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Inhibitory and stimulating effect of magnesium on vanadate-induced lipid peroxidation under *in vitro* conditions[†]

Agnieszka Ścibior[a,b*], Dorota Gołębiowska[a], Irmina Niedźwiecka[a] & Agnieszka Adamczyk[a]

[a] Laboratory of Physiology & Animal Biochemistry, Department of Zoology & Invertebrate Ecology, Institute of Environmental Protection, The John Paul II Catholic University of Lublin, 102 Kraśnicka Ave, 20-718 Lublin, Poland

[b] Laboratory of Oxidative Stress, Centre of Interdisciplinary Research, The John Paul II Catholic University of Lublin, 102 Kraśnicka Ave, 20-718 Lublin, Poland

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The behaviour of Mg related to vanadium(V)-induced lipid peroxidation (LPO) under *in vitro* conditions was examined. The studies performed on the liver supernatants (LS) obtained from control, sodium metavanadate-intoxicated, and sodium metavanadate-magnesium sulphate-administered male Wistar rats revealed and confirmed the pro-oxidative potential of V. Simultaneously, they indicated that the improved Mg status may be one of the mechanisms by which the treatment with this element may contribute to reduction of oxidative stress under the conditions of vanadate exposure. On the other hand, the results confirmed that Mg may also stimulate LPO and demonstrated that the incubation conditions and the experimental treatment of the rats from which the liver supernatants were obtained affect the intensity of the examined free radical process.

Keywords: Lipid peroxidation, Liver, Magnesium, Oxidative stress, Vanadate

Oxidative stress (OS), which is implicated in a number of human diseases, is well known to occur when the generation of free radicals (FR) and reactive oxygen species (ROS) is beyond the scavenging abilities of the antioxidant system[1].

As a transition redox-active metal (mostly occurring in cationic and anionic forms, predominantly as vanadyl – VO^{2+} and vanadate – VO_3^- ions, the +4 and +5 oxidation state, respectively), vanadium (V) may participate in reactions of FR and ROS production, ultimately leading to an increase in OS in a variety of cells and tissues[2-5]. Due to its pro-oxidative properties, V can lead to oxidative damage to various macromolecules[6]. Studies on the mechanisms of action of its compounds have indicated that V may not only generate ROS and/or FR but may also indirectly modify OS in cells, by either releasing FR-generating metals from tissues, modifying enzymatic and antioxidant defense[2], or interacting with mitochondria[7]. V redox cycling initiated by one-electron oxidation of VO^{2+} (V^{4+}) or one-electron reduction of VO_3^- (V^{5+}) with a superoxide anion radical ($O_2^{\bullet-}$) may operate as a chain reaction, providing FR and ROS[6], which in turn may attack polyunsaturated fatty acids (PUFAs) and initiate lipid peroxidation (LPO). In turn, as a non-transition element with antioxidative properties, magnesium (Mg) may modulate the pro-oxidant-antioxidant imbalance through reducing FR and ROS formation, thereby limiting the oxidative damage[8-12]. Mg deficiency has been suggested to be an important factor in enhancement of FR-induced oxidation both under *in vitro* and *in vivo* conditions[13,14] but the mechanism involved in the response to the Mg deficiency-induced increase in LPO has not been elucidated yet.

The protective effect of Mg against the sodium metavanadate ($NaVO_3$, SMV)-induced LPO in the liver was demonstrated during an 18-week administration of magnesium sulphate ($MgSO_4$, MS) to SMV-exposed rats[15]. In turn, the beneficial effect of Mg reflected in the limitation of the increase in LPO both in the liver and kidney was not observed when MS was supplied to rats together with SMV for a shorter period of time, i.e. for only 12 weeks[3,4]. On

[*]Correspondent author
Telephone: +48 - 814454604
Fax: +48 - 81445461
E-mail: cellbiol@kul.lublin.pl

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the other hand, a 12-week supplementation of rats with MS during the exposure to SMV at least partly decreased the concentration of V in the blood, normalized to some degree some parameters of the Fe status, completely prevented reduction in the erythrocyte Mg level, and restored the erythrocyte Fe concentration near the control values. Detailed analysis of those results revealed that in the rats receiving SMV in combination with MS all these changes resulted from the tendency toward the independent influence of Mg or the Mg independent action as well as from the tendency of Mg toward an interaction with V or from the V-Mg antagonistic interaction[16].

The results from previous studies[3,4,15] encouraged undertaking further investigations to explore more thoroughly the possible beneficial and unfavourable Mg impact on LPO. The effect of Mg on LPO was examined under some what different *in vitro* conditions than those used earlier[3]. In turn, LPO was evaluated in the same manner as previously – in terms of malondialdehyde (MDA) production. MDA is a parameter of FR action and a diagnostic pool for LPO, the measurement of which, although nonspecific[1], is widely used as a biomarker of this process. Analyses were performed on supernatants of the liver, the most important organ connected with metabolism and playing a crucial role in detoxification of xenobiotics, obtained from the control, SMV-exposed, and SMV-MS-administered rats. The aim of the experiment was to examine the effect of Mg on LPO under somewhat different *in vitro* conditions than those used previously[3]. Recognition of possible differences in intensification of the hepatic MDA generation between the liver supernatants pre-incubated with selected MS concentrations and subsequently incubated with selected SMV concentrations or co-incubated with MS and SMV has been an area of our interest. Moreover, attention has also been paid to whether and to what extent an earlier administration of Mg to rats in combination with SMV is able to modify the hepatic MDA generation during additional SMV exposure under the *in vitro* conditions.

Materials and Methods

Reagents—Sodium metavanadate ($NaVO_3$, SMV), magnesium sulphate ($MgSO_4$, MS), and thiobarbituric acid (TBA) were obtained from Sigma Chemicals (St. Louis, MO, USA), whereas sodium arsenite ($NaAsO_2$), trichloroacetic acid (TCA) and potassium chloride (KCl) were purchased from POCH (Gliwice, Poland). Ultra-pure water was obtained from an ultra-pure water HLP Spring 5R system* (Hydrolab, Gdańsk, Poland). All other chemicals and reagents used were of analytical grade or better and commercially available.

Collection of liver samples—All the liver samples used in this *in vitro* experiment were obtained from 48 selected outbred albino male Wistar rats. Briefly, all the animals were individually placed in stainless steel cages (1 rat/cage) kept in a room under standard laboratory conditions (20-21 °C, 55±5% RH, and 12:12 h light/dark period); every day over a 12-week period they received deionized water (Control, 16 rats), SMV solution at a concentration of 0.125 mg V/mL (16 rats) or SMV-MS solution at a concentration of 0.125 mg V/mL and 0.06 mg Mg/mL (16 rats) to drink. All the rats had unlimited access to the rodent laboratory chow (Labofeed B; Fodder and Concentrate Factory, Kcynia, Poland), deionized water as well as the SMV and SMV-MS solutions. After 12 weeks of the experiment, all the rats were sacrificed and livers were dissected, directly washed in ice-cold physiological saline solution (0.9% NaCl), and weighed. The biological material that was not used immediately was stored frozen at –20 °C or –80 ºC in a deep-freezer HFU 486 Basic * (Thermo Fisher Scientific, Germany) until further analysis. The study was approved by the 1st Local Ethical Committee for Animal Studies in Lublin (Poland).

