Increased Lipid Peroxidation in Rat Tissues by Magnesium Deficiency and Vitamin E Depletion

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Zusammenfassung

Männliche Wistar-Ratten erhielten eine Kontroll-, magnesiumarme, Vitamin-E-reduzierte und eine magnesiumarme, Vitamin-E-reduzierte Diät ad libitum für 23 Wochen. Anschließend wurde der Vitamin-E- und Malondialdehyd(MDA)-Gehalt als Indikator für die Lipidperoxidation in Leber, Niere, Herz, Muskel, Gehirn und Milz bestimmt. Mg-Mangel und in stärkerem Maße Vitamin-E-Verarmung verringerten die Vitamin-E-Gehalte in Plasma und in den Geweben. Die Vitamin-E-Verarmung erfolgte gemäß der Reihe: Leber >> Plasma > Herz > Muskel = Niere > Milz > Gehirn. Der MDA-Gehalt der Gewebe wurde durch Mg-Mangel und Vitamin-E-Verarmung erhöht. Die Zunahme des MDA-Gehaltes durch Vitamin-E-Verarmung wurde durch Mg-Mangel beträchtlich erhöht. Als Ursache kommt die Fe-Zunahme in den Geweben durch Mg-Mangel in Frage. Der Anstieg des MDA-Gehaltes erfolgte gemäß der Reihe: Leber >> Niere > Herz > Muskel = Milz = Gehirn. Wenn die Gewebehomogenate einem oxidativen Streß durch Inkubation mit Ascorbat oder Fe/Ascorbat ausgesetzt wurden, stieg der MDA-Gehalt drastisch an, besonders bei den Vitamin-E-verarmten Ratten. Dies zeigt die Rolle von Vitamin E als Radikalfänger. Nur in den Vitamin-E-verarmten Herzen verstärkte Mg-Mangel die durch oxidativen Streß hervorgerufene MDA-Bildung. Die Kombination von Mg-Mangel und Vitamin-E-Verarmung ist daher ein Risikofaktor für oxidativen Streß, der im Herzen bei Ischämie-Reperfusion eine Rolle spielen kann.

Summary

Male Wistar rats were fed control, Mg-deficient, vitamin E-reduced and Mg-deficient-vitamin E-reduced diets ad libitum for 23 weeks. Thereafter, vitamin E and malondialdehyde (MDA) content as an indicator of lipid peroxidation were measured in liver, kidney, heart, muscle, brain and spleen.

Mg deficiency and to a greater extent vitamin E-restricted nutrition reduced vitamin E contents in plasma and tissues. Vitamin E depletion occurred according to the series: liver >> plasma > heart > muscle = kidney > spleen > brain. MDA content of the tissues was increased by Mg deficiency and by reduced vitamin E nutrition. The increase in MDA content by vitamin E depletion was effectively enhanced by Mg deficiency. The reason for this may be the increase of Fe content in tissues by Mg deficiency. The increase in MDA occurred according to the sequence: liver >> kidney > heart > muscle = spleen = brain. When applying oxidative stress to the tissues by incubating the homogenates with ascorbate or Fe/ascorbate, MDA content was drastically increased particularly in the vitamin E-depleted groups, indicating the role of vitamin E as a scavenger of oxygen free radicals.

Only in the vitamin E-depleted hearts was MDA formation due to oxygen stress considerably enhanced by Mg deficiency. Thus, the combination of Mg deficiency and vitamin E reduction represents a risk factor to oxygen stress which may play a role in the heart during ischemia-reperfusion.

