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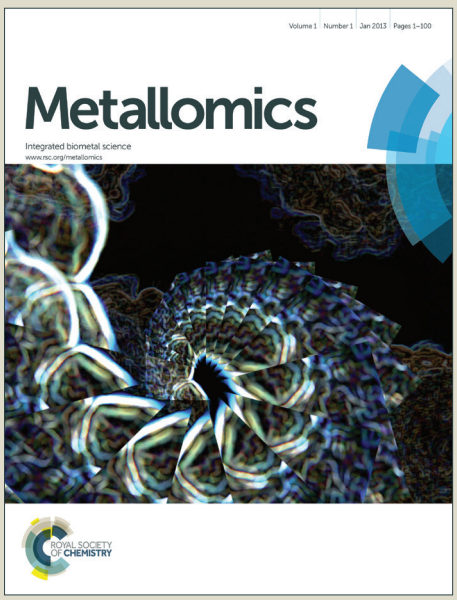

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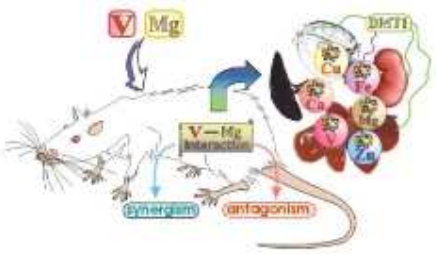

Text:

The levels of elements in rats'organs in the vanadium-magnesium interaction and a role of DMT-1 in the mechanism(s) of the SMV-induced disorders in tissue Fe homeostasis

The influence of combined magnesium and vanadate administration on the level of some elements in selected rats' organs: V-Mg interactions and the role of iron-essential protein (DMT-1) in the mechanism underlying altered tissues iron level

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Abstract

The effect of 12-week co-administration of sodium metavanadate (SMV) and magnesium sulphate (MS) on the levels of some elements in selected rats' organs and an attempt to elucidate a role of divalent metal transporter 1 (DMT-1) in the mechanism(s) of the SMV-induced disorders in some tissue Fe homeostasis were studied. SMV taken up separately or in combination with MS may pose a risk of the rise and shortage of the total hepatic and splenic Fe and Cu contents, respectively, cerebral Fe deficiency, splenic Ca deposition, and the hepatic, renal, and cerebral DMT-1 down-regulation. When administered alone, SMV may also cause the decrease in the total renal Fe and Cu contents. A visible protective effect of Mg against the renal and cerebral V accumulation and the decrease in the renal Fe and Cu contents during the SMV-MS co-administration together with our previous findings suggest a beneficial role of Mg at SMV exposure. Further, the SMV-induced fall in total iron binding capacity (TIBC), reported previously, and its correlations with the hepatic, splenic, and cerebral Fe levels allow us to suggest that diminished TIBC could be partly involved in the mechanism(s) responsible for the dramatic redistribution of Fe in those tissues. Finally, DMT-1, which potentially could participate in the hepatic non-transferrin Fe-bound uptake, does not play a significant role in this process indicating the need for studying other Fe transporters to more precisely elucidate molecular mechanism(s) underlying the hepatic Fe loading in our experimental conditions.

Key words: Bioelements, Divalent metal transporter 1, Interactions, Organs, Rats, Vanadate

1 Introduction

Vanadium (V) is a widely dispersed element.[1] It may be mutagenic, teratogenic, and potentially carcinogenic.[2-4] On the other hand, its essentiality for biological activity in most living animals and promising pharmacological properties related to diabetes, cancer and osteoporosis have also been described.[1,5]

Due to the wide varieties of uses, there is an increased risk for V poisoning. The exposure to this metal may turn out to be both occupationally and environmentally inducing harmful health effects.[6] The toxic action of V has been reported to result not only from inhibition of activity of many enzymes, or alterations in protein phosphorylation,[7,8] but also from interactions with essential elements. As vanadate (VO_3^-), it competes with phosphate at the active sites on the phosphate transport proteins, whereas as vanadyl (VO^{2+}), it competes with transition metal ions for binding sites on metalloproteins.[9] The possible competition of VO^{2+} with a simple divalent ions such as Ca^{2+}, Mn^{2+}, Zn^{2+}, and Fe^{2+} for ligand binding sites has been reported.[10] V also interferes with thiol group-containing compounds and sulphur metabolism.[11]

Due to the fact that animals and humans may suffer from toxicity of V,[1,6,12,13] which as a transition metal, that may intensify oxidative stress (OS) in the cells and tissues in some conditions by participating in the reactions involving the reactive oxygen species (ROS) formation,[3] our particular interest has been focused on searching factors which might prevent accumulation of V and its deleterious action. An agent which may interact with V due to similarity to its VO^{2+} form and protect from V damaging effects is magnesium (Mg). Mg has been reported to restore in part the antioxidant parameters and decrease OS in rats.[14] Its beneficial effects against poisoning with such metals as lead,[15,16] chromium,[17] nickel,[18] mercury,[19] arsenic,[20] and cadmium[21,22] have been published as well. Recently, we have demonstrated in an animal model that Mg may also have a protective impact against V toxicity. We showed that the supplementation of rats with Mg as magnesium sulphate ($MgSO_4$, MS) during sodium metavanadate ($NaVO_3$, SMV) intoxication, at least partly, normalized some parameters of the Fe status and limited the increase in the level of V in the blood. We revealed that all these beneficial effects of Mg demonstrated at the SMV exposure resulted from the independent action of Mg and/or from its antagonistic interaction with V, or from the clear tendency toward the above-mentioned Mg behaviour.[23] Attenuation of the pro-

oxidant potential of V by Mg in *in vivo* and *in vitro* conditions has also been observed in our experimental conditions.[24,25]

Although extensive data about the interactions between V and some elements has already been published,[26-31] the data about the V-Mg interactions is still scarce. Beside our reports [23,24,32-35] there are only single papers about this issue in the literature. [36-39] The consequences of the V-Mg interactions for health are also still poorly investigated. Therefore, the interactions between V and Mg have been an area of our interest. Their examination not only still seems to be needed, given that the chronic V intoxications correspond to environmental and/or occupational contamination by this metal, but it is very important especially for extending the knowledge of the mechanism of V effect on organisms and a potential role of Mg in prevention of its toxicity.

It is a well-known fact that examination of interactions among metals and recognition of synergistic or antagonistic effects among them may have a significant medical aspect. Moreover, the interactive effects revealed among the elements may be used to elucidate the cellular mechanisms of the response to combinations of metals. Further, it is also clear that the therapeutic or toxic effect of the elements is very often dependent on not only the dose, time of exposure and concentrations, but also on the synergistic or antagonistic cooperation among them, and that uptake of one element in the amount exceeding the physiological level may be highly toxic and cause release, redistribution, and urinary elimination of other elements, thereby disrupting all the metabolic processes, in which they perform regulatory functions. It is also generally known that essential metal deficiencies do exert health effects associated with toxic metal exposures and that adequate dietary essential metals are necessary for prevention and intervention of metal toxicities.[40] Furthermore, the processes in the body that regulate the absorption and transport of essential elements have been suggested to be related to the processes that determine the body levels of toxic metals.[41] Therefore, taking all the above into account and the fact that exploration of the role of any element in an organism