Analytical procedures—Lipid peroxidation (LPO) was estimated in liver supernatants (LS) by measuring the MDA production in the thiobarbituric acid (TBA) reaction. All details concerning this method and preparation of LS for MDA determination have been reported earlier[3]. Therefore, only a description of incubation of LS I, II and III obtained from the control, SMV-intoxicated and SMV-MS-administered rats, respectively, with the two chosen concentrations of SMV separately and together with the selected concentrations of MS has been provided. Part of LS I, II and III was first pre-incubated with MS at the concentrations of 0.4, 0.8, 1.0, 1.5, 5.0,

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50, or 100 mM (Mg pre-incubation) and subsequently incubated with SMV (as an inductor of LPO) at the concentrations of 0.2 mM or 0.4 mM. Another part of the same LS I, II and III was co-incubated with 0.4, 0.8, 1.0, 1.5, 5.0, 50, or 100 mM MS and 0.2 or 0.4 mM SMV (without Mg pre-incubation), whereas some other part of LS I, II and III was only incubated with 0.2 or 0.4 mM SMV. Finally, part of the above-mentioned supernatants was incubated without any MS and SMV concentrations (LPO spontaneous).

Statistical analysis—The results were processed with the Statistica version 9.0 PL for Windows. To assess the distribution patterns in the data, the Shapiro-Wilk normality test was performed. The homogeneity of variances was carried out employing Levene's test. The presence of outliers from a normal distribution was checked with Grubbs' test and standardization of the data. The Student's *t*-test and Wilcoxon test were used to compare two dependent samples when the data met or not the assumptions of ANOVA, respectively. However, the non-parametric Mann-Whitney *U* test was applied to compare two unpaired groups when the data did not meet the assumptions of ANOVA. All the results were presented as mean $(\bar{x})$ ± SEM. A *P* value of 0.05 or less was assumed as a criterion for a statistically significant difference.

Results

MDA level in LS I, II and III incubated and not with 0.2 or 0.4 mM SMV—Spontaneous production of MDA in LS II and III was significantly higher, compared with that observed in LS I (Fig. 1). MDA generation in all the kinds of the supernatants (LS I, II and III) incubated with 0.2 or 0.4 mM SMV was also markedly higher in comparison with the spontaneously formed MDA found in the above-mentioned supernatants (Fig. 1). LS I, II and III incubated with 0.4 mM SMV exhibited markedly elevated the MDA levels as well compared with those demonstrated during incubation with 0.2 mM SMV (Fig. 1). Moreover, production of MDA in LS III incubated with 0.2 or 0.4 mM SMV was distinctly lower than that observed in LS II incubated under the same experimental *in vitro* conditions (Fig. 1).

MDA level in LS I, II and III pre-incubated with MS and subsequently incubated with SMV or co-incubated with MS and SMV—The pre-incubation of LS I with all the MS concentrations used (0.4, 0.8, 1.0, 1.5, 5.0, 50, and 100 mM) and the subsequent incubation thereof with two SMV concentrations (0.2 or 0.4 mM; Fig. 2A and B, respectively) led to elevated generation of MDA compared with that found in LS I incubated with 0.2 or 0.4 mM SMV alone. Only in the case of pre-incubation of LS I with 0.4, 1.5 or 5.0 mM MS and the subsequent incubation thereof with 0.4 mM (Fig. 2B) the elevated MDA level was not significant in comparison with that found in LS I incubated with only 0.4 mM SMV. However, when LS I were co-incubated with all the above-mentioned MS concentrations and with 0.2 or 0.4 mM SMV (Fig. 2C and D, respectively) without Mg pre-incubation, the MDA production remained in the range of the values observed for the MDA level in LS I incubated with 0.2 or 0.4 mM SMV alone. Only in the case of incubation of LS I with 0.4 or 1.0 mM MS and 0.4 mM SMV (Fig. 2D) was the formation of MDA significantly lower compared with that found in LS I incubated with 0.4 mM SMV alone. Moreover, the co-incubation of LS I with 0.4, 0.8, 1.0, 1.5, 5.0, 50, or 100 mM MS and 0.2 (Fig. 2C) or 0.4 (Fig. 2D) mM SMV resulted in lower production of MDA in comparison with that observed during the

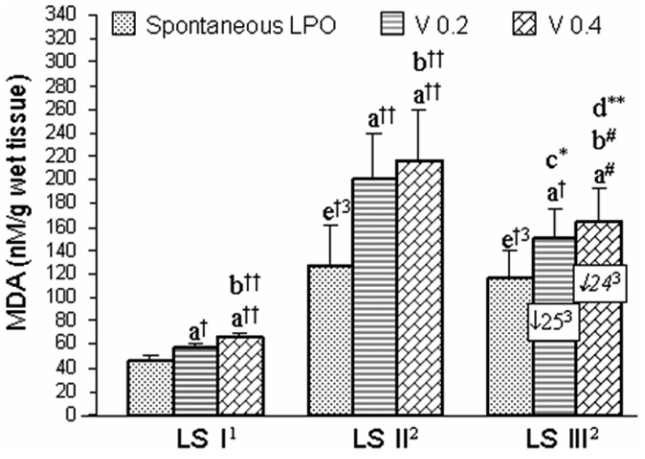

Fig. 1—MDA level in LS I, II and III obtained from the control, SMV-intoxicated and SMV-MS-administered rats, respectively, which were and not incubated with 0.2 mM or 0.4 mM $NaVO_3$. Differences (^{1,2,3}*t*-test, Wilcoxon test and U Mann-Whitney test, respectively) are indicated by: ^{a}versus spontaneous LPO; ^{b}versus V 0.2 mM; ^{c,d}versus LS II incubated with V 0.2 or V 0.4 mM, respectively; ^{e}versus spontaneous LPO for LS I. *P* values: †< 0.05; ††< 0.01; $^{\#}$< 0.001; *= 0.20; **= 0.14. The numerical values in the bars indicate a percentage decrease (↓) in the MDA level, compared with that found in LS II not incubated (underline normal) and incubated with 0.2 mM $NaVO_3$ (normal alone) or with 0.4 mM $NaVO_3$ (italic alone). Values are mean ± SE.

pre-incubation of LS I with the aforementioned MS concentrations and the subsequent incubation thereof with 0.2 (Fig. 2A) or 0.4 (Fig. 2B) mM SMV.

When LS II were pre-incubated with all the selected MS concentrations (0.4, 0.8, 1.0, 1.5, 5.0, 50, or 100 mM) and subsequently incubated with the two chosen SMV concentrations (0.2 or 0.4 mM; Fig. 3A and B, respectively), the level of MDA was lower compared with that found in LS II incubated with 0.2 or 0.4 mM SMV alone. Markedly limited MDA formation was found when LS II were pre-incubated with 0.4 or 0.8 mM MS and subsequently incubated with 0.2 mM SMV (Fig. 3A). However, when LS II were co-incubated with the aforementioned MS concentrations and 0.2 or 0.4 mM SMV (Fig. 3C and D, respectively) without Mg pre-incubation, the level of MDA turned out to be higher compared with that observed in LS II pre-incubated with all the MS concentrations used (Fig. 3A and B) and that found in LS II incubated with 0.2 or 0.4 mM SMV alone.