Résumé

Des rats Wistar mâles ont reçu pendant 23 semaines une alimentation témoin, une alimentation pauvre en magnésium, une alimentation pauvre en vitamine E ou une alimentation carencée à la fois en magnésium et en vitamine E. Nous avons ensuite déterminé la concentration de vitamine E et de dialdéhyde malonique (DAM), lequel reflète la peroxydation des lipides, dans le foie, les reins, les muscles, le cerveau et la rate. La carence en magnésium et surtout la carence en vitamine E ont induit une diminution des taux plasmatiques et tissulaires de vitamine E. L'appauvrissement en vitamine E suivait la séquence: foie >> plasma > coeur > muscles = reins > rate > cerveau. le déficit en magnésium et la carence en vitamine E ont induit une augmentation de la concentration tissulaire de DAM. L'augmentation de la concentration de DAM induite par la carence en vitamine E était fortement accentuée par la carence en magnésium. Ce phénomène peut peut-être s'expliquer par l'augmentation de la concentration tissulaire de fer induite par la carence en magnésium. L'augmentation de la concentration de DAM suivait la séquence: foie >> reins > coeur > muscles = rate = cerveau. Quand les homogénats tissulaires ont été exposés à un stress oxydativ par incubation avec de l'acide ascorbique ou du fer/acide ascorbique, la concentration de DAM a considérablement augmenté, surtout chez les rats carencés en vitamine E, ce qui prouve le rôle de la vitamine E comme capteur de radicaux. La carence en magnésium n'a accentué la synthèse de DAM induite par le stress oxydatif que dans les coeurs carencés en vitamine E. Autrement dit, la coexistence d'une carence en magnésium et d'une carence en vitamine E constitue un facteur de risque pour le stress oxydatif, qui peut jouer un rôle dans le coeur lors de l'ischémie - reperfusion.

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Introduction

In preceding experiments Mg deficiency increased lipid peroxidation (LPO) and reduced vitamin E content in plasma and tissues [1, 2]. LPO was highest when rats were fed an Mg-deficient diet in combination with a reduced vitamin E content. However, the steady state level of MDA content in the tissues was very low [1, 2]. This result might have been caused

1. by permanent metabolism and renal excretion of MDA and
2. by the action of antioxidative mechanisms (vitamin E, vitamin C, superoxide dismutase, catalase, peroxidase, glutathione peroxidase, glutathione).

Therefore, in order to test the pathogenetic risk of oxygen radicals and the protective antioxidant capacity of the tissues we subjected the tissues to oxidative stress by incubating tissue homogenates in vitro with Fe/ascorbate, which increases the rate of oxygen radical formation and LPO (for lit. see [3]). Fe/ascorbate was chosen since in Mg deficiency the Fe content in the tissues is increased [1, 2].

Materials and Methods

Having been approved by local authorities and the Animal Protection Committee the experiment was performed with male Wistar rats (Interfauna, Tuttlingen, Germany) weighing 200g. Male rats were taken, because it has been reported that male rats are more susceptible to hepatotoxicity by oxygen free radicals [4]. The rats were fed a control or Mg-deficient diet, which were additionally reduced by vitamin E according to tab. 1. The diets (pellets) were obtained from Ssniff (Soest, Germany). The composition of the control diet has been described in detail [5]. Mg-deficient and vitamin E-reduced diets were produced by omission of Mg and vitamin E from the mineral or vitamin mix. The rats were fed the diets and demineralized water ad libitum for 23 weeks.

Subsequently, the rats were anesthetized by i.p. injection of 60 mg/kg pentobarbital. Blood, liver, kidneys, heart, brain, muscle and spleen were taken, and the tissues were frozen in liquid nitrogen and stored at –20 °C. Heparinized blood was centrifuged at 1000 g for 5 min. The concentration of Mg, Ca, Fe and Zn in plasma were measured by atomic absorption spectrophotometry (AAS, Philips, SP9). Parts of the tissues were freeze-dried and powdered. Powdered tissue was ashed in the Plasma Processor 200-E (Technics, München, Germany). The ash was dissolved in 0.1 N HCl and Fe was measured by AAS. For non-hemoglobin (Hb)-bound Fe, Hb was determined in spleen by means of the cyanomethemoglobin method and Hb-bound Fe was substracted from total Fe.

Vitamin E in food, plasma and tissue was determined by its fluorescence in hexane extracts according to *Taylor* et al. [6]. For calibration D,L α-tocopherol (Serva) was used.