(cell metabolism) is pointless if its relationship with various micro- and macroelements is not taken into consideration, as only a combination thereof is vital in crossing the organism protective barriers, we undertook the studies to check on a rat model a) whether and to what extent the effect of a 12-week co-administration of SMV and MS alters the total content/concentration of V and Mg and other macro- and microelements (Ca, Fe, Zn, and Cu) in the selected rats' organs, b) which changes in the levels of the above mentioned elements are modified by an independent action of V, Mg, or/and by the V-Mg interaction, c) what kind of interaction revealed between V and Mg with respect to the indices tested is, d) to what degree the effects of administration of SMV and MS in combination differ from those observed under the administration of each compound separately, e) to what extent Mg (at the selected level) co-applied with SMV will be able to limit the accumulation of V in the rats' examined organs, and finally f) whether iron important protein - the divalent metal transporter 1 (DMT-1) plays a role in the mechanism(s) underlying disorders in homeostasis of tissues Fe levels in our experimental conditions. According to our knowledge, no similar reports which would describe changes in the parameters examined under the conditions of simultaneous administration of V and Mg at the selected level and reveal the type of V-Mg interaction referring to those indices have been published up until now. In the literature data about changes (increase or decrease) or their absence in the levels of such elements as Mg, Ca, Fe, Zn, and Cu in some organs/tissues at administration of various V or Mg compounds has been published for different species of animals (also for rats) (Table 1), [26,34,38,39,42-56] however, it was difficult to compare the results presented in this report with those described by other researchers because of different experimental conditions used.

2 Materials and methods

2.1 Reagents

$NaVO_3$ and $MgSO_4$ were obtained from Sigma Chemical (St. Louis, USA), whereas all the reagents used for digestion of the rats' examined tissues and for determination of some elements by the atomic absorption spectrometry (AAS) method in those tissues were acquired from Merck (Darmstadt, Germany): nitric acid (HNO_3, 65% suprapure); from Sigma Chemical (St. Louis, USA): the caesium chloride lanthanum chloride buffer (CsClLaCl) and Triton X-100; from Inorganic ™ Ventures (Christiansburg, USA): stock V, Mg, Fe, Zn, and Cu atomic absorption standard solutions; and from Spectracer (UK): a stock of Ca atomic absorption standard solution. The kits for determination of the levels of the divalent metal transporter 1 (DMT-1) in some rats' tissues homogenates (E94426Ra) were, in turn, derived from Uscn Life Science Inc. (Wuhan, China). The physiological buffered saline (PBS) was purchased from POCH (Gliwice, Poland). Ultra-pure water was received from an ultra-pure water HLP Spring 5R system [1] (Hydrolab, Gdańsk, Poland).

2.2 Instrumentation

Samples of some rats' internal organs (liver, kidney, spleen, and cerebral hemisphere) for quantitative determination of selected elements were firstly digested using the model Speedwave Four microwave digestion system [2] (Berghof, Germany) which was equipped with a temperature and pressure sensor in each vessel. V, Mg, Fe, Zn, Cu, and Ca in the above-mentioned organs were determined by Flame or Graphite Furnace Atomic Absorption Spectrometry (F-AAS and GF-AAS) using the SpectrAA Z2000 TANDEM atomic absorption spectrometer [3] (Hitachi, Japan) equipped with a Zeeman background corrector. The level of DMT-1 in the liver, kidney and cerebral hemisphere was, in turn, assessed using an ELISA

[1-7] They were bought as part of the Project entitled: "Building of the Centre of Interdisciplinary Research" realized within the framework of the Operational Programme "Development of Eastern Poland" 2007-2013, Priority I: Modern Economy, Action I.3. The Advancement of Innovation, co-financed by the European Regional Development Fund.

microplate reader Synergy 2 [4] equipped with an automated microplate strip washer ELx50 [5] (BIO-TEK Instruments Inc., USA).

2.3 Animal protocol

The biological material used in this study was obtained from the selected 40 outbred albino male Wistar rats used in our previous experiment[23] (conducted in accordance with the experimental protocol approved by the 1st Local Ethical Committee for Animal Studies in Lublin). All the rats were divided into 4 groups (10 rats per group) and received every day over a 12-week period in special bottles with the scale: *Group I* (*Control*): deionised water; *Group II*: water solution of $NaVO_3$ (SMV) at concentration of 0.125 mg V per mL (pH = 7.2); *Group III*: water solution of $MgSO_4$ (MS) at concentration of 0.06 mg Mg per mL (pH = 5.7); *Group IV*: water solution of SMV-MS (pH = 7.1) at the same concentrations as in Groups II and III for V and Mg, respectively. SMV, MS and SMV-MS were supplied as drinking solutions. Each rat in Groups I-IV had the same bottle and each of them received the same volume of the deionised water and underwent the above-mentioned solutions throughout the experimental time. The stock solutions were replaced by freshly prepared solutions every 2 days. No changes in their pH were observed during the storage period. All the animals were maintained individually in stainless steel cages under controlled conventional conditions provided previously[23] and had *ad libitum* access to fresh fluids and a standard diet (Labofeed B, Fodder and Concentrate Factory, Kcynia, Poland). Volumes of deionised water (Group I) and the SMV, MS, and SMV-MS solutions (Groups II-IV, respectively) consumed by each rat were measured each day during the whole experiment. The consumption of food was also monitored daily, whereas body weight was checked weekly throughout the experimental period. More details about the experiment had already been described by us in our earlier report[23].

After the 12-week SMV or/and MS administration, the liver, both kidneys, spleen, and brain were removed from all anesthetized rats. They were immediately washed in ice-cold physiological saline (0.9% NaCl) and weighed. The cerebral hemisphere was gently separated from the cerebellum, and then both were rewashed in 0.9% NaCl and reweighed. The parts of those organs were stored frozen at –80 °C in deep freezer HFU 486 Basic [6] (Thermo Fisher Scientific, Germany) until the time of mineralization. The rest of biological material was also frozen at –20 °C or –80 ºC until further biochemical analysis.

2.4 Decomposition of some internal organs

In order to determine V, Mg, Ca, Fe, Zn, and Cu in the liver, kidney, spleen, and cerebral hemisphere, 0.5 g of the mentioned organs were wet-mineralized in the presence of 5 ml of 65% HNO_3 in Teflon Fluor Modified (TFM) vessels. Appropriate microwave digestion programs for digestion of the above-mentioned biological material were used. Blank solutions, *i.e.* 1 ml of ultra-pure water plus 5 ml of 65% HNO_3 were placed in five TFM vessels and digested following the same procedures as for the liver, kidney, spleen, and cerebral hemisphere samples in order to check contaminations from the reagents and containers that were in contact with the sample solution. More details about digestion of the rats' tissues had already been provided in our previous reports.[23,57]

2.5 The measurement of selected elements in some rats' organs by AAS method

All the operating parameters of the SpectrAA Z2000 TANDEM atomic absorption spectrometer with details of measurements of V, Mg, Fe, Zn, Cu, and Ca in the liver, kidney, spleen, and cerebral hemisphere had already been provided by us in our other papers.[23,57] Only the limits of detections (DL, LOD) for elements investigated in particular rats' organs, not mentioned previously, have been added in the present report and placed in the Results section. The total hepatic, renal and splenic V, Mg, Ca, Fe, Zn, and Cu contents were

calculated on the basis of the concentrations of those metals in the liver, kidney, and spleen and the weights of the mentioned organs.