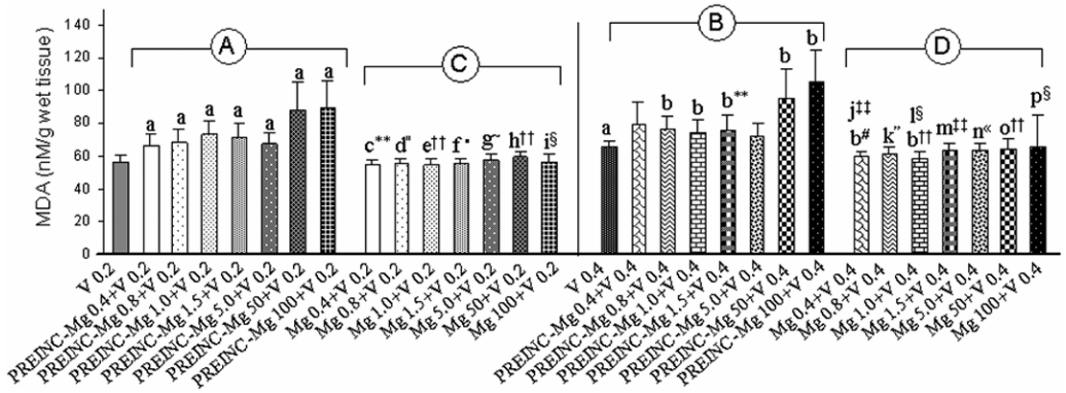

Fig. 2—MDA level in LS I obtained from the control rats, which were first pre-incubated with the selected concentrations of $MgSO_4$ and subsequently incubated with 0.2 mM (A) or 0.4 mM (B) $NaVO_3$, and which were co-incubated with the selected concentrations of $MgSO_4$ and with 0.2 mM (C) or 0.4 mM (D) $NaVO_3$ but without $MgSO_4$ pre-incubation. Differences (Student *t*-test or Wilcoxon test) are indicated by: [a]versus V 0.2 mM; [b]versus V 0.4 mM; [c]versus PREINC-Mg 0.4+V 0.2; [d]versus PREINC-Mg 0.8+V 0.2; [e]versus PREINC-Mg 1.0+V 0.2; [f]versus PREINC-Mg 1.5+V 0.2; [g]versus PREINC-Mg 5.0+V 0.2; [h]versus PREINC-Mg 50+V 0.2; [i]versus PREINC-Mg 100+V 0.2; [j]versus PREINC-Mg 0.4+V 0.4; [k]versus PREINC-Mg 0.8+V 0.4; [l]versus PREINC-Mg 1.0+V 0.4; [m]versus PREINC-Mg 1.5+V 0.4; [n]versus PREINC-Mg 5.0+V 0.4; [o]versus PREINC-Mg 50+V 0.4; [p]versus PREINC-Mg 100+V 0.4. *P* values: [§]< 0.01; [††]< 0.05; [”]= 0.05; [•]= 0.08; [#]= 0.09; [‡‡]= 0.10; [**]= 0.11; ["]= 0.12; [˜]= 0.13; [“]> 0.05. Values are mean ± SE.

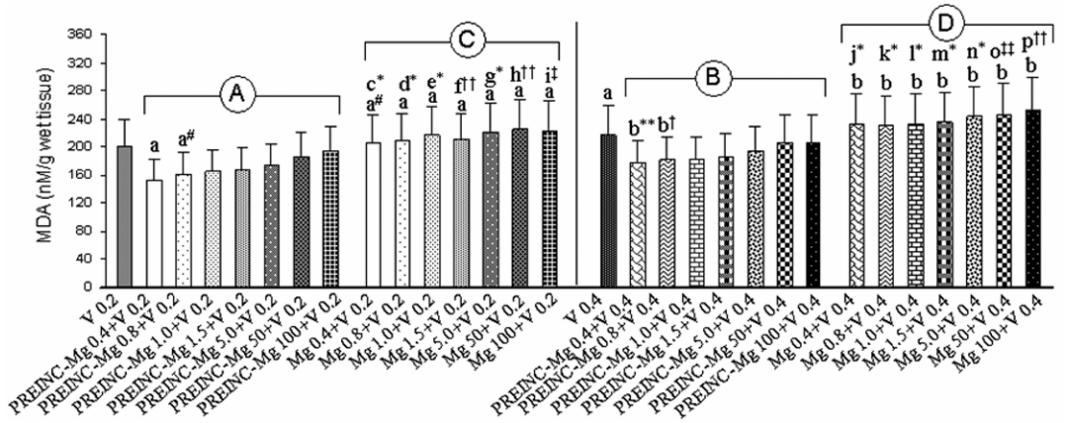

Fig. 3—MDA level in LS II obtained from the SMV-intoxicated rats, which were first pre-incubated with the selected concentrations of $MgSO_4$ and subsequently incubated with 0.2 mM (A) or 0.4 mM (B) $NaVO_3$, and which were co-incubated with the selected concentrations of $MgSO_4$ and with 0.2 mM (C) or 0.4 mM (D) $NaVO_3$ but without $MgSO_4$ pre-incubation. Differences (Wilcoxon test) are indicated by: [a]versus V 0.2 mM; [b]versus V 0.4 mM; [c]versus PREINC-Mg 0.4+V 0.2; [d]versus PREINC-Mg 0.8+V 0.2; [e]versus PREINC-Mg 1.0+V 0.2; [f]versus PREINC-Mg 1.5+V 0.2; [g]versus PREINC-Mg 5.0+V 0.2; [h]versus PREINC-Mg 50+V 0.2; [i]versus PREINC-Mg 100+V 0.2; [j]versus PREINC-Mg 0.4+V 0.4; [k]versus PREINC-Mg 0.8+V 0.4; [l]versus PREINC-Mg 1.0+V 0.4; [m]versus PREINC-Mg 1.5+V 0.4; [n]versus PREINC-Mg 5.0+V 0.4; [o]versus PREINC-Mg 50+V 0.4; [p]versus PREINC-Mg 100+V 0.4. *P* values: [*]< 0.001; [††]< 0.05; [‡]= 0.06; [#]= 0.09; [‡‡]= 0.10; [**]= 0.11; [†]= 0.19. Values are mean ± SE.

Enhanced MDA production was found in LS III when these supernatants were first pre-incubated with MS in all the selected concentrations (0.4, 0.8, 1.0, 1.5, 5.0, 50, or 100 mM) and subsequently incubated with 0.2 or 0.4 mM SMV (Fig. 4A and B) compared with that observed in LS III incubated with 0.2 or 0.4 mM SMV alone. Moreover, elevated MDA formation was also demonstrated in the same supernatants when they were co-incubated with the aforementioned MS concentrations and 0.2 or 0.4 mM SMV without Mg pre-incubation (Fig. 4C and D) in comparison with that found in LS III during incubation thereof with only 0.2 or 0.4 mM SMV alone.

Comparison of the MDA level between LS II and III co-incubated with MS and SMV—In LS III incubated with 0.4, 0.8, 1.0, 1.5 or 5.0 mM MS and with 0.4 mM SMV, the MDA production was distinctly lowered (Fig. 5D III) compared with that found in LS II incubated under the same *in vitro* conditions (Fig. 5D II) but these differences turned out to be insignificant. The percentage decrease or increase in the MDA level for the selected liver supernatants is presented in Tables 1 and 2.

Discussion

Despite the fact that a lot has already been written about the effect of Mg on LPO assessed by MDA/TBARS production, especially about its protective impact described in humans as well as both in *in vivo* and *in vitro* studies[17-37] (Table 3), there are no data available on the influence of Mg on SMV-induced LPO apart from our studies[3,4,15]. The scarce data on this issue and the results obtained from earlier experiments[3,15] encouraged more thorough investigations into the behaviour of Mg with respect to the pro-oxidant potential of V under specific *in vitro* conditions. The lack of similar reports renders the current results impossible to discuss widely. Therefore, the discussion rests only on the results from the present study and some previous findings[3].