Malondialdehyde (MDA) was determined by a variation of the thiobarbituric acid (TBA) method [7, 8]. A 20% homogenate of the tissue in 150 mM KCl was diluted 1:1 with 5% trichloroacetic acid and centrifuged for 5 min at 13000 g. 500 μl TBA (1%, pH 7) was added to 500 μl supernatant and heated at 95 °C for 15 min. After cooling, the probes were extracted with 3 ml 1-butanol by vortexing for 30 sec and centrifugation at 2100 g for 15 min. MDA in the butanol phase was measured fluorometrically (Perkin Elmer LS 50, excitation: 532 nm, emission: 553 nm, slit width: 5 nm). The calibration curve was prepared with malonaldehyde tetraethylacetal (Sigma), which was treated in the same way. For oxidative stress aliquots of the homogenates (2 mg protein/ml) were incubated in 150 mM NaCl, 25 mM Hepes, pH 7.4, for 30 min (muscle and kidney) or 60 min for the other tissues in the presence of 250 μM ascorbate without or with 12.5 μM $FeSO_4$. After addition of 100 μl 25% trichloroacetic acid the tests were centrifuged for 5 min at 13000 g. In the supernatant MDA was measured as described above. Protein was determined with the BCA Protein Reagent (Pierce, Rockford, IL, USA).

Tab. 1: Mg and vitamin E content of control (A), Mg-deficient (B), vitamin E-reduced (C) and vitamin E-reduced plus Mg-deficient (D) diet.
Mean ± SEM of 4 determinations.

	Mg mmol/kg	Vitamin E μmol/kg
A	42.2 ± 2.0	262 ± 10
B	2.2 ± 0.1	267 ± 8
C	42.0 ± 2.2	57 ± 9
D	2.0 ± 0.1	56 ± 6

Results

General observations

Fig. 1 shows that reduction of vitamin E in the diet caused a slight non-significant decrease in growth rate. After 5 weeks of Mg deficiency in groups B and D body weight was reduced. At the same time food intake in the Mg-deficient groups was reduced. Food intake in the Mg-sufficient groups (A, C) amounted to 27–30 g/day and to 10–12 g/day in the Mg-deficient groups (B, D) (fig. 2). During the experiment some of the

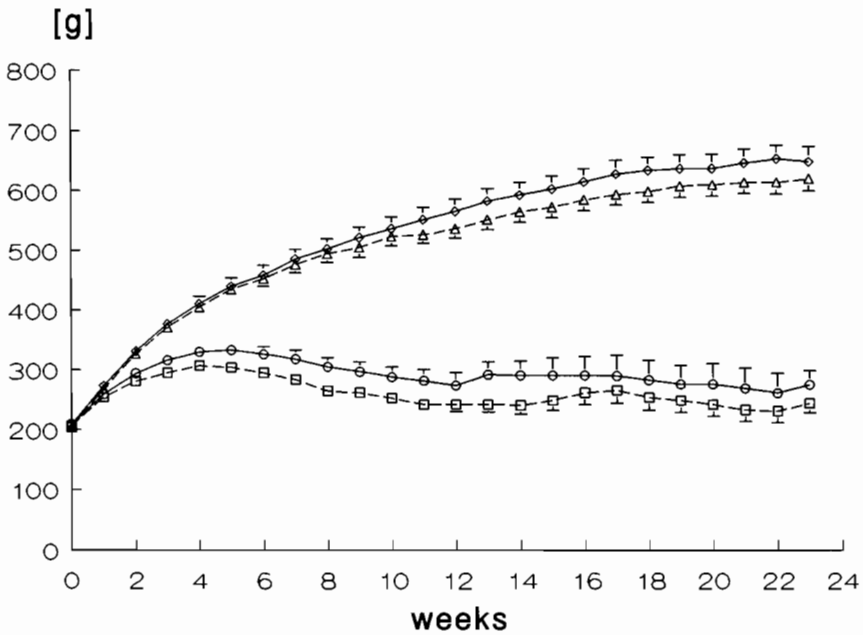

Fig. 1: Growth of rats fed various diets. ◇, control; △, vitamin E-reduced diet; ○, Mg-deficient diet; □, Mg-deficient plus vitamin E-reduced diet.
Mean ± SEM. When SEM was not indicated, it was smaller than the symbol.