2.6 Determination of DMT-1 in some internal organs

The level of DMT-1 in the liver, kidney and cerebral hemisphere was assessed by traditional method using rat-specific commercial enzyme-linked immunosorbent assay (ELISA) kits. The ELISA tests were performed according to the manufacturer's protocols. Before the measurements homogenates of the liver, kidney, and cerebral hemisphere were prepared. Briefly, 150 mg of the above-mentioned organs were homogenized in 1 mL of ice-cold PBS. Next, the resulting suspension was subjected to two freeze-thaw cycles to further break the cell membranes. After that, the homogenates were centrifuged with cooling (5000 x g, 5 min, 4 °C) using a centrifuge Heraeus Megafuge 11R [7] (Thermo Fisher Scientific, Germany). The optimal factors of dilution for samples were chosen when necessary. The samples were diluted with PBS (pH 7.4 ± 0.2) and immediately used for analysis. The level of DMT-1 in the samples was determined by comparing the optical density of the samples to the standard curve. Finally, the level of DMT-1 in the rats' examined organs was normalized per weight of tissue.

2.7 Statistical analysis

The statistical analysis of the data was performed with the Statistica and SPSS, version 9.0 and 14.0 PL for Windows, respectively. The distribution of the data was tested with Shapiro Wilk's normality test. When the data had normal distribution, the homogeneity of variances was verified employing Levene's test. Hartley's Fmax, Cochran's C, and Bartlett's tests were carried out when the hypothesis of equal variances was rejected by Levene's test. Next, the two-way analysis of variance (two-way ANOVA; factors of V and Mg, test F) was employed. If the two-way ANOVA indicated the significant effect of V, Mg or the V-Mg interaction,

subsequent post hoc comparisons between the four individual groups were performed. The Tukey multiple range test or the T3 Dunnett test were carried out when the data had normal distribution but the variances were or were not homogenous, respectively. Moreover, when the two-way ANOVA test showed the trend toward V-Mg interaction or the significant interactive effect between V and Mg, subsequent calculations were done in order to describe the kind of the interaction that had been revealed. To this end, the sum of the effects of V and Mg action administered separately was compared with the effect of both co-applied elements. Thus, when co-administration of V and Mg produced smaller (V+Mg effect<V effect+Mg effect) or greater (V+Mg effect>V effect+Mg effect) effect than the sum of the action of V and Mg applied separately, the interaction was recognized as antagonistic or synergistic, respectively.[58] In addition, graphs confirming the interaction between V and Mg or the trend toward the V-Mg interaction were included in the paper as well and illustrated in Figure 2A-E. The Student's t-test for independent samples was also applied for detection of significant differences between the V doses consumed by the rats in Groups II and IV and Mg doses ingested by the rats in Groups III and IV. The *P* value of 0.05 or less was taken as a criterion for statistically significant difference. Pearson's correlation analysis for all the rats was applied to assess the relationships among the measured variables. Moreover, correlations among some indices of Fe status such as total iron binding capacity (TIBC) and transferrin saturation (TS)[23] and the total content/concentration of Fe in the liver, kidney, spleen, and cerebral hemisphere (this report) were estimated as well. Correlations were considered statistically significant at $P < 0.05$.

3 Results

3.1 Some basic indices

The fluid and food intake as well as body weight were expressed in terms of percentage rate of the control group and illustrated in Figure 1A, 1C, and 1D, respectively, whereas the V and

Mg doses (expressed in terms of mg per kg body weight per 24 h) consumed by the rats from Groups II-IV were presented in Figure 1B.

In the rats receiving SMV separately (Group II) or in combination with MS (Group IV), fluid and food intake as well as body weight decreased, compared with the control (Group I) and MS supplemented animals (Group III) (Figure 1A, 1C, and 1D, respectively). All these changes were only a result of the independent action of V (Table 2). The V doses consumed by the rats from Groups II and IV were similar, whereas the Mg doses ingested by the rats from Groups III and IV were not at the same level. The rats receiving the SMV-MS solution took up less Mg than those supplemented with MS alone (Figure 1B).

The V and Mg concentrations were selected on the basis of previous data[32] and some literature results.[37,59] Based on the concentration of V in the blood and urine,[23,57] the level of this element may reflect the exposure that is likely to occur in human life.[60,61] Its level is also within the broad range of doses that were used for demonstrating its antidiabetic activity[59,62] and for analysing its pharmacokinetic behaviour[63] on an animal model. The level of Mg in drinking water, that is critical for its concentration in the body due to higher Mg bioavailability from water than from food,[64] was, in turn, selected to be not too high, as Mg as $MgSO_4$ (being a popular Mg-inorganic salt widely used in *in vivo* studies and in clinical practice) has been found to induce dose-limiting diarrhoea.[65]

3.2 V, Mg, Ca, Fe, Cu, and Zn levels in some internal organs

The total hepatic, renal and splenic V, Mg, Ca, Fe, Zn, and Cu contents were presented in terms of percentage rate of the control group in Figure 3A-C and in Figure 4A-C. In Figures 3D and 4D the concentrations of all the above mentioned elements in the cerebral hemisphere, expressed in terms of percentage rate of the control group as well, were illustrated.

In the SMV intoxicated and SMV-MS co-administered rats (Groups II and IV, respectively), the total content of V in the liver, kidney, and spleen and its concentration in the cerebral hemisphere were significantly higher, compared with those found in the control (Group I) and MS supplemented (Group III) animals (Figure 3A-D). In the rats in Group III, the total content of this metal in the kidney (Figure 3B) and spleen (Figure 3C) was, in turn, markedly lowered and elevated, respectively, in comparison with Group I, whereas its concentration in the cerebral hemisphere was unchanged (Figure 3D). Moreover, in the same group of rats (Group III) the total hepatic, renal, and splenic V content as well as its cerebral concentration were significantly diminished, compared with Groups II and IV. Further, the data presented in Figure 3B and 3D showed that the supplementation with MS during the exposure to SMV (Group IV) decreased the level of V in the kidney and cerebral hemisphere, by 29.5% and 39% respectively, in comparison with Group II. Thus, these findings provided evidence about the protective Mg action against the increased level of V in both examined organs. As the results of the two-way ANOVA analysis suggest, the fall in the renal and cerebral V level in the rats of Group IV was induced by the independent action of Mg and by its interaction with V (Table 2, Figure 2A and 2B).

The total content of Mg in the liver of the rats in Groups II, III, and IV was unaltered, compared with Group I, but in the rats in Groups II and IV its total hepatic content was markedly lowered, compared with that demonstrated in the animals of Group III (Figure 4A). Further, the total content of Mg in the kidney (Figure 4B) and spleen (Figure 4C) as well as its concentration in the cerebral hemisphere (Figure 4D) remained unchanged between the investigated groups of rats, although in the case of the rats in Groups II and IV the total renal (Figure 4B) and splenic (Figure 4C) Mg content showed the tendency toward the increase and decrease, respectively, in comparison with Groups I and III. The two-way ANOVA indicated that the changes in the total hepatic and splenic Mg content in the SMV-MS co-applied rats were due to the independent action of V only. The alterations in the total renal Mg content

were, in turn, a consequence of the clear trend toward the independent action of V (Table 2). However, no significant V, Mg or VxMg effects were shown by the two-way ANOVA with respect to the changes in the concentration of Mg in the cerebral hemisphere of the SMV-MS co-administered rats (Table 2).

