear erythemas – one of the typical symptoms of acute Mg deficiency – of each animal were registered. Animals were anesthetized with 6 mg/100 g b.w. pentobarbital (Ceva, Bad Segeberg). Samples were stored in the freezer until further preparation.

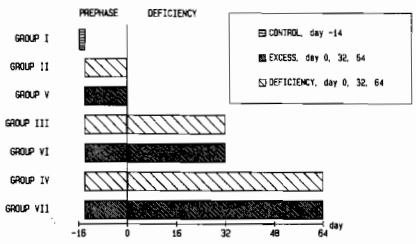

Fig. 1: Experimental design.

Analysis

After three days of lyophilization bones and soft tissues were ashed two times for 24 hours at 550°. 1 ml of HCl was added to each sample after each oven treatment. Electrolyte content was determinded in diluted plasma, bone and soft tissue samples via atomic absorption spectrophotometry (AAS, Perkin Elmer 1100).

Statistics

Normal distribution (Kolmogoroff Smirnow Test) and Analysis of Variance (ANOVA) was tested. With given homogeneity of variance Scheffe-Test was used for detecting significant differences ($p < 0.01$) between the treatments (*Schubö* and *Uehlinger*, 1984). Dose-effect-relations were proven via Chi^2 and Litchfield-Wilcoxon-Test (1948).

Results

Although for the supplemented groups (V, VI on VII) on day 0 a ca. 4 % lower body weight (b.w.) was registered, their b.w. was at termination (d 64) with 566 g about 3% higher than that of marginal fed groups (II, III, and IV). The high salt concentration was accompanied by rising drinking-water consumption. Both resulted in diarrheas, which seemed to be responsible for the lower b.w. Since diarrheas dissappeared after one week, it can be concluded that animals adapted to the high Mg dose. Presence of ear erythemas were already registered in marginal fed animals during the first week. These groups (III and IV) achieved 50%-frequency on d 12 (confidence interval 10.–15. d) while for MAH supplemented groups (VI and VII) d 21 (19.–24. d) was determined (fig. 2). Appearance of erythemas coincided with remarkable noise and stress sensitiviness. Erythemas changed to skin ulcera which healed during the deficiency period, but remained hairless.

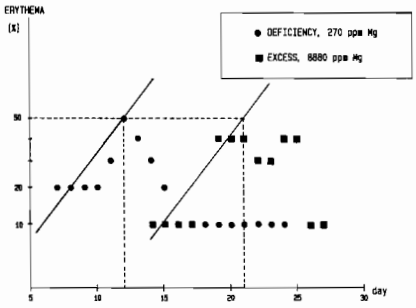

Fig. 2: 50%-Frequency of ear erythema appearance of rats with Mg loaded and unloaded stores.

Plasma

Plasma-Mg reflected the Mg content in the diet (fig. 3). Significant differences were determined between Mg-excess and marginal groups ($p < 0.001$). In all groups lowest Mg concentrations were already reached shortly after half-time of deficiency period.

With decreasing Mg in the plasma rising Ca concentrations were found. These findings are confirmed by other investigations using the rat model. We found a negative correlation between both parameters ($r = -0.62$, $p < 0.001$). Values for sodium (Na) and potassium (K) varied strongly, which made a clear interpretation difficult.

Bones

Fig. 4 depicts the Mg concentration of the four different bones investigated on three different time points of the

experiment. While femur started with a higher Mg content (220 mmol/kg), similar baseline values were analysed for pelvis, cranum, and costa (190 mmol/kg). During the supplementation period Mg of femur and pelvis increased less (1.4 %) compared to cranum and costa (10 and 18 %).
Significant differences were determined concerning the mobilization capacity. While on d 64 in the femur of group IV 135 mmol/kg was measured, Mg concentrations of pelvis (90 mmol/kg), cranum (87), and costa (77) contained on an average 85 mmol/kg. Mg concentrations of all bones correlated positively ($p < 0.001$) with the plasma-Mg.