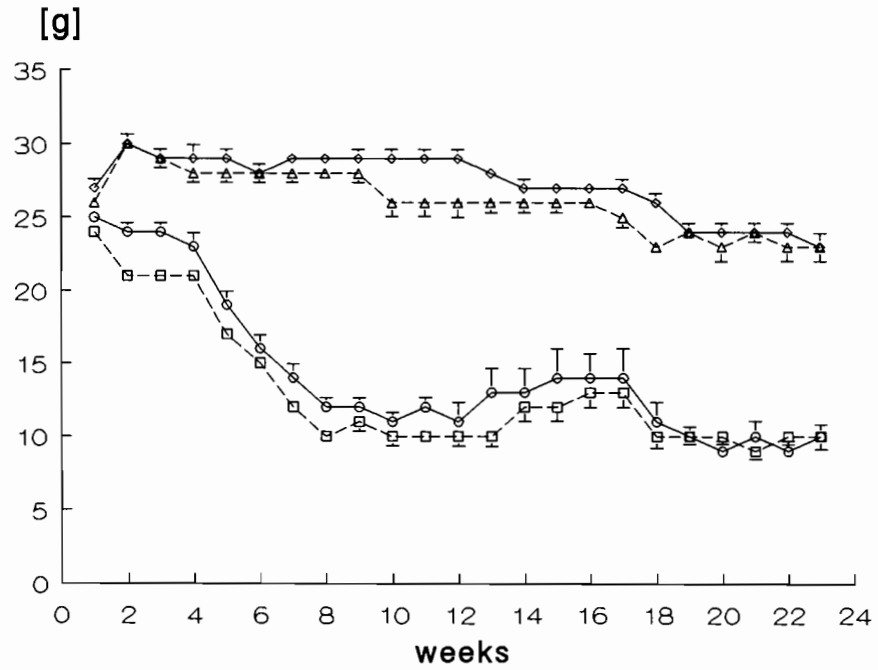

Fig. 2: Food consumption of rats fed various diets. See legend to fig. 1.

Tab. 2: Concentrations of Mg, Ca, Fe, Zn and protein in plasma of rats fed control (A), Mg-deficient (B), vitamin E-reduced (C) and vitamin E-reduced plus Mg-deficient (D) diets.
Mean ± SEM; N, number of rats in each group. Significant difference to group A as revealed by unpaired Student's t-test. a, $p < 0.05$; b, $p < 0.01$; c, $p < 0.001$.

Group	N	Mg mmol/l	Ca mmol/l	Fe µmol/l	Zn µmol/l	Protein g/l
A	10	0.76 ± 0.03	2.40 ± 0.02	36.9 ± 1.6	17.6 ± 0.6	66.3 ± 1.0
B	6	0.27 ± 0.03[c]	2.41 ± 0.04	44.8 ± 3.0[a]	11.0 ± 0.6[c]	52.7 ± 1.4[c]
C	9	0.69 ± 0.03	2.40 ± 0.02	34.5 ± 2.6	19.2 ± 0.4	65.8 ± 0.9
D	12	0.27 ± 0.04[c]	2.49 ± 0.05	40.2 ± 4.1	11.6 ± 0.6[c]	48.8 ± 1.5[c]

Tab. 3: Fe content in rat tissues fed various diets (mmol/kg dry weight).
See legent to tab. 2.

	Spleen non-Hb-Fe[x]	Liver Fe	Kidney Fe	Heart Fe	Muscle Fe	Brain Fe
A	137.2 ± 12.0	9.0 ± 0.8	6.71 ± 0.29	5.84 ± 0.13	1.24 ± 0.14	1.80 ± 0.06
B	402.0 ± 56.0[c]	37.2 ± 5.4[c]	7.43 ± 0.36	7.28 ± 0.36[b]	1.87 ± 0.19[a]	1.71 ± 0.09
C	154.9 ± 12.2	10.2 ± 1.1	6.78 ± 0.20	5.66 ± 0.32	1.29 ± 0.06	1.63 ± 0.04
D	367.1 ± 35.1[c]	33.9 ± 4.7[c]	8.50 ± 0.34[b]	6.72 ± 0.17[c]	1.89 ± 0.11[b]	1.75 ± 0.06

[x] Because of the high blood content of spleen, hemoglobin (Hb) was measured and Hb-bound Fe was substracted from total Fe content. In the other tissues Hb-bound Fe was neglected.