The total content of Ca in the liver of the rats in Groups II, III, and IV unaltered markedly, compared with Group I (Figure 4A), only in the rats of Groups II and IV the total hepatic content of this element significantly decreased and increased, respectively, compared with Groups IV and II. The total content of Ca in the spleen of the rats in Groups II and IV distinctly tended to be higher, in comparison with Group I (Figure 4C), whereas no considerable differences in the changes in the concentration of Ca in the cerebral hemisphere among the examined groups of rats were shown (Figure 4D). Further, the total renal Ca content remained significantly unchanged between the groups, although in the rats in Group II its content in the kidney tended to be lower, in comparison with Group I, whereas in the rats of Group IV it was kept within the range of the values represented by the control group (Figure 4B). The results of the two-way ANOVA revealed that the changes in the total renal Ca content in the rats of Group IV were a consequence of visible trend toward the interaction between V and Mg (Table 2, Figure 2E). The two-way ANOVA also suggested that the elevated content of Ca in the liver of the rats in Group IV resulted from the independent action of Mg and from the interaction between V and Mg (Table 2, Figure 2D), whereas the changes in the total content of Ca in the spleen of the same group of rats were only due to the independent action of V (Table 2). However, no V or/and Mg effects toward the alterations in the concentration of Ca in the cerebral hemisphere of the SMV-MS co-administered rats were indicated (Table 2).

The total content of Fe in the liver (Figure 4A) and spleen (Figure 4C) of the rats in Groups II and IV increased, whereas its concentration in the cerebral hemisphere of the same groups of rats decreased (Figure 4D), in comparison with Group I. The hepatic (Figure 4A)

and cerebral (Figure 4D) level of this element in the rats of Groups II and IV was also elevated and diminished, respectively, compared with Group III. The total content of Fe in the kidney of the rats in Group II decreased, as compared with Groups I and III, whereas in the rats of Group IV its total renal content returned to be kept within the range of the values represented by the control group (Figure 4B). Thus, these results show that the supplementation with MS during the exposure to SMV may limit the decrease in the total renal Fe content. As the two-way ANOVA suggests the changes in the total content of Fe in the kidney of the rats in Group IV were caused by the clear trend toward the interaction between V and Mg (Table 2, Figure 2C). The two-way ANOVA also revealed that the increase in the total content of Fe in the liver and spleen of the rats in Group IV as well as the decrease in the concentration of Fe in the cerebral hemisphere of the same group of rats were only influenced by the independent action of V (Table 2).

The total content of Cu in the liver (Figure 4A) and spleen (Figure 4C) of the rats in Groups II and IV decreased, compared with Groups I and III. The decreased total Cu content was also demonstrated in the kidney of the rats from Group II, compared with Groups I and III (Figure 4B). However, in the rats of Group IV the total renal content of this microelement did not change significantly, compared with Group I (Figure 4B), which may suggest that the administration of MS together with SMV may limit the decrease in the total Cu content in the kidney too. The concentration of Cu in the cerebral hemisphere in all the tested animal groups remained, in turn, at the similar level (Figure 4D), although the results of the two-way ANOVA demonstrated that the changes in the cerebral Cu concentration in the rats of Group IV were a consequence of the independent action of V only (Table 2). The two-way ANOVA analysis also showed that the limitation of the decrease in the total renal Cu content in the rats in Group IV might result from the trend toward the independent action of Mg (Table 2). Moreover, it also revealed that the changes in the total content of Cu in the liver resulted from

the independent action of V and Mg, whereas the alterations in the total content of this microelement in the spleen were due to the independent action of V only (Table 2).

The total content of Zn in the liver of all the tested animal groups did not alter markedly compared with Group I, only in the rats in Group II its total hepatic content decreased significantly, in comparison with Group III (Figure 4A). The total content of Zn in the kidney and spleen did not also change markedly among the groups (Figure 4B and 4C), although in the rats of Group II visible trend toward the increase in its total renal content was observed (Figure 4B). Clear trend toward the increase in the concentration of Zn in the cerebral hemisphere was also shown in the animals in Groups III and IV, compared with Group I (Figure 4D). The two-way ANOVA revealed that the changes in the total hepatic and splenic Zn content in the rats of Group IV were a consequence of the independent action of V only, whereas the alterations in the cerebral Zn concentration in the same group of rats were only due to the independent action of Mg (Table 2). However, no significant V or/and Mg effects toward the alterations in the total content of Zn in the kidney of the SMV-MS co-administered rats were indicated (Table 2).

DL for V, Cu, Mg, Ca, Fe, and Zn in the cerebral hemisphere was 0.29 μg per L, 1.12 μg per L, $1x10^{-5}$ mg per L, 0.03 mg per L, 10 μg per L, and $1x10^{-4}$ mg per L, respectively; DL for Mg in the liver and spleen was $6x10^{-5}$ and $1x10^{-5}$ mg per L; DL for Ca in the liver and spleen was 0.36 and 0.36 mg per L; DL for Fe in the kidney was $2.2x10^{-2}$ mg per L; and DL for Zn in the liver and spleen was $2.2x10^{-3}$ and $1x10^{-3}$ mg per L. The analytical quality of the element measurements was checked with the use of Certified Reference Material (CRM) such as Bovine Liver 1577c (NIST), the analysis of which confirmed the reliability of the proposed approach. V, Mg, Fe, Zn, Cu, and Ca levels (mean ± SD, N = 5) determined by us in the reference liver (10.18 ± 1.63 μg per kg, 657.35 ± 20.19 mg per kg, 193.92 ± 4.32 mg per kg, 180.34 ± 8.68 mg per kg, 268.56 ± 4.66 mg per kg and 133.42 ± 15.21 mg per kg, respectively) well agreed with the values provided by the producers for the liver (8.17 ± 0.66

μg per kg, 620.0 ± 42.0 mg per kg, 197.94 ± 0.65 mg per kg, 181.1 ± 1.0 mg per kg, 275.2 ± 4.6 mg per kg and 131.0 ± 10 mg per kg, respectively).

3.3 DMT-1 level in selected internal organs

The levels of DMT-1 in the examined rats' organs were presented in terms of percentage rate of the control group and illustrated in Figure 5A-C. The level of DMT-1 in the liver and cerebral hemisphere of the rats in Groups II and IV were distinctly, but not significantly, lowered, compared with the rats of Groups I and III, whereas the level of DMT-1 in the kidney of the rats in Group II and IV was significantly diminished, compared with that found in the rats of Groups I and III (Figure 5A-C). The two-way ANOVA demonstrated that the decrease in the level of DMT-1 in the above-mentioned organs of the rats receiving SMV in combination with MS resulted from the independent action of V only (Table 2).

3.4 Correlations of variables

Significant positive and negative correlations and trends toward them were revealed, among others, among the hepatic, renal, or cerebral V level, and the hepatic, renal, or cerebral levels of some macro- and/or microelements (Table 3). They were also found among the hepatic, renal, or cerebral DMT-1 level and the hepatic, renal, or cerebral Fe or V levels as well as among TIBC or TS and the level of Fe in the liver, spleen, and cerebral hemisphere (Table 3). The relationships among the hepatic, renal, and cerebral DMT-1 levels were also indicated (Table 4).