As expected, the incubation of LS I with exogenous SMV concentrations stimulated MDA generation (Fig. 1), which confirmed previous results[3]. This may be explained by the strong pro-oxidative properties of V and its ability to

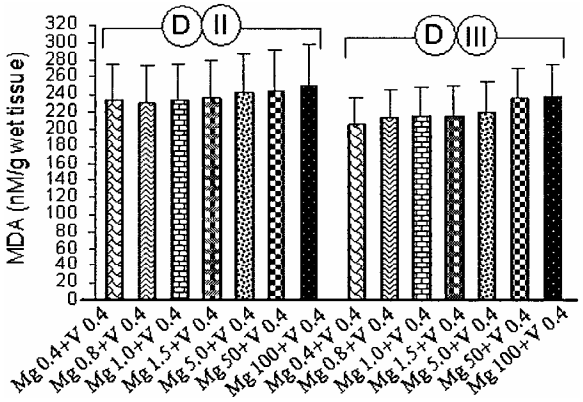

Fig. 5—MDA level in LS II (D II) and III (D III) obtained from the SMV and SMV-MS-administered rats, respectively, which were co-incubated with 0.4 mM $NaVO_3$ and with the selected concentrations of $MgSO_4$ without $MgSO_4$ pre-incubation. Values are mean ± SE.

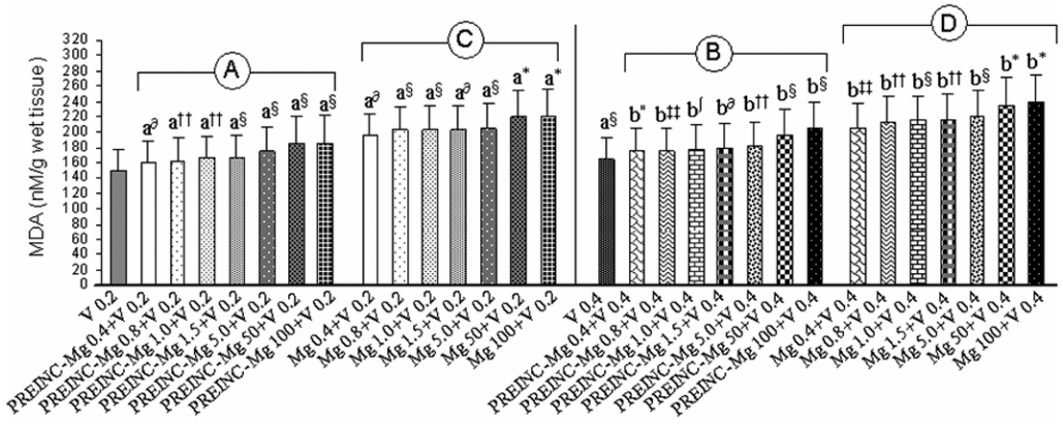

Fig. 4—MDA level in LS III obtained from the SMV-MS-administered rats, which were first pre-incubated with the selected concentrations of $MgSO_4$ and subsequently incubated with 0.2 mM (A) or 0.4 mM (B) $NaVO_3$, and which were co-incubated with the selected concentrations of $MgSO_4$ and with 0.2 mM (C) or 0.4 mM (D) $NaVO_3$ but without $MgSO_4$ pre-incubation. Differences (Wilcoxon test) are indicated by: [a]versus V 0.2 mM; [b]versus V 0.4 mM. *P* values: [*]< 0.001; [§]< 0.01; [††]< 0.05; [∂]= 0.05; [ʃ]= 0.09; [‡‡]= 0.10; ["]= 0.16. Values are mean ± SE.

Table 1—Percentage (%) decrease or increase in the MDA level for LS II and III

LS II obtained from the SMV-intoxicated rats

LS II (A) compared with LS II V 0.2		LS II (B) compared with LS II V 0.4	
PREINC-Mg 0.4+V 0.2	↓ 23††	PREINC-Mg 0.4+V 0.4	↓ 18.2**
PREINC-Mg 0.8+V 0.2	↓ 18.8$^{\#}$	PREINC-Mg 0.8+V 0.4	↓ 16††
PREINC-Mg 1.0+V 0.2	↓ 16.6$^{\text{«}}$	PREINC-Mg 1.0+V 0.4	↓ 15.7$^{\text{«}}$
PREINC-Mg 1.5+V 0.2	↓ 16.2$^{\text{«}}$	PREINC-Mg 1.5+V 0.4	↓ 14$^{\text{«}}$
PREINC-Mg 5.0+V 0.2	↓ 13.5$^{\text{«}}$	PREINC-Mg 5.0+V 0.4	↓ 10.3$^{\text{«}}$
PREINC-Mg 50+V 0.2	↓ 6.6$^{\text{«}}$	PREINC-Mg 50+V 0.4	↓ 4.5$^{\text{«}}$
PREINC-Mg 100+V 0.2	↓ 2.5$^{\text{«}}$	PREINC-Mg 100+V 0.4	↓ 4.3$^{\text{«}}$
LS II (C) compared with LS II V 0.2		**LS II (D) compared with LS II V 0.4**	
Mg 0.4+V 0.2	↑ 3.9$^{\#}$	Mg 0.4+V 0.4	↑ 7.8§
Mg 0.8+V 0.2	↑ 4.7††	Mg 0.8+V 0.4	↑ 6.7§
Mg 1.0+V 0.2	↑ 8.9††	Mg 1.0+V 0.4	↑ 8§
Mg 1.5+V 0.2	↑ 5††	Mg 1.5+V 0.4	↑ 8.8§
Mg 5.0+V 0.2	↑ 10.6*	Mg 5.0+V 0.4	↑ 12.2*
Mg 50+V 0.2	↑ 13*	Mg 50+V 0.4	↑ 13.2*
Mg 100+V 0.2	↑ 12§	Mg 100+V 0.4	↑ 15.7*

V 0.2 Compared with V 0.4

8.5§

LS III obtained from the SMV-MS-administered rats

LS III (A) compared with LS III V 0.2		LS III (B) compared with LS III V 0.4	
PREINC-Mg 0.4+V 0.2	↑ 7$^{\text{”}}$	PREINC-Mg 0.4+V 0.4	↑ 6$^{\text{"}}$
PREINC-Mg 0.8+V 0.2	↑ 9††	PREINC-Mg 0.8+V 0.4	↑ 6.4‡‡
PREINC-Mg 1.0+V 0.2	↑ 11††	PREINC-Mg 1.0+V 0.4	↑ 8$^{\#}$
PREINC-Mg 1.5+V 0.2	↑ 12§	PREINC-Mg 1.5+V 0.4	↑ 9$^{\text{”}}$
PREINC-Mg 5.0+V 0.2	↑ 17§	PREINC-Mg 5.0+V 0.4	↑ 11††
PREINC-Mg 50+V 0.2	↑ 24§	PREINC-Mg 50+V 0.4	↑ 19§
PREINC-Mg 100+V 0.2	↑ 24.5§	PREINC-Mg 100+V 0.4	↑ 24§
LS III (C) compared with LS III V 0.2		**LS III (D) compared with LS III V 0.4**	
Mg 0.4+V 0.2	↑ 31††	Mg 0.4+V 0.4	↑ 25‡‡
Mg 0.8+V 0.2	↑ 35§	Mg 0.8+V 0.4	↑ 30††
Mg 1.0+V 0.2	↑ 36§	Mg 1.0+V 0.4	↑ 31§
Mg 1.5+V 0.2	↑ 35††	Mg 1.5+V 0.4	↑ 31.5††
Mg 5.0+V 0.2	↑ 37§	Mg 5.0+V 0.4	↑ 34§
Mg 50+V 0.2	↑ 46*	Mg 50+V 0.4	↑ 43*
Mg 100+V 0.2	↑ 47*	Mg 100+V 0.4	↑ 45*