Tab. 4: Vitamin E content in plasma (µmol/l) and tissues of rats (µmol/kg wet weight) fed control (A), Mg-deficient (B), vitamin E-reduced (C) and Mg-deficient plus vitamin E-reduced (D) diets.
Mean ± SEM of 6 rats in each group. Significant difference to group A as revealed by unpaired Student's t-test. a, $p < 0.05$; b, $p < 0.01$; c, $p < 0.001$.

Group	A	B	C	D
Plasma	78.2 ± 6.8	42.0 ± 2.2[c]	12.2 ± 0.8[c]	9.3 ± 0.5[c]
Liver	51.6 ± 4.6	14.7 ± 4.6[c]	3.2 ± 0.2[c]	0.5 ± 0.5[c]
Heart	166.8 ± 7.8	107.2 ± 7.1[c]	31.6 ± 2.8[c]	22.2 ± 0.7[c]
Spleen	181.7 ± 10.1	148.9 ± 7.0[a]	41.3 ± 1.5[c]	36.6 ± 1.3[c]
Kidney	82.5 ± 3.6	72.0 ± 4.2[a]	15.7 ± 0.8[c]	12.9 ± 0.8[c]
Muscle	65.1 ± 4.6	46.6 ± 5.7[a]	13.9 ± 1.2[c]	10.4 ± 1.0[c]
Brain	73.2 ± 1.6	56.9 ± 1.3[c]	29.6 ± 1.9[c]	24.5 ± 1.5[c]

Mg-deficient rats exhibited weight loss and the rats with lowest body weight died (1 of 10 rats in group B, 7 of 22 rats in group D). Therefore, from the 12th to 17th week all rats received 1.5 mM $MgCl_2$ in the drinking water. From the 17th to the 20th week the rats received demineralized water and 2 of 9 surviving rats in the Mg-deficient group B and 5 of 15 surviving rats in group D were s.c. injected twice per week with 0.5 ml 20 mM $MgCl_2$. From the 20th to the 23rd week there was no Mg supplementation. Mg substitution induced a transient increase in food consumption and a transient stop of body weight reduction (figs. 1, 2). Mg supplementation prevented death of the injected rats.
When analyzing plasma values at the end of the experiment, there was no significant difference between transiently injected and non-injected Mg-deficient rats.
Measurement of vitamin E and incubation experiments were performed with tissues of rats which were not injected with $MgCl_2$.

Plasma electrolytes and plasma protein (tab. 2)
Mg-deficient diet caused a drastic reduction of plasma Mg and a significant reduction of plasma Zn. Plasma Fe was somewhat increased parallel to tissue Fe (see below). Since Zn in plasma is bound to protein (33% to α_2 macroglobulin, 59% to albumin, 7% to transferrin and 1% to amino acids), the reduction of plasma Zn concentration can be explained in part by the reduction of plasma protein concentration. Additionally, there may be some intracellular Zn uptake in the Mg-deficient groups (B, D) due to induction of metallothionein.

Fe content of tissues (tab. 3)
For correlation of lipid peroxidation to Fe content, Fe content of the tissues was measured. Fe content in all tissues with the exception of brain, was increased by Mg deficiency or by Mg deficiency plus vitamin E reduction. Vitamin E reduction alone (group C) had no significant effect on Fe content. This result shows that Fe content was only increased by Mg deficiency. Fe content was highest in spleen (non hemoglobin-Fe), followed by liver,

Tab. 5: Malondialdehyde (μmol/kg wet weight) in liver. See legend to tab. 4.