4 Discussion

This paper presents a part of the results originating from the *in vivo* studies performed on outbred male Wistar rats undertaken to evaluate the effects of 12-week V and Mg administration separately or in combination (as SMV and MS, respectively) on various

parameters, the alterations of which were measured, among others, in the body fluids and some tissues of those animals. The main hypothesis tested in the studies was whether and to what extent additional Mg supplementation, at the selected level, may limit V toxicity during SMV intoxication. Another important aspect of these studies, not previously described in the literature, was to examine whether alterations in the tested indices in the rats after combined SMV and MS administration were influenced by the independent action of V, Mg or/and by their mutual interaction as well as whether the possible protective influence of Mg during SMV exposure resulted from Mg independent action or/and was a consequence of its interaction with V. The present report reveals for the first time the effects and kind of mutual interaction between V and Mg in terms of the total hepatic, renal, and splenic contents of chosen macro- and microelements and of their cerebral concentration in male Wistar rats. It also draws attention to the probable mechanisms underlying SMV-induced disorders in homeostasis of Fe in selected tissues, and illustrates for the first time some relationships between examined indices.

The alterations in the fluid and food intake and body weight gain, in rats after SMV exposure have already been discussed by us;[66] therefore, this issue has been disregarded in this report. Instead, only a short comment concerning the consumption of the V and Mg doses will be provided. We may conclude that Mg could change the effects of the action of V because of similar V intake by the rats from Groups II and IV. However, the extent to which V might modify the influence of Mg is difficult to estimate because the consumption of Mg in the rats supplemented with MS during the SMV exposure, as compared with that observed in the rats receiving MS alone, significantly decreased.

The evidence provided by the deliverables show that supplementation with Mg during the exposure to SMV may limit the increase in the total renal V content and in its cerebral concentration as well as normalize the SMV-induced disorders in the total renal Fe and Cu contents. The two-way ANOVA with detailed analysis of the results allow us to conclude that

these positive effects observed in the SMV-MS co-administered rats might result from the independent influence of Mg or the trend toward that Mg action and/or from its antagonistic interaction with V or the tendency toward that Mg behaviour (Table 2).

It is known that the VO^{2+} and Mg^{2+} ions may compete for binding sites on metalloproteins and for small ligands because of similarity of VO^{2+} to Mg^{2+}.[67] Thus, competition between them for the transport process is possible. It has been reported that most of the vanadium(V^{+5}) undergoes one-electron reduction to form VO^{2+} (V^{+4}) in the gastrointestinal tract and that as soon as vanadate enters the cell, vanadate is favorably reduced to the vanadyl form. Moreover, endogenous reducing agents ensure that both the +4 and the +5 oxidation states are present in the serum/plasma.[68] Since interactions among elements may take place at different stages of their metabolism (at the stage of distribution in the organism too),[40] examination of the effect of SMV and MS co-administration on elements transport pathways and identification of transporter molecules might be an important point in elucidation of the molecular mechanism(s) of V and Mg action.

Beside the V-Mg antagonistic interaction, the independent influence of Mg on the significant reduction of V concentration in the cerebral hemisphere in the rats after concomitant SMV and MS administration is also worthy of notice. Mg, the changes of which in the brain have been reported to have serious functional consequences,[69] is known to be a very important element for the central nervous system (CNS). However, its role and mechanisms are still not well understood. When in excess, V, which crosses the blood brain barrier, may lead to many neuropathologic consequences.[70,71] It has been proposed that most of the neurotoxic effects of this metal are due to ROS generation.[72] Our findings clearly show that Mg may reduce V toxicity in the cerebral hemisphere as co-administration of Mg (as MS) with SMV limits V accumulation. Therefore, explanation of the mechanism(s) of this beneficial Mg action revealed under the SMV exposure seems to be important and advisable.

Further, it is a well known fact that too high Mg dose could cause adverse Mg balance evoked by disturbance of equilibrium between Mg and its main antagonist – calcium (Ca).[73] In our experimental conditions, no significant disorders in the level of Ca in the rats' organs tested were found during the supplementation with Mg alone. However, when Mg was administered in combination with V the notable (but not significant compared with the control group) increase in Ca deposition was demonstrated in the liver of the SMV-MS co-applied rats (Figure 4A). A similar, but less expressed effect, has also been observed for the kidney in the same group of animals (Figure 4B). The elevated total hepatic and renal Ca content in the rats supplemented with Mg during the SMV exposure seem to result from the V-Mg synergistic interaction (Table 2) that has also been described by Matsuda et al.[37] but with respect to improvement of insulin sensitivity and glycogen synthesis in diabetic rats. Explanation of the exact mechanism(s) of the enhanced hepatic and renal Ca accumulation as well as the splenic Ca deposition, which in the SMV-MS co-administered rats only resulted from the independent action of V, require further studies.

As it had been previously highlihgted,[23] excess Fe deposition in the liver of the SMV and SMV-MS administered rats probably resulted from incoming dietary Fe through stimulated intestinal Fe absorption, plausibly modulated by the mechanism referred to as the erythroid regulator,[74] due to ineffective erythropoiesis that consequently led to the development of microcytic and hypochromic anaemia in those animals.[23] It is understandable that during the SMV exposure the liver might have a function of clearing the blood from incoming Fe that as a form of a non-transferrin (Tf) bound iron (NTBI) might be present in the plasma of the SMV-exposed rats in which TIBC, TS and the plasma Fe level were lowered, elevated, and unchanged, respectively.[23] It is known that primarily the liver, which acts as a reservoir of Fe, can readily take up an amount of circulating NTBI recognized as a contributor to hepatic Fe overload. It has also been reported that NTBI appears in the plasma during Fe loading - when the plasma Fe level is in excess of the binding capacity of Tf, and

that it is taken up independently of the Tf receptor (TfR), through a Tf independent mechanism.[75,76]

The liver is particularly susceptible to Fe-related disorders.[77] Beside NTBI it can take up the Tf-bound Fe (TBI) by the divalent metal transporter 1 (DMT-1),[78,79] the regulation of which is tissue specific.[80] This proton-coupled ferrous-ion (Fe^{2+})-preferring transporter is present not only in the liver but also in such organs as the pancreas, heart, spleen, brain and kidney,[41,77,81-83] and it has been suggested to be a candidate for Fe homeostasis.[84] On the basis of our previous[23] and current results we may conclude that the hepatic Fe loading demonstrated in the rats receiving SMV separately or in combination with MS reflected in its elevated concentration in the liver[23] and its enhanced total content in this organ (Figure 4A) did not result from the increase in the hepatic DMT-1 expression, because the level of this transporter in the liver of those animals was distinctly lowered, compared with the control group (Figure 5A). Thus, the present results clearly show that DMT-1 iron-essential protein does not play a significant role in the hepatic Fe overload during SMV exposure and suggest that the liver may have other Fe uptake mechanism. Contrary to the liver and spleen, the kidney and cerebral hemisphere of the SMV-intoxicated rats and also the cerebral hemisphere of the SMV-MS co-administered rats suffered from Fe deficiency; whereas the kidney of the latter group of animals had the total Fe content in the range of the values represented by the control group (Figure 4B and 4D). Normalization of the total renal Fe content observed in the rats supplemented with MS during the SMV exposure might be a consequence of the clear trend toward the independent action of Mg (Table 2), the behaviour of which seems to be important because the kidney, likewise, the brain, requires Fe for its metabolic processes.[83,85-87] The decreased cerebral Fe concentration in the SMV-intoxicated and SMV-MS co-administered rats and the distinct fall in the cerebral DMT-1 level (by 44% and 30% respectively), which has been suggested to play a direct role in the brain Fe uptake,[83] with a parallel tendency toward the positive correlation between the cerebral DMT-1 and Fe levels