(A) and (B)= LS pre-incubated with different concentrations of $MgSO_4$ and subsequently incubated with 0.2 mM or 0.4 mM $NaVO_3$, respectively; (C) and (D)= LS co-incubated with different concentrations of $MgSO_4$ and with 0.2 mM or 0.4 mM $NaVO_3$, respectively, without Mg pre-incubation; V 0.2 and V 0.4= LS incubated only with 0.2 mM or 0.4 mM $NaVO_3$, respectively. *P* values: *< 0.001; §< 0.01; ††< 0.05; $^{\text{«}}$>0.05; $^{\text{”}}$= 0.05; $^{\#}$= 0.09; ‡‡= 0.10; **= 0.11; $^{\text{"}}$= 0.16; ††= 0.19; $^{\text{«}}$> 0.05

↓= decrease; ↑= increase

induce OS[6,38-45], which is illustrated in Fig. 6. It is well known that V may behave as a pro-oxidant[6] and stimulate LPO, which may cause oxidative destabilization of the cell membrane, decrease its fluidity, increase permeability, and affect its electrical potential, transport and the activity of membrane-bound enzymes, consequently leading to impairment of functions and alterations of cell integrity. It has been suggested that increased LPO and peroxidative damage may be significant factors underlying V toxicity[46], and that excessive unregulated LPO may lead to pathological disorders and diseases. Evidence has been provided that LPO and its products, which due to own chemical reactivity can make modifications of macromolecules, are the basis of the cardiotoxic and neurotoxic effects of V[47] and may be responsible for the inflammatory and early fibrotic changes in the lung[48] and predictive of renal dysfunction[49]. On the other hand, the physiological levels and biological effects of LPO and its products

Table 2—Percentage (%) decrease in the MDA level for LS II obtained from the SMV-intoxicated rats

(A) compared with (C)		(B) compared with (D)	
	Mg 0.4+V 0.2		Mg 0.4+V 0.4
PREINC-Mg 0.4+V 0.2	↓ 26[§]	PREINC-Mg 0.4+V 0.4	↓ 24.2[§]
	Mg 0.8+V 0.2		Mg 0.8+V 0.4
PREINC-Mg 0.8+V 0.2	↓ 22.5[§]	PREINC-Mg 0.8+V 0.4	↓ 21.2[§]
	Mg 1.0+V 0.2		Mg 1.0+V 0.4
PREINC-Mg 1.0+V 0.2	↓ 23.5[§]	PREINC-Mg 1.0+V 0.4	↓ 22[§]
	Mg 1.5+V 0.2		Mg 1.5+V 0.4
PREINC-Mg 1.5+V 0.2	↓ 20[††]	PREINC-Mg 1.5+V 0.4	↓ 21[§]
	Mg 5.0+V 0.2		Mg 5.0+V 0.4
PREINC-Mg 5.0+V 0.2	↓ 22[§]	PREINC-Mg 5.0+V 0.4	↓ 20[§]
	Mg 50+V 0.2		Mg 50+V 0.4
PREINC-Mg 50+V 0.2	↓ 17.4[††]	PREINC-Mg 50+V 0.4	↓ 15.6[‡‡]
	Mg 100+V 0.2		Mg 100+V 0.4
PREINC-Mg 100+V 0.2	↓ 13[‡]	PREINC-Mg 100+V 0.4	↓ 17.3[††]

(A) and (B)= LS II pre-incubated with different concentrations of $MgSO_4$ and subsequently incubated with 0.2 mM or 0.4 mM $NaVO_3$, respectively; (C) and (D)= LS II co-incubated with different concentrations of $MgSO_4$ and with 0.2 mM or 0.4 mM $NaVO_3$, respectively, without Mg pre-incubation. *P* values: [§]< 0.01; [††]< 0.05; [‡]= 0.06; [‡‡]= 0.10

↓= decrease

have not been well elucidated yet[45]. Till now, their real powerful biological role in cell signalling under both pathological and physiological conditions, mainly in cell cycle regulation, has been suggested[1].

The clearly but insignificantly lowered (by 25 and 24 %) hepatic MDA formation in LS III (obtained from the SMV-MS-administered rats) exogenously incubated with 0.2 or 0.4 mM SMV, respectively, compared with LS II (obtained from the SMV-intoxicated rats) incubated with the same exogenous SMV concentrations (Fig. 1), allow a suggestion that the limited MDA generation in LS III might be a consequence of earlier administration of Mg in combination with SMV to rats. The endogenously administered Mg in conjunction with SMV may also be the reason for the distinctly lowered MDA level in LS III compared with that found in LS II during their co-incubation with exogenous 0.4, 0.8, 1.0, 1.5 or 5.0 mM MS and 0.4 mM SMV (without Mg pre-incubation). (Fig. 5D II and III). The above-mentioned findings allow a conclusion that endogenously administered Mg may, to some degree, limit LPO under the conditions of SMV exposure. The protective impact of Mg against the SMV-induced increase in the hepatic LPO may also be confirmed by the fall in the level of MDA generated during the pre-incubation of LS II with exogenous Mg concentrations, especially with 0.4 or 0.8 mM MS, which turned out to be the most effective in limiting the MDA formation in these supernatants during their later incubation with exogenous 0.2 mM SMV (Fig. 3A). The latter results suggest that pre-incubation with some exogenous Mg concentrations may stabilize the plasma membrane which was thereby able to withstand the LPO induced exogenously by SMV. The markedly higher MDA generation found in LS II during their co-incubation with addition of exogenous concentrations of MS and SMV (Fig. 3C and D) may confirm this conclusion. However, similar effects have not been observed in LS I (Fig. 2A and B), in which significantly reduced MDA generation was only demonstrated during co-incubation thereof with exogenous 0.4 mM SMV and 0.4 or 1.0 mM MS without Mg pre-incubation (Fig. 2D). The visible trends toward a decrease in the hepatic LPO in the presence of some MS concentrations had also been found previously[3].

The beneficial Mg action reflected in the limitation of the intensity of LPO under the conditions of SMV exposure (Figs 1, 2D, 3A, 3B, and 5D) could be a possible outcome of its anti-peroxidative properties[50]. Mg has also been suggested to activate the synthesis of membrane phospholipids and play a pivotal role in regulating the membrane structure and maintaining membrane integrity[51,52]. The probable mechanisms underlying the inhibitory effect of Mg on LPO and the scavenging activity of this element[8,12,25,31,32,34,36,50,52-56] are presented in Fig. 7. At this stage of the studies, it is difficult to explain the exact mechanism underlying the beneficial Mg action. Given the similarity of the VO^{2+} ion to the Mg^{2+} ion[57], competition between V and Mg for binding sites to membrane should not be excluded.