Group	No incubation	Incubation with ascorbate	Incubation with Fe/ascorbate
A	0.73 ± 0.20	10.67 ± 1.68	19.22 ± 2.17
B	3.07 ± 0.84[a]	21.56 ± 3.45[a]	28.44 ± 3.32[a]
C	27.47 ± 2.73[c]	996.8 ± 45.1[c]	1192 ± 35.5[c]
D	33.78 ± 3.91[c]	921.2 ± 127[c]	1201 ± 18.6[c]

Tab. 6: Malondialdehyde (μmol/kg wet weight) in kidney. See legend to tab. 4.

Group	No incubation	Incubation with ascorbate	Incubation with Fe/ascorbate
A	6.94 ± 1.18	76.8 ± 10.0	58.5 ± 6.8
B	11.91 ± 1.65[a]	100.0 ± 11.4	67.3 ± 7.6
C	43.06 ± 3.11[c]	1125 ± 42.5[c]	1455 ± 64.8[c]
D	59.05 ± 5.97[c]	988.3 ± 81.8[c]	1302 ± 54.6[c]

Tab. 7: Malondialdehyde (μmol/kg wet weight) in heart. See legend to tab. 4.

Group	No incubation	Incubation with ascorbate	Incubation with Fe/ascorbate
A	1.66 ± 0.18	5.28 ± 0.67	7.71 ± 0.71
B	2.40 ± 0.20[a]	11.35 ± 0.79[c]	13.32 ± 0.93[c]
C	5.82 ± 0.60[c]	132.1 ± 23.7[c]	538.4 ± 127[c]
D	10.78 ± 1.22[c]	1030 ± 172[c]	1107 ± 153[c]

Tab. 8: Malondialdehyde (μmol/kg wet weight) in brain. See legend to tab. 4.

Group	No incubation	Incubation with ascorbate	Incubation with Fe/ascorbate
A	1.45 ± 0.08	134.0 ± 11.5	1680 ± 123
B	1.52 ± 0.16	215.1 ± 25.5[a]	1765 ± 114
C	1.90 ± 0.17[a]	458.5 ± 34.7[c]	1965 ± 70
D	2.55 ± 0.23[b]	524.6 ± 28.4[c]	2023 ± 40[a]

Tab. 9: Malondialdehyde (μmol/kg wet weight) in muscle. See legend to tab. 4.

Group	No incubation	Incubation with ascorbate	Incubation with Fe/ascorbate
A	1.59 ± 0.09	124.7 ± 10.0	229.0 ± 33.0
B	1.76 ± 0.31	167.0 ± 29.4	287.6 ± 24.3
C	2.19 ± 0.23[a]	314.3 ± 31.9[c]	867.9 ± 50.9[c]
D	2.80 ± 0.31[b]	315.4 ± 50.8[b]	619.4 ± 69.6[c]

Tab. 10: Malondialdehyde (μmol/kg wet weight) in spleen. See legend to tab. 4.

Group	No incubation	Incubation with ascorbate	Incubation with Fe/ascorbate
A	6.05 ± 0.96	45.1 ± 3.2	48.6 ± 4.8
B	7.82 ± 1.07	80.9 ± 9.2[b]	82.0 ± 9.5[a]
C	8.20 ± 0.95	89.9 ± 5.8[c]	108.4 ± 15.1[b]
D	7.89 ± 0.96	159.1 ± 12.1[c]	285.7 ± 41.4[c]

indicating the role of these tissues in Fe metabolism. The high increase of Fe in spleen and liver can be explained by an increased rate of erythrocyte destruction in Mg deficiency and the subsequent transfer of released Fe to the liver.

Vitamin E content of plasma and tissues (tab. 4)

Vitamin E content was high in heart, as also found in preceding experiments [1, 2], and in spleen. In plasma and all tissues, vitamin E content was reduced by Mg deficiency and by vitamin E reduction. The strongest reduction of vitamin E was found in liver followed by plasma, heart, kidney, muscle, spleen and brain. The lowest percentage of vitamin E reduction was found in the brain. Vitamin E in liver was almost completely removed by feeding the vitamin E-reduced diet. This result indicates that vitamin E in liver is particularly active and can supply other tissues with vitamin E. The same result was obtained by other authors [9].