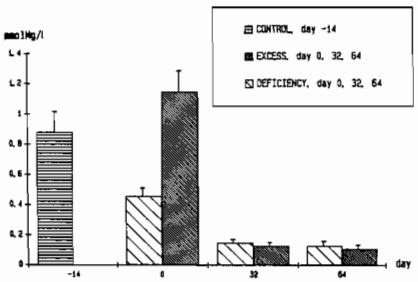

Fig. 3: Plasma-Mg concentrations of controls, deficiency, and excess groups at the different time points of the experiment.

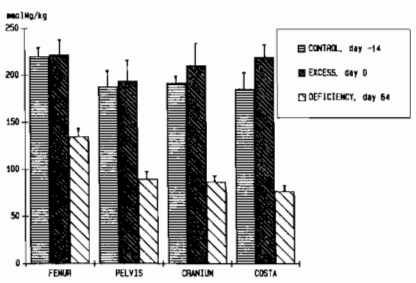

Fig. 4: Mg concentrations of four different bones of controls, group V (d 0), and group IV (d 64).

Concerning the Ca (fig. 5) highest concentrations at baseline were determined for the pelvis and cranum. No significant differences were obtained between the two treatments after prephase. More important, different effects of Mg deficiency on Ca content were observed. Ca concentrations increased in costa in both Mg-loaded and unloaded groups (+10 %) during deficiency. In the femur only excess groups increased Ca. constantly (+2 %). Marginal Mg fed animals remained uneffected. On the other hand pelvis and cranum mobilized Ca during Mg deficiency. Loss of Ca was higher in cranum of group IV (–12 %) than of group VII (–6 %), but equal amounts of Ca were deprived in pelvis (–10 %).

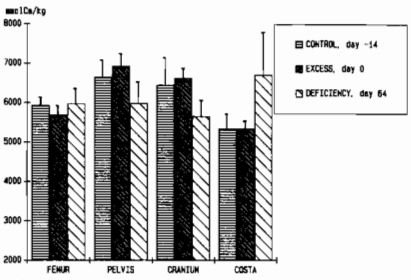

Fig. 5: Ca concentrations of four different bones of controls, group V (d 0), and group IV (d 64).

Soft Tissues

In the organs electrolyte shifts described in earlier studies by *Günther* (1981) were present. Most prevalent were the effects in the myocardial tissue. Surprisingly, no clear differences between the two dietary regimens could be detected on d 0. If all groups were pooled, Mg content decreased after a deficiency period on an average of 5 %. As expected rising Ca concentrations were prevalent (17 %) in all groups, but statistical analysis could not reveal significant differences between the groups. The altering Ca concentrations influenced strongly Na and K. After 64 d of deficiency Na concentrations in Mg excess and marginal loaded animals were on an average 53 % higher than control values. On the other hand K content decreased. Lowest concentrations were also determined at experimental termination. However, differences between dietary treatment could not be revealed. Hepatical tissue did not show a loss of Mg, but group VII had 19 % higher values than after loading. No clear interpretations were possible concerning hepatical Na and K content. Due to inconsistency and high standard deviations clear effects on electrolytes in aortical tissue could not be interpretated. However, rising Na characterized persistent Mg deficiency and Mg content correlated positively with K content ($p < 0.001$, $r = 0.62$). Although no homogeneity of variances could be determinded, means of Mg concentrations of the kidneys reflected mainly the content of the dietary intake. In marginal supplied animals Ca accumulation could be already observed by macroscopic inspection. However, calcification was not as excessive as observed in studies of *Classen* et al. (1988) in younger rats under similar conditions. Supplemen-tation seemed to prevent Ca enrichment, since in group VII (d 64) similiar values as control groups were measured. Values of Na and K were inconsistent and seem to be uneffected by the treatment. Tab. 1 lists percental changes in soft tissues after 64 d of Mg deficiency in comparison to controls (group I).

Discussion

The study was designed to create within 14 d two different rat populations: one with "latent Mg deficiency"

Tab. 1: Percental changes of electrolyte content in soft tissues of ca. 1 year old SD rats after 64 days of Mg deficiency (75 ppm Mg). Controls (group I) are 100 %. Rats were pretreated for 14 days either with marginal (270 ppm) or with excessive (8880 ppm) dietary Mg.