Table 3—Studies on the influence of Mg administration on the level of MDA/TBARS

Compounds	Species of animals/Subject description	Concentration/Dose	Route	Period of administration/ Treatment	Organs/blood/ supernatants	Ref.
		Animal studies				
$MgSO_4$ + DMM	S-D rats (♂)	10 mg/kg + 10 mg/kg	orally	3 d after 72 h DMM intoxication	↓ in the liver, kidney, brain	17
Fru-$MgCl_2$	W rats (♂)	1.0 %	diet	4 wk	↓ in the plasma	18
$MgSO_4$ + L-NAME	P S-D rats (♀)	600 mg/kg body wt./24 h + 50 mg/kg body wt./24 h	dw	from 11th to 19th d of pregnancy	↙ in the plasma	19
$MgSO_4$	S-D rats (♂)	50 and 100 mg/kg	iv	after R	↓ in the lung	20
$MgSO_4$	S-D rats (♀)	600 mg/kg	ip	20 min before IRI	↓ foetal skin	21
$MgSO_4$	S-D rats (♀)	600 mg/kg	ip	after BI	↓ in the brain	22
$MgSO_4$	rats (♂)	600 mg/kg	sc	1 h after SCI	↓ in the spinal cord	23
$MgSO_4$		300 and 600 mg/kg body wt.		single injection	↓ in the liver	24
$MgSO_4$ + $CdCl_2$	W rats	300 and 600 mg/kg body wt. + 2.5 mg/kg body wt.	ip	5 and 10 d	↓ in the liver, kidney	24
$MgSO_4$	W-D rats (♂)	0.9 mg/mL	dw	4 wk	↓ in the plasma, liver	25
$MgSO_4$	P rats	600 mg Mg/kg	diet	30 min before IRI	↓ in the brain	26
$MgSO_4$	NZ rabbits (♂)	100 mg/kg	iv	5 min	↓ in the brain	27
$MgSO_4$	NZ rabbits	100 mg/kg	iv	after HT	↓ in the brain	28
$MgSO_4$	Japanese quails	600 mg Mg/kg	diet	30 d	↓ in the plasma	29
MgO	Japanese quails	1.0 and 2.0 g Mg/kg	diet	42 d	↓ in the serum, liver	30
Mg-PROT		1.0 and 2.0 g Mg/kg			↓ in the serum, liver	30
MgO	Broiler chicks	2.0 g Mg/kg	diet	42 d	↓ in the liver	31
Mg-PROT		2.0 g Mg/kg			↓ in the liver	31
$MgCl_2$ + 1 % sugar solution	*Drosophila melanogaster* (♂)	0.1, 0.25 and 0.5 M	diet	24 h	↙ in the fly supernatants	32
		Human studies				
$MgSO_4$	Neonates	0.5 mEq kg^{-1}/d	TPN	just after birth according to PFP	↓ in the erythrocytes	33
$MgSO_4$	PEPW	4 g followed by a maintenance dose of 1 g/h	iv	24 h	↓ in the erythrocyte ghosts	34
$MgSO_4$	PEW	4 g followed by a maintenance dose of 1 g/h	iv	at delivery and 24 h postpartum	↓ in the serum	35
Compounds	Cell cultures	Concentration	Incubation time		MDA/TBARS level	Ref.
		In vitro studies				
$MgSO_4$	MixNeurGCC	3 mM	26 h		↓	36
$MgCl_2$		2 and 4 mmol/dm^3	1, 2 and 5 h		↓	37
$MgCl_2$ + Et	RHepatC	2 and 4 mmol/dm^3 + 150 mmol/dm^3	5 h		↓	37

S-D rats= Sprague-Dawley rats; P S-D rats= pregnant Sprague-Dawley rats; W rats= Wistar rats; W-D rats= Wistar diabetic rats; P rats= pregnant rats; NZ rabbits= New Zealand rabbits; PEPW= preeclamptic pregnant women; PEW= preeclamptic women; $MgSO_4$= magnesium sulphate; DMM= dimetyl mercury; Fru-Mg= fructose fed Mg supplemented; L-NAME= L-nitro-L-arginine methyl ester; $MgCl_2$= magnesium chloride; $CdCl_2$= cadmium chloride; MgO= magnesium oxide; Mg-PROT= magnesium proteinate; TPN= total parenteral nutrition; PFP= parenteral feeding protocol; Et= ethanol; R= reperfusion; SCI= spinal cord injury; IRI= ischemia reperfusion injury; BI= brain injury; HT= head trauma; MixNeurGCC= mixed neuronal and glial cortical cultures; RHepatC= rat hepatocyte cultures; dw= drinking water; ip= intraperitoneal; iv= intravenous; sc= subcutaneous; d= day; wk= week; mo= month; h= hour; min= minutes
↓= decrease; ↙= a trend toward a decrease

On the other hand, an unfavourable influence of exogenous Mg concentrations on the hepatic LPO reflected in the enhanced MDA generation has also been revealed. These effects have been demonstrated in the case of LS I pre-incubated with exogenous Mg concentrations and incubated later with exogenous 0.2 or 0.4 mM SMV (Fig. 2A and B) as well as in the case of LS III under both kinds of incubation conditions used (Fig. 4A-D). Thus, the above-mentioned findings point to a stimulating effect of Mg

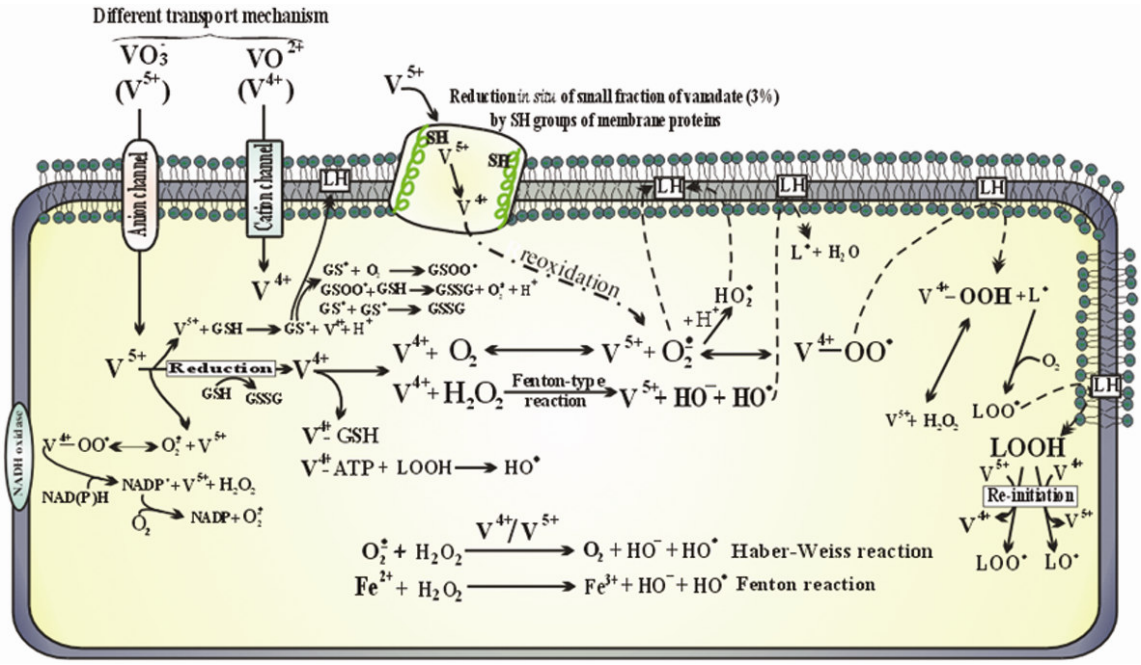

Fig. 6—Some mechanisms underlying V toxicity illustrated on the basis of the selected literature data[6,38-45]. VO_3^-= vanadate ion (V^{5+}= +5 oxidation state); VO^{2+}= vanadyl ion (V^{4+}= +4 oxidation state); $O_2^{\bullet-}$= superoxide radical; $HO^{\bullet}$= hydroxyl radical; LH= polyunsaturated lipid; $L^{\bullet}$= lipid alkyl radical; $LO^{\bullet}$= lipid alkoxyl radical; $LOO^{\bullet}$= lipid peroxyl radical; LOOH= lipid hydroperoxide; V^{4+}-$OO^{\bullet}$= peroxy-vanadyl-type intermediate; V^{4+}-OOH= vanadyl hydroperoxide (pervanadate); GSH= reduced glutathione; GSSG= oxidized glutathione; $GS^{\bullet}$= glutathionyl radical; $GSOO^{\bullet}$= glutathionyldioxyl radical; NADPH= reduced nicotinamide adenine dinucleotide phosphate.

