The influence on magnesium deficiency on the development of gastric stress ulcers in rats*)

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Zusammenfassung

41 weibliche Sprague-Dawley Ratten (38 g) erhielten während 21 Tagen eine Mg-Mangeldiät (58 ppm Mg) und deionisiertes Wasser mit einem Zusatz von 2 bzw. 30 mmol Mg/l als $MgCl_2$ (Mangel- bzw. Kontrollgruppe). Nach 24 h Hungern wurde ein 18-h-Immobilisations-Streß erzeugt. — Die Mangeltiere zeigten vermindertes Wachstum, aber keine Beeinflussung des absoluten und relativen Thymus-Gewichtes; der Mg-Gehalt war im Knochen um 58 % erniedrigt, der Ca-Gehalt im Herzen um 50 % erhöht. Streß-Ulzera des Magens fanden sich in beiden Gruppen. Die makroskopische und quantitative mikroskopische Auswertung, die unter Blindbedingungen erfolgte, ergab übereinstimmend, daß Mg-Mangel die Ausbildung von Streß-Ulcera signifikant verstärkt.

Summary

Forty-one female Sprague-Dawley rats (38 g) were kept on a Mg-deficient diet (58 ppm Mg) and deionized water supplemented with 2 or 30 mmol Mg/l as $MgCl_2$ (= deficient and control group). After fasting for 24 hr, the animals were exposed to an 18-hr immobilization stress. — In the deficient group, gain of body weight was reduced, while thymus weights (abs. and rel.) were not influenced; bone Mg was decreased by 58 % and myocardial Ca was increased by 50 %. Gastric stress ulcers were found in both groups. Gross and quantitative microscopic evaluation, performed under blind conditions, revealed that the development of stress ulcers was significantly aggravated by Mg-deficiency.

Résumé

41 rats femelles Spraque-Dawley (38 g) ont été maintenus pendant 21 jours à un régime déficitaire en magnésium (58 ppm de Mg) et avec de l'eau deionisée, avec un complément de 2 ou 30 mmol/l de $MgCl_2$ respectivement (groupe déficitaire et groupe de contrôle). Après un jeûne de 24 heures, nous avons provoqué un stress par une immobilisation de 18 heures. Les animaux avec déficit ont présenté une croissance réduite, mais il n'y pas eu d'influence sur les poids absolu et relatif du thymus; la teneur en Mg dans l'os a été réduite de 58 %, la teneur en Ca dans le cœur accrue de 50 %. Des ulcères de l'estomac provoqués par le stress ont été trouvés dans les deux groupes. L'évaluation macroscopique et microscopique quantitative qui a été effectuée dans des conditions avec insu, a révélé de façon concordante que le déficit magnésique renforce significativement la formation des ulcères gastriques.

*) Results presented at the 3rd International Symposium on Magnesium, Baden-Baden, 1981.

Introduction

In numerous animal experiments and several studies in man it has been shown that the manifestation of acute stress reactions is profoundly influenced by the actual Mg status [for reviews see 2 and 5]: In general, the development of stress-induced disturbances is hastened by Mg deficiency, whereas preventive and protective effects can be observed after correcting the deficit or by supplying additional Mg. In previous studies we have demonstrated that in rats maintained on a standard diet and starved for 24 hr, significantly less frequent and less severe gastric lesions were found on exposure to an 18-hr immobilization stress when they were simultaneously treated with S. C. injections of Mg [6]. Therefore it seemed promising to study whether under comparable conditions Mg depletion would aggravate these alterations.

From the work of *Hans Selye* [7] it is known that the effects of different stressors are usually additive in proportion to their relative potency, while potentiating factors sensitize certain organs, e. g. the heart muscle, to acute stress, but exhibit only negligible „intrinsic" stressor activity or none at all. With this in mind we decided to carry out additional experiments to ascertain whether Mg depletion per se must be regarded as a stressor or as a potentiating factor, under the conditions of this study.

Material and methods

Female Spraque-Dawley rats (Süddeutsche Versuchstierfarm, Tuttlingen) were used in all experiments and kept under controlled conditions (22—24° C, 40—60 % relative humidity, 12-hr light/dark cycles and about 11 room-air changes/hr). They were given feed pellets (C 1035, Altromin GmbH, Lage) *ad lib.* unless otherwise indicated. According to the manufacturer, the semisynthetic diet was adjusted to the requirements of rats; only

the Mg content was altered as indicated in the respective experiments. — Demineralized water enriched with 0, 2 or 30 mmol/l Mg (as $MgCl_2$) was offered *ad lib.* In addition to the main experiment, two other series were performed.