did not allow us to exclude the contribution of DMT-1 to the decrease of the level of Fe in this organ. The lowered total renal Fe content and the renal DMT-1 level demonstrated in the SMV-intoxicated rats (Figures 4B and 5B, respectively) and a) the strong negative correlation between the renal V accumulation and the renal DMT-1 level and the lack of relationship between the renal Fe and DMT-1 levels (Table 4) as well as b) normalized total renal Fe content in the SMV-MS co-administered rats (Figure 4B) together with the results obtained for the hepatic Fe content mentioned above in this report suggest to perform additional analysis of other Fe transporters to more precisely recognize the regulatory mechanisms associated with the disorders of tissue Fe homeostasis developing under our experimental conditions. Therefore, further research which will provide insight into the potential contribution of other proteins involved in Fe metabolism will be continued, especially given the fact that the biological functions of metal transporters in peripheral tissues that could facilitate understanding the metal-metal interactions are still not clear. Taking into account lowered TIBC (being indirect measure of circulating Tf),[88] demonstrated previously in the SMV exposure,[23] and relationships or their absence showed in this report between the DMT-1 and Fe levels in the tested organs (Table 4) as well as the significant correlations among TIBC and the hepatic, splenic, and cerebral Fe levels (Table 4, Scheme 1), we may state that one of probable causes of the altered levels of Fe in the rats' examined organs in our experimental conditions might be the reduced level of Tf, the lack of which has been reported to result in dramatic redistribution of tissue Fe.[89]

The clear hepatic, renal, and cerebral DMT-1 down-regulation (Figure 5A-C) and the significant main effect of V on the level of this metal transporter in the examined organs, revealed by the two-way ANOVA (Table 2), together with the significant negative correlations among the total hepatic or renal V content and the hepatic or renal DMT-1 level as well as with the trend toward the negative correlation between the cerebral V and DMT-1 levels may, in turn, suggest that V interferes with DMT-1 expression in the examined tissues.

However, the mechanism(s) of the V effect, that has been observed, are not known yet. It has been suggested that DMT-1 transports vanadyl ion (VO^{2+}) and serves V metabolism.[78] However, the data about regulation of DMT-1 by V is very scarce and only single reports on this issue are currently available in the literature. Down-regulation of the isoform of DMT-1, that lacked iron-response element (-IRE) (-IRE DMT-1), with parallel decrease in Fe uptake has been found in the transformed human bronchial epithelial cells (BEAS-2B) and in the lung of rats after vanadyl administration,[90] whereas, up-regulation of DMT-1 after vanadium pentoxide (V_2O_5) exposure with parallel increase in intracellular level of V has been demonstrated in dopaminergic neuronal cells (N27).[91]

Finally, the lowered total Cu content in the liver and spleen of the SMV-exposed and SMV-MS co-administered rats and in the kidney of the SMV-intoxicated animals might at least in part arise from deficit of the plasma Cu binding protein, ceruloplasmin (Cp), which carries > 95% of total plasma Cu and transports it into the liver and other tissues, thus becoming a candidate for a tissue source.[92-94] This hypothesis will be tested by us in the near future because it is well known that Cp deficiency may also result in aberrant Fe distribution and Fe accumulation in the liver cells and macrophages as well as may impair the ability to transport Fe from macrophage and liver stores.[93,95] At the present stage of our study, the significant correlations found among the tissues V and Cu or Fe levels allow us to point to the influence of V (as SMV) on the disorders of Cu and Fe homeostasis in the rats' tested organs. Moreover, the results obtained also provided evidence that supplementation with Mg during the SMV exposure may normalize the SMV-induced fall in the total renal Cu content, and that this effect may be a consequence of the clear trend toward the independent influence of Mg; therefore, a mechanism involved in that Mg action requires further studies.

5. Conclusions

The results of this study provide evidence that V (as SMV) taken up by the rats separately or in combination with Mg (as MS) for 12 weeks may lead to disturbances in homeostasis of some essential elements in rats' examined organs. On the other hand, they also clearly showed that Mg applied in combination with SMV was able to partly protect against the renal and cerebral V accumulation and normalize the total renal Cu and Fe contents. Beside the above-mentioned effects, the obtained results extend the knowledge in the research field concerning the combined V and Mg administration providing, for the first time, evidence of the independent action of both elements and of their antagonistic or synergistic interactions (in the selected experimental conditions) with respect to all the indices illustrated in this report. Moreover, they also indicated that in the mechanism underlying the SMV-induced hepatic Fe overload, DMT-1 did not take part, and together with our previous results[23] allowed us to suggest that the tissue Fe disturbances might have been partly caused by diminished Tf level, reflected by lowered TIBC found during SMV exposure.[23] Finally, our findings also pointed to the need for continuation of the studies on combined V and Mg administration to a) more precisely elucidate the molecular mechanism(s) responsible for the disorders of Fe homeostasis in the rats' liver, b) know and understand better the action of Mg in the conditions of combined administration with V, and c) determine the safe Mg dose which would reduce more effectively tissue V accumulation and thereby better protect against the deleterious effects of the V dose studied. Moreover, the inclusion of female rats in order to consider the influence of sex and hormones on the parameters examined will be an important area of our future research.

Conflicts of interest statement

There are no conflicts of interest of authors.

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Table 1 The influence of V and Mg administration on the level of the selected elements in some organs of the experimental animals

Vanadium compounds [a]	Species of animals [b]	Concentration/Dose	Route [c]	Experimental period [d]	Organs/Tissues				References
					liver	kidney	muscle/ spleen	adipose tissue/ heart	
			Magnesium (Mg)						
NH_4VO_3	S-D rats (♂)	0.5 ppm	dw	32 wk	↔				42
$NaVO_3$	W rats (♂)	0.125 mg V/mL (12.9 mg V/kg body weight/d)	dw	12 wk	↑	↑			34
BMOV	W rats (♂)	1 mg V/d	dw	5 wk	↔	↔	↔/-	↔/-	38
BMOV	DM W rats (♂)	1 mg V/d	dw	5 wk	↔	↔	-/↓	-/↔	39
BMOV	DM W rats (♂)	3 mg V/d	dw	5 wk	↔	↔	-/↓	-/↔	39
			Calcium (Ca)						
NH_4VO_3	S-D rats (♂)	0.5 ppm	dw	32 wk	↔				42
			Iron (Fe)						
NH_4VO_3	S-D rats (♂)	0.5 ppm	dw	21 wk	↔				43
NH_4VO_3	S-D rats (♂)	0.5 ppm	dw	32 wk	↔				42
NH_4VO_3	weanling W-K rats (♂)	1 μg/g	with diet	8 wk	↑				44
$NaVO_3$	S-D rats dams (♀) and pups	75 μg V/g diet	with diet	3 wk	↔				45
$NaVO_3$	S-D rats (♀)	1.2 mM/80 mM NaCl	dw	26 d	↔	↔			46
$NaVO_3$	W rats (♂)	0.100 mg V/mL (~8.6 mg V/kg body weight/d)	dw	12 wk	↑	↔			47
NH_4VO_3		1.2 mM			↔	↔			48
$VOSO_4$	W rats (♂)	1.2.mM	dw	12 wk	↔	↓			48
BMOV		1.2 mM			↔	↓			48
NH_4VO_3	weanling Hartley guinea pigs (♂)	0.5 μg V/g diet	with diet	21 wk	↔				49
$VOCl_2$	broiler chicks (♀)	10, 20 or 40 ppm	with diet	3 wk	↔	↔			26
			Zinc (Zn)						
NH_4VO_3	S-D rats (♂)	0.5 ppm	dw	32 wk	↔				42
NH_4VO_3	weanling W-K rats (♂)	1 μg/g	with diet	8 wk	↓				44
$NaVO_3$	S-D rats dams (♀)	75 μg V/g diet	with diet	3 wk	↔				45
	and pups				↑				45
$NaVO_3$	S-D rats (♀)	1.2 mM/80 mM NaCl	dw	26 d	↔	↔			46
$NaVO_3$	W rats (♂)	0.100 mg V/mL (~8.6 mg V/kg body weight/d)	dw	12 wk	↔	↔			47
NH_4VO_3		1.2 mM			↔	↔			48
$VOSO_4$	W rats (♂)	1.2.mM	dw	12 wk	↔	↔			48
BMOV		1.2 mM			↔	↓			48
NH_4VO_3	S-D rats (♂)	0.5 ppm	dw	20 wk	↔				50
NH_4VO_3	weanling Hartley guinea pigs (♂)	0.5 μg V/g diet	with diet	21 wk	↔				49
			Cooper (Cu)						
NH_4VO_3	S-D rats (♂)	0.5 ppm	dw	32 wk	↔				43
NH_4VO_3	S-D rats (♂)	0.5 ppm	dw	20 wk	↔				50
NH_4VO_3	weanling W-K rats (♂)	1 μg/g	with diet	8 wk	↔				44
$NaVO_3$	S-D rats dams (♀) and pups	75 μg V/g diet	with diet	3 wk	↔				45