Lipid peroxidation (LPO)

LPO without incubation (tabs. 5-10)

LPO was measured as malondialdehyde (MDA). Dependent on the tissue, MDA was increased:

1. due to Mg deficiency by the factor 1.1 (in brain) to 4.2 (in liver),
2. due to vitamin E reduction by the factor 1.3 (in brain) to 38 (in liver) and
3. due to Mg deficiency plus vitamin E reduction by the factor 1.3 (in spleen) to 46 (in liver) (tab. 11).

The increase in MDA by Mg deficiency, vitamin E reduction and combined treatment was parallel in all tissues, according to the series: liver > kidney > heart > muscle = spleen = brain. This series corresponds to the percentage of vitamin E reduction in the tissues by the various treatments (tab. 12).

As shown in tab. 11, vitamin E depletion had a much higher effect on MDA formation than Mg deficiency. However, the increase in MDA formation by vitamin E depletion was strongly enhanced by additional Mg deficiency. These results indicate that Mg defi-

Tab. 11: Increase of malondialdehyde (by factor) in various tissues during Mg deficiency (B/A), vitamin E depletion (C/A) and Mg deficiency plus vitamin E depletion (D/A). Values (No incubation) were taken from tabs. 5-10.

Group	Liver	Kidney	Heart	Muscle	Spleen	Brain
B/A	4.2	1.72	1.45	1.11	1.29	1.05
C/A	37.6	6.20	3.51	1.38	1.36	1.31
D/A	46.2	8.50	6.49	1.76	1.30	1.76

Tab. 12: Decrease in vitamin E (by factor) in various tissues during Mg deficiency (A/B), vitamin E depletion (A/C) and Mg deficiency plus vitamin E depletion (A/D). Values were taken from tab. 4.

Group	Liver	Heart	Muscle	Kidney	Spleen	Brain
A/B	3.5	1.56	1.40	1.15	1.22	1.29
A/C	16.1	5.28	4.68	5.25	4.40	2.47
A/D	103	7.51	6.26	6.39	4.96	2.99

ciency may become effective particularly in vitamin E deficiency.

Lipid peroxidation by oxidative stress

To find optimal conditions for oxidative stress, tissue homogenates from normal rats were incubated with 250 μM ascorbate and 0, 12.5, 25 and 50 μM $FeSO_4$ for various times. Under these conditions, maximal MDA was formed with 12.5 μM Fe after 60 min in liver, heart, brain and spleen, whereas in muscle and kidney MDA formation was already maximal after 30 min (not shown).

After incubation of the tissue homogenates with Fe/ascorbate, MDA content was drastically increased in the tissues of all groups. MDA content (LPO) was particularly enhanced in the vitamin E-reduced groups C and D, and the increase in LPO was highest in brain according to the series: brain (factor 1000) > muscle (factor 390) > heart (factor 100) > liver (factor 40) = kidney (factor 30) = spleen (factor 30). Since the ascorbate- and Fe/ascorbate-induced increase in MDA formation was particularly expressed in groups C and D, in which Fe content was very different and almost identical to Fe content of groups A (A ≈ C) and B (B ≈ D), the increase in MDA formation was due to vitamin E reduction. As a control, tissue homogenates were incubated in parallel with ascorbate alone. As can be seen from tabs. 5–10, already addition of ascorbate alone increased LPO. In liver, kidney, spleen and heart (group D) ascorbate alone was nearly as effective as addition of Fe/ascorbate. This may indicate that these tissues contain a high amount of weakly-bound Fe which can react with ascorbate to induce an almost maximal LPO, whereas in brain and muscle addition of Fe was necessary to induce a maximal rate of LPO. After incubation with Fe/ascorbate maximal rates of LPO in brain were much higher than in the other tissues. Therefore, besides available active Fe, other factors may determine the rate of LPO. This may be the content of unsaturated fatty acids. Spleen showed the lowest increase in MDA. This may be explained by the result that vitamin E content was highest in spleen.