Parameter	Dietary Mg: 270 resp. 75 ppm	Dietary Mg: 8880 resp. 75 ppm
Heart Mg	– 4	– 6
Ca	+ 16	+ 17
Na	+ 49	+ 57
K	– 31	– 35
Liver Mg	– 2	+ 18
Ca	+ 33	+ 39
Na	– 11	– 8
K	+ 21	+ 7
Aorta Mg	– 25	– 26
Ca	– 34	– 28
Na	+ 44	+ 116
K	+ 17	– 3
Kidneys Mg	0	+ 2
Ca	+ 333	+ 18
Na	+ 21	+ 18
K	0	+ 16

and one with loaded Mg stores. Latent deficiency was present, since at d 0 plasma-Mg (0.46 mmol/l) was about half the values of control animals and all bones investigated responded with decreasing Mg content (% change, mean of all bones: –10 %). In contrast loaded animals had both elevated plasma-Mg (1.15 mmol/l) and bone-Mg (% change, mean of all bones: +8 %). The loading-treatment resulted in a retardation of acute deficiency symptoms for about 9 d. In young rats *Classen* et al. (1988) found under similar conditions a nearly identical time delay, but ear erythemas appeared about 5 d later and remained for a longer period in older rats. Studies of *Smith* and *Nisbet* (1968; 1972) confirm these findings.

The bones investigated showed different loading capacities for Mg. Surprisingly, femora of supplemented rats increased Mg content by only 1 %. Young rats increased Mg content after loading by 37 %. Mobilization during Mg deficiency was also less in adult loaded and unloaded rats (35 % and 39 % vs. 88 and 66 %). Such storage and mobilization capacities of young rats could not be determined in any bone investigated of adult rats. Costa bone showed greatest increase (+18 %) and deprivation (59 % in unloaded animals, 53 % in loaded). Similar high storage (+10 %) and mobilization (–55 %, resp. –44 %) were found in cranum bone, while in pelvis bone storage was less (+4 %), but mobilization good (–52 %, resp. 45 %). Obviously, exchangeable pools of Mg decrease with aging. These findings have been already described by *Alfrey* et al. (1974). Like in young rats, plasma-Ca concentrations increased with decreasing Mg. In bones only the costa responded with increasing Ca depositions, femora seemed to remain uneffected and cranum and costa deprived in Ca. These results demonstrate different metabolic responses of different bones during Mg-loading and deficiency.

In soft tissues clear effects of Mg deficiency, especially secondary electrolyte shifts, could be determined in myocardial tissue. Although Mg concentrations remained mainly uneffected by supplementation, Ca concentration increased already with latent deficiency (d 0), but excessive calcifications found by *Stein* (1988) in hypertensive rats could not be found. According to *Fleckenstein* et al. (1987) Mg deficiency causes an increased uptake of Ca into intracellular Ca stores (sarcoplasmic reticulum), which affects contractility of the heart muscle and thus energy homoeostasis. After 32 d of Mg deficiency clear decreasing K and increasing Na concentrations could be determined, but effects could not be delayed with MAH supplementation. The Mg deficiency-induced change in electrolyte contents is mainly produced by increasing cell permeabilitiy (*Günther*, 1981).

Results give evidence that older animals are able to create additional Mg stores in bones if treated 14 d with an excessive dietary Mg (MAH). Acute deficiency symptoms could be postponed for about 9 d. The high dietary MAH level was well tolerated after adaption. Initial diarrheas disappeared after one week although water consumption remained elevated.

Results also demonstrate that it is recommended for investigations concerning storage and mobilization capacities of Mg to perform additional electrolyte measurements of costa, pelvis or cranum bones in adult, resp. old rats, since exchange rate of Mg and Ca in femora was low. Mg deficiency by itself alone did not seem to be responsible for excessive Ca accumulation in hepatical, aortical and myocardial tissues, but significant electrolyte shifts could be determined. It remains to be investigated if additional risk factors like hypertension, stress, Ca supplements, or K deficiency at persistent Mg deficiency may aggravate electrolyte shifts in old rats.

Intake of Mg in elderly tends to be suboptimal. Decreased intestinal Mg absorption with progressive age provides evidence for the fact that Mg requirements may be higher in this population, especially when treated with diuretics, laxative agents, and/or heart glycosides. Supplementation of MAH is therefore recommended especially before periods of insufficient dietary supply or increased requirements, resp. loss.

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