$NaVO_3$	S-D rats (♀)	1.2 mM/80 mM NaCl	dw	26 d	↔	↔			46
$NaVO_3$	weanling S-D rats (♂)	10, 20, 40 or 80 μg V/g diet	with diet	3 wk	↔				51
$NaVO_3$	W rats (♂)	0.100 mg V/mL (~8.6 mg V/kg body weight /d)	dw	12 wk	↔	↔			47
NH_4VO_3					↔	↔			48
$VOSO_4$	W rats (♂)	1.2 mM	dw	12 wk	↔	↔			48
BMOV					↔	↔			48
$NaVO_3$	W rats (♂)	3 mg/kg	*per os*	2-4 wk	↔				52
				6 and 8-12 wk	↓				52
$VOSO_4$	W rats (♂)	3.75 mg/kg	*per os*	2 and 4 wk	↔				52
				6 and 8-12 wk	↓				52
NH_4VO_3	weanling Hartley guinea pigs (♂)	0.5 μg V/g diet	in diet	21 wk	↔				49

Magnesium compounds[a]	Species of animals[b]	Concentration/Dose	Route[c]	Period[d]	Organs				References
					liver	kidney	spleen	brain	
			Magnesium (Mg)						
$MgCO_3$	W rats (♂)	20 mg Mg/kg body weight/d	i.g.	4 wk	↔	↔	↔	↔	53
$MgCl_2$	W rats (♂)	1000 ppm	dw	21 d	↔	↔			54
$MgCl_2$	W rats (♂)	300 mg/L	dw	6 wk	↑	↑		↑	55
Mg-L-Aspart-hCl	S-D rats (♂)	9000 ppm	in diet	11 d	↔				56
			Calcium (Ca)						
$MgCO_3$	W rats (♂)	20 mg Mg/kg body weight/d	i.g.	4 wk	↓	↔	↔	↑	53
$MgCl_2$	W rats (♂)	300 mg/L	dw	6 wk	↑	↑		↑	55
Mg-L-Aspart-hCl	S-D rats (♂)	9000 ppm	in diet	11 d	↔				56

[a] NH_4VO_3: ammonium metavanadate; $NaVO_3$: sodium metavanadate; $VOSO_4$: vanadyl sulphate; BMOV: bis(maltolato)oxovanadium; $VOCl_2$: vanadyl chloride; $MgCO_3$: magnesium carbonate; $MgCl_2$: magnesium chloride; Mg-L-Aspart-hCl: magnesium-L-aspartathydrochloride. [b] S-D rats: Sprague-Dawley rats; W rats: Wistar rats; DM W rats: diabetic streptozotocin rats; W-K rats: Wistar-Kyoto rats. [c] dw: through drinking water; i.g.: intragastric. [d] wk: weeks; d: days. ↓ - decrease; ↑ - increase; ↔ without changes.

Table 2 V and Mg main and interactive effects on selected parameters measured in male Wistar rats receiving SMV in combination with MS for 12 weeks

Variables [a]	Two-way ANOVA analysis [b]			Kind of interaction revealed or a trend toward interaction [c]
	Main effect of V	Main effect of Mg	Interactive effect of V and Mg	
Fluid-I	$F = 102.214$, $P = 0.000$	NS	NS	-
Food-I	$F = 134.765$, $P = 0.000$	NS	NS	-
BWG	$F = 22.086$, $P = 0.000$	NS	NS	-
V_L	$F = 859.160$, $P = 0.000$	NS	NS	-
V_K	$F = 326.391$, $P = 0.000$	$F = 5.552$, $P = 0.024$	$F = 5.312$, $P = 0.027$	Antagonistic
V_S	$F = 726.322$, $P = 0.000$	NS	NS	-
V_{CH}	$F = 146.158$, $P = 0.000$	$F = 22.090$, $P = 0.000$	$F = 16.663$, $P = 0.000$	Antagonistic
Mg_L	$F = 7.447$, $P = 0.010$	NS	NS	-
Mg_K	$F = 3.023$, $P = 0.091$	NS	NS	-
Mg_S	$F = 4.302$, $P = 0.045$	NS	NS	-
Mg_{CH}	NS	NS	NS	-
Ca_L	NS	$F = 6.761$, $P = 0.013$	$F = 7.880$, $P = 0.008$	Synergistic
Ca_K	NS	NS	$F = 2.437$, $P = 0.128$	Synergistic
Ca_S	$F = 5.893$, $P = 0.020$	NS	NS	-
Ca_{CH}	NS	NS	NS	-
Fe_L	$F = 17.096$, $P = 0.000$	NS	NS	-
Fe_K	$F = 4.151$, $P = 0.049$	NS	$F = 3.771$, $P = 0.060$	Antagonistic
Fe_S	$F = 14.832$, $P = 0.000$	NS	NS	-
Fe_{CH}	$F = 34.293$, $P = 0.000$	NS	NS	-
Cu_L	$F = 25.527$, $P = 0.000$	$F = 5.711$, $P = 0.022$	NS	-
Cu_K	$F = 16.070$, $P = 0.000$	$F = 2.801$, $P = 0.103$	NS	-
Cu_S	$F = 36.524$, $P = 0.000$	NS	NS	-
Cu_{CH}	$F = 4.383$, $P = 0.043$	NS	NS	-
Zn_L	$F = 8.329$, $P = 0.007$	NS	NS	-
Zn_K	NS	NS	NS	-
Zn_S	$F = 7.954$, $P = 0.008$	NS	NS	-
Zn_{CH}	NS	$F = 4.658$, $P = 0.038$	NS	-
DMT-1_L	$F = 5.729$, $P = 0.022$	NS	NS	-
DMT-1_K	$F = 39.698$, $P = 0.000$	NS	NS	-
DMT-1_{CH}	$F = 4.353$, $P = 0.044$	NS	NS	-

[a] Fluid-I, Food-I, BWG, V, Fe, Cu, Zn, Mg, and Ca: fluid intake, food intake, body weight gain, vanadium, iron, copper, zinc, magnesium, and calcium, respectively. [b] Data is presented as F values and the levels of significance (*P*); NS: no significant effect. [c] Antagonism: the effect of V and Mg in combination < sum of V effect alone and Mg effect alone; Synergism: the effect of V and Mg in combination > sum of V effect alone and Mg effect alone. [L, K, S, CH] Liver, kidney, spleen, and cerebral hemisphere, respectively.