Discussion

In the present experiment, in which the period of Mg deficiency and vitamin E reduction was extended from 11 to 23 weeks, preceding results [11] were confirmed and extended to more tissues. Mg deficiency reduced vitamin E content and increased MDA content in all tissues.

When comparing these results of the two experiments, vitamin E and consequently MDA content were changed to a greater extent during the longer experimental period.

An unspecific mechanism as reason for the increased LPO in Mg deficiency, e.g. by reduced food intake (fig. 2), can be excluded. Some authors found inhibition of LPO by starvation, whereas in other experiments malnutrition of rats had no significant effect on MDA content of a postmitochondrial liver fraction [10]. The increase in MDA by vitamin E reduction alone and in combination with Mg deficiency was particularly expressed in liver, kidney and heart due to the reduction of vitamin E. This result shows that vitamin E plays a major role in protecting these organs against LPO. However, in vivo MDA content in all tissues was low compared to the dramatic increase in MDA in the tissues after incubation in vitro with ascorbate or Fe/ascorbate. There was already a small increase in MDA when incubation was performed without ascorbate and without Fe (not shown). The increase in MDA after incubation in vitro may be caused:

1. by the elimination of MDA metabolism and renal excretion of MDA,
2. by the dilution of water-soluble antioxidants due to tissue homogenisation and
3. by interaction of tissue Fe with ascorbate (see below).

The drastic increase of MDA after incubation with ascorbate or with Fe/ascorbate was particularly expressed in the vitamin E-reduced animals (groups C, D). Hence, in these groups there was a drastically increased oxygen radical formation under oxidative stress due to vitamin E depletion.

Ascorbate is known to be a hydrophilic antioxidant. In experiments with rat liver microsomes, ascorbate by itself had no effect on LPO. However, in combination with Fe^{3+} and particularly with Fe^{2+}, 10 μM Fe^{2+} and 200 μM ascorbate, induced a drastic increase in LPO [11]. (Only at concentrations above 2 μM had ascorbate an antioxidant effect [11].) Ascorbate may enhance LPO by reduction of Fe^{3+} to Fe^{2+}, since in the mechanism of oxygen radical formation according to the Fenton reaction Fe^{2+} is oxidized to Fe^{3+}, and an optimal Fe^{3+}/Fe^{2+} ratio is needed to induce LPO [12]. Moreover, ascorbate can be oxidized according to the reaction: ascorbate + O_2 → dehydroascorbate + H_2O_2 [11], H_2O_2 being the substrate in the Fenton reaction. The use of ascorbate in the formation of oxygen radicals had the advantage that Fe, accumulated in the tissue during Mg deficiency, became active in oxidative stress. The increased MDA formation after incubation with ascorbate alone shows that the tissues contained weakly-bound Fe which could react with ascorbate to induce MDA formation. Moreover, ascorbate may be involved in the release of a small amount of Fe from ferritin [13] or hemosiderin [14]. Weakly-bound Fe represents an Fe pool which is bound to low-molecular-mass substances and can induce the formation of oxygen radicals [15]. The weakly Fe-binding substances (probably citrate, amino acids and nucleotides [16]) and the size of this Fe pool are not defined [15, 16]). Therefore, MDA formation (LPO) cannot be correlated to total Fe content of the tissues.

Although the steady-state level of MDA in the tissues by vitamin E reduction was considerably enhanced by Mg deficiency, LPO seems to play no significant pathogenetic role in vivo in Mg deficiency. As shown in figs. 1 and 2, growth and food consumption of the vitamin E-reduced animals (group C) was not significantly reduced and the vitamin E-reduced rats (group C) did not show any pathological symptoms.

The dramatic increase of MDA formation after incubation in vitro with ascorbate or with Fe/ascorbate shows that vitamin E reduction represents a risk factor in pathological events which function via LPO. Particularly when oxidative stress was applied to hearts of Mg-deficient plus vitamin E-depleted

rats (group D) a drastic rise in MDA was induced. Thus, in the heart the combination of Mg deficiency and vitamin E reduction may be a risk factor to oxidative stress which may function in ischemia-reperfusion [17].

Acknowledgement

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