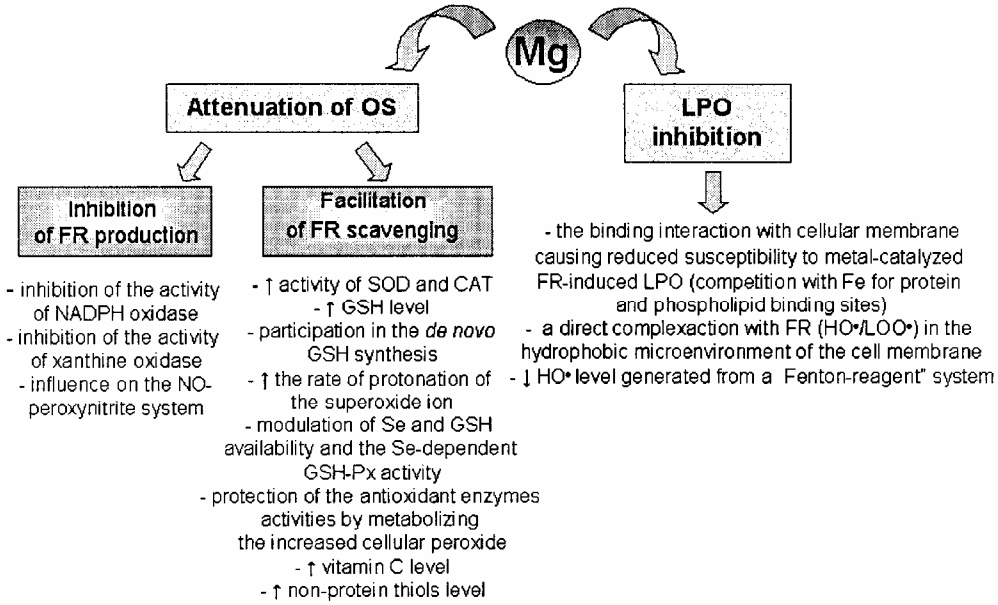

Fig. 7—Possible mechanisms of the antioxidant Mg action illustrated on the basis of some literature data[8,12,25,31,32,34,36,50,52-56]. OS= oxidative stress; FR= free radicals; LPO= lipid peroxidation; NADPH= reduced nicotinamide adenine dinucleotide phosphate; SOD= superoxide dismutase; CAT= catalase; GSH= reduced glutathione; GHS-P× = glutathione peroxidase; $HO^{\bullet}$= hydroxyl radical; $LOO^{\bullet}$= lipid peroxyl radical. ↑= increase; ↓= decrease.

on LPO, which was also shown in a previous *in vitro* experiment[3].

In conclusion, the results of the present study show that Mg may be beneficial to counteract the V pro-oxidant potential but on the other hand they also reveal its stimulating action on the hepatic LPO. Additionally, they provide evidence that also the incubation conditions and the experimental treatment of the rats from which the liver supernatants were obtained affect the intensity of LPO.

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References

1 Guéraud F, Atalay M, Bresgen N, Cipak A, Ecki P M, Huc L, Jouanin I, Siems W & Uchida K, Chemistry and biochemistry of lipid peroxidation products, *Free Rad Res*, 44 (2010) 1098.

2 Evangelou A M, Vanadium in cancer treatment, *Crit Rev Oncol Hematol*, 42 (2002) 249.

3 Ścibior A, Zaporowska H & Niedźwiecka I, Lipid peroxidation in the liver of rats treated with V and/or Mg in drinking water, *J Appl Toxicol*, 29 (2009) 619.

4 Ścibior A, Zaporowska H & Niedźwiecka I, Lipid peroxidation in the kidney of rats treated with V and/or Mg in drinking water, *J Appl Toxicol*, 30 (2010) 487.

5 Ścibior A & Zaporowska H, Effects of combined vanadate and magnesium treatment on erythrocyte antioxidant defence system in rats, *Environ Toxicol Pharmacol*, 30 (2010) 153.

6 Byczkowski A Z & Kulkarni A P, Oxidative stress and pro-oxidant biological effects of vanadium, in *Vanadium in the environment part II: Health effects*, edited by J O Nriagu (John Wiley & Sons, New York) 1998, 235.

7 Yang X G, Yang X D, Yuan L, Wang K & Crans D C, The permeability and cytotoxicity of insulin-mimetic vanadium compounds, *Pharm Res*, 21 (2004) 1026.

8 Afanas'ev I B, Suslova T B, Cheremisina Z P, Abramova N E & Korkina L G, Study of antioxidant properties of metal aspartates, *Analyst*, 120 (1995) 859.

9 Hong Y C, Paik S R, Lee H J, Lee K H & Jang S M, Magnesium inhibits nickel-induced genotoxicity and formation of reactive oxygen, *Environ Health Perspect*, 105 (1997) 744.

10 Maulik D, Zanelli S, Numagami Y, Ohnishi S T, Mishra O P & Delivoria-Papadopoulos M, Oxygen free radical generation during in-utero hypoxia in the fetal guinea pig brain: the effects of maturity and of magnesium sulfate administration, *Brain Res*, 817 (1999) 117.

11 Mak I T, Komarov A M, Kramer J H & Weglicki W B, Protective mechanisms of Mg-gluconate against oxidative endothelial cytotoxicity, *Cell Mol Biol*, 46 (2000) 1337.

12 Murthi S B, Wise R W, Weglicki W B, Komarov A M & Kramer J H, Mg-gluconate provides superior protection against postischemic dysfunction and oxidative injury compared to Mg-sulfate, *Mol Cell Biochem*, 245 (2003) 141.

13 Dickens B F, Weglicki W B, Li Y S & Mak I T, Magnesium deficiency *in vitro* enhances free radical-induced intracellular oxidation and cytotoxicity in endothelial cells, *FEBS Lett*, 311 (1992) 187.

14 Bussière F I, Gueux E, Rock E, Girardeau J P, Tridon A, Mazur A & Rayssiguier Y, Increased phagocytosis and production of reactive oxygen species by neutrophils during magnesium deficiency in rats and inhibition by high magnesium concentration, *Br J Nutr*, 87 (2002) 107.

15 Ścibior A, Gołębiowska D & Niedźwiecka I, Magnesium can protect against vanadium-induced lipid peroxidation in the hepatic tissue, *Oxid Med Cell Longev*, Special Issue: *Lipid peroxidation products in human health and disease (LPPHHD)*, 2013 (2013) 11.

16 Ścibior A, Adamczyk A, Gołębiowska D & Niedźwiecka I, Effect of 12-week vanadate and magnesium co-administration on chosen haematological parameters as well as on some indices of iron and copper metabolism and biomarkers of oxidative stress in rats, *Environ Toxicol Pharmacol*, 34 (2012) 235.

17 Shukla S, Singh V & Joshi D, Modulation of toxic effects of organic mercury by different antioxidants, *Toxicol Int*, 14 (2007) 67.

18 Olatunji L A & Soladoye A O, Increased magnesium intake prevents hyperlipidemia and insulin resistance and reduces lipid peroxidation in fructose-fed rats, *Pathophysiology*, 14 (2007) 11.

19 Cinar M, Yildirim E, Yigit A A, Macun H C & Duru O, Effects of magnesium sulphate on the oxidative stress, biochemical and hematological parameters in pregnant rats with L-name induced preeclampsia, *Fresenius Environ Bull*, 20 (2011) 1942.

20 Kao M C, Jan W C, Tsai P S, Wang T Y & Huang C J, Magnesium sulfate mitigates lung injury induced by bilateral lower limb ischemia-reperfusion in rats, *J Surg Res*, 171 (2011) 97.

21 Kaptanoglu A F, Arca T & Kilinc K, Magnesium sulfate protects fetal skin from intrauterine ischemia reperfusion injury, *Arch Dermatol Res*, 304 (2012) 529.