Main experiment

A total of 41 rats with an initial body weight of 38 g were randomly allocated to 2 groups of 21 and 20 animals, and kept on a Mg-deficient diet (58 ppm Mg) during 21 days with demineralized water enriched with 2 mmol/l Mg (= deficient group) or 30 mmol/l Mg (= control group). The animals were starved during 24 hr on day 22 and subjected to immobilization during 18 hr on day 23. All parameters listed below were taken from each animal.

Additional experiment No 1

Thirty-seven rats with an initial body weight of 42 g were randomly allocated to two groups. Nineteen animals were maintained on a Mg-deficient diet (58 ppm Mg) and demineralized water *ad lib.* during 13 days. Since 4 animals died of convulsions, the survivors received 2 mmol/l Mg beginning on day 14. — The controls (n = 18) received the same diet, but 30 mmol/l Mg in their drinking water. Before being sacrificed on day 23, groups of the same size were starved for 18, 24 and 42 hr. — All stomachs were grossly inspected. Histology was performed in 3 rats chosen randomly from each group. The weight of the thymus glands was determined in each rat and calculated with reference to 100 g of body weight.

Additional experiment No 2

Eighty-five rats with an initial body weight of 68 g were randomly allocated to 8 groups of 10 to 15 animals and kept on a diet with about 160, 260, 360, 560, 950, 1540, 3500 or 6850 ppm Mg (added as magnesium aspartate hydrochloride) and demineralized water *ad lib.* during 26 to 27 days. The animals were starved for 24 hr before being sacrificed. The stomachs were grossly inspected. The weight of the thymus glands was determined and calculated with reference to 100 g of body weight.

Immobilization stress was produced during 18 hr by strapping the animals in the prone position to a board by means of adhesive tape attached to the paws. Immediately after this period, the animals were sacrificed by decapitation. The *stomachs* were excised, opened along the greater curvature using fine scissors, rinsed with physiological saline and carefully inspected for ulcerations (which never occurred in the non-glandular forestomach). The severity of the lesions was estimated in terms of an arbitrary scale ranging from 0 to 4:

0 = no lesions
1 = 1 to 2 small punctiform ulcers
2 = 3 to 5 small punctiform ulcers
3 = several large longish ulcers
4 = multiple large longish and small ulcers

Histology

After coding, 10 sections of 6 µm were taken from each corpus at distances of 0.5 to 0.6 mm and stained according to *Goldner* [3]. Evaluation was performed under blind conditions at 100-fold magnification, taking into account per corpus:

a) the severity of all lesions detectable in the 10 sections in terms of an arbitrary scale ranging from 0 to 4 (see above);
b) the sum of the diameters (in µm) and depths (in µm) of all ulcerations encountered in the 10 sections, which were measured by means of a calibrated ocular.

For *electrolyte determinations* the ventricles were excised from the still beating heart, cut open and quickly rinsed in bidistilled water. The right femur was also removed. The tissues were dried at 110° C during 48 hr, weighed, ashed at 500° C and prepared for electrolyte determinations (AAS, Perkin Elmer 432) in the usual way. All values were calculated on the dry weight.

Statistical evaluation was done using the modified Mann-Whitney U-test [4], the T^2-test described by *Hotelling*, a multivariate t-test [7], or Student t-test.

Results

A. Main experiment: As compared with the controls, gain in body weight was slightly reduced in the animals kept on the Mg-deficient diet (148 ± 10 g versus 127 ± 11 g on day 21; $p < 0.001$; correspondingly, food consumption per rat during the whole observation period was also decreased (207 g versus 193 g/rat) in this group, whereas water consumption was not altered. Typical erythema of the ears, paws and tails appeared during days 6 to 17 in the Mg-deficient group, but neither convulsions nor mortality occurred. The relative thymus weights did not differ signifi-

cantly in both groups (0.402 ± 0.066 versus 0.396 ± 0.073). Bone-Mg was highly significantly decreased by 58 % in the Mg-deficient group (5.16 ± 0.30 versus 2.17 ± 0.23 mg Mg/g), whereas cardiac Ca-content was increased by 50 % in these animals (0.191 ± 0.021 versus 0.287 ± 0.183 mg Ca/g). Stress ulcers were seen in rats of both groups. The incidence and severity of gastric lesions was however highly significantly increased in the Mg-deficient group, taking into account both the results of the gross and of the microscopical evaluation (see Table 1; p. < 0.001).

In accordance with these observations, the quantitative evaluation of diameters and depths also revealed a highly significant aggravation of gastric ulcerations by Mg deficiency (p. < 0.001, see Table 2).