22 Turkoglu O F, Eroglu H, Okutan O, Tun M K, Bodur E, Sargon M F, Öner L & Beskonakli E, A comparative study of treatment for brain edema: magnesium sulphate versus dexamethasone sodium phosphate, *J Clin Neurosci*, 15 (2008) 60.

23 Süzer T, Coskun E, Islekel H & Tahta K, Neuroprotective effect of magnesium on lipid peroxidation and axonal function after experimental spinal cord injury, *Spinal Cord*, 37 (1999) 480.

24 Boujelben M, Ghorbel F, Vincent C, Makni-Ayadi F, Guermazi F, Croute F & El-Feki A, Lipid peroxidation and HSP72/73 expression in rat following cadmium chloride administration: interactions of magnesium supplementation, *Exp Toxicol Pathol*, 57 (2006) 437.

25 Hans C P, Chaudhary D P & Bansal D D, Effect of magnesium supplementation on oxidative stress in alloxanic diabetic rats, *Mag Res*, 16 (2003) 13.

26 Solaroglu A, Dede F S, Gelisen O, Secilmis O & Dede H, Neuroprotective effect of magnesium sulfate treatment on

fetal brain in experimental intrauterine ischemia reperfusion injury, *J Matern Fetal Neonatal Med*, 24 (2011) 1259.

27 Bariskaner H, Ustun M E, Ak A, Yosunkaya A & Ulusoy H B, Effects of magnesium sulfate on tissue lactate and malondialdehyde levels after cerebral ischemia, *Pharmacology*, 68 (2003) 162.

28 Üstün M E, Gürbilek M, Ak A, Vatansev H & Duman A, Effects of magnesium sulfate on tissue lactate and malondialdehyde levels in experimental head trauma, *Intensive Care Med*, 27 (2001) 264.

29 Kucuk O, Zinc in a combination with magnesium helps reducing negative effects of heat stress in quails, *Biol Trace Elem Res*, 123 (2008) 144.

30 Sahin N, Onderci M, Sahin K, Cikim G & Kucuk O, Magnesium proteinate is more protective than magnesium oxide in heat-stressed quail, *J Nutr*, 135 (2005) 1732.

31 Guo Y, Zhang G, Yuan J & Nie W, Effects of source and level of magnesium and vitamin E on prevention of hepatic peroxidation and oxidative deterioration of broiler meat, *Anim Feed Sci Tech*, 107 (2003) 143.

32 Matkovics B, Kiss I & Kiss S A, The activation by magnesium treatment of anti-oxidants eliminating the oxygen free radicals in *Drosophila melanogaster in vivo*, *Mag Res*, 10 (1997) 33.

33 Gulczynska E, Gadzinowski J, Wilczynski J & Zylinska L, Prenatal $MgSO_4$ treatment modifies the erythrocyte band 3 in preterm neonates, *Pharmacol Res*, 53 (2006) 347.

34 Abad C, Teppa-Garrán A, Proverbio T, Piñero S, Proverbio F & Marín R, Effect of magnesium sulfate on the calcium-stimulated adenosine triphosphatase activity and lipid peroxidation of red blood cell membranes from preeclamptic women, *Biochem Pharmacol*, 70 (2005) 1634.

35 Ariza A C, Bobadilla N, Fernández C, Muñoz-Fuentes R M, Larrea F & Halhali A, Effects of magnesium sulfate on lipid peroxidation and blood pressure regulators in preeclampsia, *Clin Biochem*, 38 (2005) 128.

36 Regan R F, Jasper E, Guo Y & Panter S S, The effect of magnesium on oxidative neuronal injury *in vitro*, *J Neurochem*, 70 (1998) 77.

37 Niedworok E, Wardas M, Stec M & Dołowy M, Ocena procesu peroksydacji lipidów modyfikowanego jonami magnezu z etanolem w izolowanych hepatocytach szczura, *Nowiny Lek*, 71 (2002) 295.

38 Schöneich C, Asmus K D, Dillinger U & Bruchhausen F, Thiyl radical attack on polyunsaturated fatty acids: a possible route to lipid peroxidation, *Biochem Biophys Res Commun*, 161 (1989) 113.

39 Shi X L, Sun X Y & Dalal N S, reaction of vanadium (V) with thiols generates vanadium (IV) and thiyl radicals, *FEBS Lett*, 271 (1990) 1885.

40 Minotti G & Aust S D, Redox cycling of iron and lipid peroxidation, *Lipids*, 27 (1992) 219.

41 Sakurai H, Vanadium distribution in rats and DNA cleavage by vanadyl complex: implication for vanadium toxicity and biological effects, *Environ Health Perspect*, 102 (1994) 35.

42 Zhang B, Ruan L, Chen B, Lu J & Wang K, Binding of vanadate to human erythrocyte ghosts and subsequent events, *BioMetals*, 10 (1997) 291.

43 Cam M C, Brownsey R W & McNeill J H, Mechanisms of vanadium action: insulin-mimetic or insulin-enhancing agent ?, *Can J Physiol Pharmacol*, 78 (2000) 829.

44 Jelikić-Stankov M, Uskoković-Marković S, Holclajtner-Antunović I, Todorović M & Djurdjević P, Compounds of Mo, V and W in biochemistry and their biomedical activity, *J Trace Elem Med Biol*, 21 (2007) 8.

45 Niki E, Lipid peroxidation: physiological levels and dual biological effects, *Free Rad Biol Med*, 47 (2009) 469.

46 Elfant M & Keen C L, Sodium vanadate toxicity in adult and developing rats, *Biol Trace Elem Res*, 14 (1987) 193.

47 Donaldson J & LaBella F, Prooxidant properties of vanadate *in vitro* on catecholamines and on lipid peroxidation by mouse and rat tissues, *J Toxicol Environ Health*, 12 (1983) 119-126.

48 Zychlinski L, Byczkowski J Z & Kulkarni A P, Toxic effects of long-term intratracheal administration of vanadium pentoxide in rats, *Arch Environ Contam Toxicol*, 20 (1991) 295.

49 Donaldson J, Hemming R & LaBella F, Vanadium exposure enhances lipid peroxidation in the kidney of rats and mice, *Can J Physiol Pharmacol*, 63 (1985) 196.

50 Mak I T, Chmielinska J J, Kramer J H & Weglicki W B, AZT-induced cardiovascular toxicity: attenuation by Mg-supplementation, *Cardiovasc Toxicol*, 9 (2009) 78.

51 Zdanowicz M M & Barletta M A, Magnesium protection against anthracycline toxicity *in vitro*, *Magnes Res*, 4 (1991) 105.

52 Laires M J & Monteiro C, Exercise and magnesium, in *New perspectives in magnesium research. Nutrition and health*, edited by Y Nishizawa, H Morii & J Durlach (Springer, London) 2007, 173.

53 Schaich K M, Metals and lipid oxidation. Contemporary issues, *Lipids*, 27 (1992) 209.

54 Garcia L A, Dejong S C, Martin S M, Smith R S, Buettner G R & Kerber R E, Magnesium reduces free radicals in an *in vivo* coronary occlusion-reperfusion model, *J Am Coll Cardiol*, 32 (1998) 536.

55 Zhang Y, Davies L R, Martin S M, Bawaney I M, Buettner G R & Kerber R E, Magnesium reduces free radical concentration and preserves left ventricular function after direct current shocks, *Resuscitation*, 56 (2003) 199.

56 Vernet P, Britan A, Gueux E, Mazur A & Drevet J R, Dietary magnesium depletion does not promote oxidative stress but targets apical cells within the mouse caput epididymidis, *Biochem Biophys Acta*, 1675 (2004) 32.

57 Badmaev V, Prakash S & Majeed M, Vanadium: a review of its potential role in the fight against diabetes, *J Altern Complement Med*, 5 (1999) 273.