B. Additional experiment No. 1: When weanling rats were fed the severely Mg-depleted regimen for 13 days — inducing convulsions and 21 % mortality — the survivors (receiving 2 mmol/l Mg in their drinking-water from days 14 to 23) gained less body weight (−50 g) than controls, and food intake as well as water consumption were reduced. A significant involution of the thymus had taken place, as shown by decreased weights (0.43 ± 0.05 versus 0.38 ± 0.04; p < 0.01). After different periods of starvation (18 to 42 hr), no lesions were detectable in the gastric mucosa of controls on gross and histological evaluation. On the other hand, ulcerations were seen in the Mg-deficient group, regardless of the starvation period. The gross findings were confirmed under the microscope and are summarized in Table 3.

C. Additional experiment No. 2: When rats were fed different Mg concentrations for nearly 4 weeks — including groups with suboptimal supply — only some erythema developed in animals receiving the 160-ppm and 260-ppm Mg diets. Neither convulsions nor mortality occurred and gain in body weight as well as food and water consumption were of the same order. After 24 hr of starvation the thymus weights ranged between 0.28 and 0.31 (NS) and no gastric lesions were detectable on careful gross examination.

Discussion

The results presented in this paper as well as our earlier findings [6] were obtained by qualitative gross examination of the gastric mucosa of rats and confirmed by quantitative determinations of the size of the lesions, under blind conditions. This would suggest that the development of stress ulcers is hastened by Mg deficiency on the one hand and inhibited by pharmacological doses of Mg, on the other hand, at least in the rat. Since Mg containing antacids are widely used with convincing success in human therapy, our animal data would appear to establish that these drugs, besides buffering excess acid, might also influ-

Tab. 1: Incidence and severity of gastric ulcerations (0 to 4) on gross and microscopical evaluation in rats exposed to Mg-deficiency, 24-hr starvation plus immobilization stress.

Group	Severity of ulcerations				
	0	1	2	3	4
a) *Gross evaluation*					
Controls	6/20	5/20	7/20	2/20	0/20
Mg deficiency	1/21	3/21	11/21	2/21	4/21
b) *Microscopical evaluation* (blind conditions)					
Controls	1/20	4/20	6/20	8/20	1/20
Mg deficiency	1/21	0/20	1/21	12/21	7/21

Tab. 2: Quantitative evaluation (mean ± standard error) of gastric ulcerations; for details see Table 1.

Group	n	Total diameter (μm)	Total depth (μm)
Control	20	2295.6 ± 464.7	1343.4 ± 272.6
Mg deficiency	21	4965.4 ± 936.9	2688.6 ± 405.5

Table 3: Incidence and severity of gastric lesions (0 to 4) in rats exposed to severe Mg depletion plus starvation

Group	Severity of ulcerations				
	0	1	2	3	4
Controls	18/18	0/18	0/18	0/18	0/18
Mg deficiency	9/15	3/15	2/15	0/15	1/15

ence the actual Mg status, either by correcting a pre-existent deficit or/and by inducing mild hypermagnesemia, which has been shown to exert beneficial effects in diverse stress models [for review see 2]. *Bowen* and *Fleming* [1] have studied several blood electrolytes, the acid-base status and other biochemical parameters in soldiers with and without acute stress ulcers. However, no significant correlation was encountered between these parameters and the severity of gastric lesions. Unfortunately, Mg was not considered in these studies; nevertheless, it might play an important role in the development of stress ulcers in man, too.

The present experiments also show that severe Mg depletion producing convulsions in young rats must be regarded as a potent stressor leading to an involution of the thymus and to the development of gastric ulcers. However, in the main experiment only a moderate degree of Mg deficiency was induced without central nervous disturbances. In these animals the involution of the thymus gland by immobilization stress was not aggravated, in contrast to a marked Ca overload of the heart muscle and the occurrence of more and larger gastric ulcerations. Taking also into consideration that no mucosa damage nor an involution of the thymus could be observed in rats with mild, marginal Mg deficiency, it seems reasonable to suppose that the Mg deficiency induced in the main experiment must rather be regarded as a potentiating factor than as an additional stressor, with respect to the gastric mucosa.

Recently, the role of Mg in the gastrointestinal tract has been reviewed by *Tansy* and *Kendall* [9]. Although the data presented there do not offer a direct interpretation of our results, two facts should be pointed out:

a) early or late stages of Mg deficiency in rats are not associated with elevations in titrable gastric acid output, and

b) Mg can inhibit Ca-induced acid secretion in man.

The first statement supports our assumption that moderate and marginal Mg deficiency does not affect directly the gastric mucosa, and the second finding points to the possibility that via stress or increased catecholamine levels, Ca fluxes might be elevated, which could trigger ulcerogenic pathomechanism(s) on the one hand and which might be modulated by actual Mg status, on the other. More detailed studies concerning these questions are in progress.

Acknowledgement

The authors thank Mrs *D. Leibfried* for skillfully preparing the histological sections and Dr. *F. Laibig* (Institut für angewandte Mathematik und Statistik, Universität Hohenheim) for supervising the statistical evaluation of the results.

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