Table 3 Correlation coefficients for the compared variables

Variables	V_L	Variables	V_K	Variables	V_{CH}
Mg_L	**0.393** *	Mg_K	**0.346** *	Mg_{CH}	-
Fe_L	**0.571** ‡	Fe_K	**-0.334** *	Fe_{CH}	**-0.536** ‡
Cu_L	**-0.594** ‡	Cu_K	**-0.556** ‡	Cu_{CH}	**-0.387** *
Ca_L	-	Ca_K	-	Ca_{CH}	-
Zn_L	**-0.420** †	Zn_K	-	Zn_{CH}	-

Variables	DMT-1_L	Variables	DMT-1_K	Variables	DMT-1_{CH}
Fe_L	-	Fe_K	-	Fe_{CH}	*0.290* #c
V_L	**-0.389** *	V_K	**-0.677** ‡	V_{CH}	*-0.237* #d

Variables	TIBC[f]	Variables	TIBC[f]	Variables	TIBC[f]	Variables	TIBC[f]
Fe_L	**-0.535** ‡	Fe_S	**-0.434** †	Fe_K	-	Fe_{CH}	**0.485** †

Variables	TS[f]	Variables	TS[f]	Variables	TS[f]	Variables	TS[f]
Fe_L	*0.309* #a	Fe_S	**0.655** ‡	Fe_K	-	Fe_{CH}	*-0.297* #b

Data is presented as the correlation coefficients (r) and the levels of statistical significance (P). The significant correlations and tendencies toward them are highlighted as embolden and by means of italics, respectively. TIBC, TS, L, S, K, and CH: total iron binding capacity, transferrin saturation, liver, spleen, kidney, and cerebral hemisphere, respectively. ‡ $P < 0.001$; † $P < 0.01$; * $P < 0.05$; # $P > 0.05$; #a $P = 0.052$; #b $P = 0.06$; #c $P = 0.07$; #d $P = 0.14$. [f] Previous results (Ścibior et al., 2012). ⁻ Lack of relationship among measured variables.

Table 4 Correlation coefficients for DMT-1 in the tested rats' organs

Variables	$DMT\text{-}1_L$	$DMT\text{-}1_K$	$DMT\text{-}1_{CH}$
$DMT\text{-}1_L$	1	**0.731** ‡	**0.821** ‡
$DMT\text{-}1_K$	**0.731** ‡	1	**0.733** ‡
$DMT\text{-}1_{CH}$	**0.821** ‡	**0.733** ‡	1

Data is presented as the correlation coefficients (r) and the levels of statistical significance (P). The significant correlations are highlighted as embolden. $^{L, K, CH}$ Liver, kidney, and cerebral hemisphere, respectively. $^{‡}P < 0.001$.

Legends for Figures 1-5 and for Scheme 1

Figure 1. Fluid intake (A), V and Mg doses consumed by the rats of Groups II, III and IV (B), food intake (C), and body weight gain (D) in the Control Group, SMV intoxicated, MS supplemented and SMV-MS co-administered rats. Differences in the groups are indicated by: [a]vs. control (Group I), [b]vs. SMV (Group II), [c]vs. MS (Group III), and [d]vs. SMV-MS (Group IV) ([1]the Tukey test, [2]the T3 Dunnett test or [3]the t-test).

Figure 2. The graphs of the interaction between V and Mg concerning the V level in the kidney (A) and cerebral hemisphere (B); the Fe level in the kidney (C); and the Ca level in the liver (D) and kidney (E) expressed in terms of percentage rate of the Control Group. V+, V-, Mg+, Mg-, presence (+) or lack (-) of V or Mg; V+ and Mg+ (Group IV); V+ and Mg- (Group II); V- and Mg+ (Group III), and V- and Mg- (Group I).

Figure 3. The content of V in the liver (A), kidney (B), spleen (C) and its concentration in the cerebral hemisphere (D) expressed in terms of percentage rate of the Control Group. Differences in the groups are indicated by: [a]vs. control (Group I), [b]vs. SMV (Group II), [c]vs. MS (Group III), and [d]vs. SMV-MS (Group IV) ([1]the Tukey test or [2]the T3 Dunnett test). Numerical values in the bars indicate percentage rate of decrease (↓) in the V level, compared with the SMV-exposed rats (bold alone).

Figure 4. The contents of Mg, Ca, Fe, Cu, and Zn in the liver (A), kidney (B), spleen (C), and their concentrations in the cerebral hemisphere (D) expressed in terms of percentage rate of the Control Group. Differences in the groups are indicated by: [a]vs. control (Group I), [b]vs. SMV (Group II), [c]vs. MS (Group II), and [d]vs. SMV-MS (Group IV) ([1]the Tukey test or [2]the T3 Dunnett test). $^{*}P = 0.050$; $^{†}P = 0.055$; $^{‡}P = 0.058$; $^{††}P = 0.062$; $^{∂}P = 0.068$; $^{\#}P = 0.079$; $^{‡‡}P = 0.087$; $^{§}P = 0.097$; $^{•}P = 0.106$.

Figure 5. The level of DMT-1 in the liver (A), kidney (B), and cerebral hemisphere (C) expressed in terms of percentage rate of the Control Group. Differences in the groups are indicated by: [a]vs. control (Group I), [b]vs. SMV (Group II), [c]vs. MS (Group III), and [d]vs. SMV-MS (Group IV) ([1]the Tukey test).

Scheme 1. Disturbances in Fe homeostasis during the SMV exposure illustrated on the basis of our previous and present findings. V, Fe, SMV, TIBC, TS, and DMT-1: vanadium, iron, sodium metavanadate, total iron binding capacity, transferrin (Tf) saturation, and divalent metal transporter 1, respectively.

Figure 1

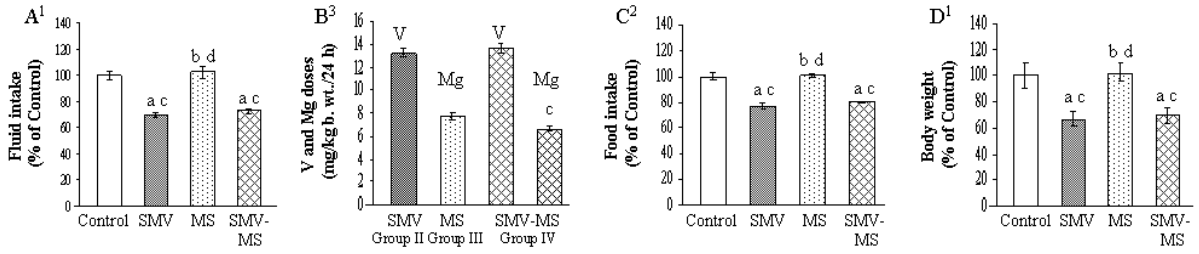


Figure 2

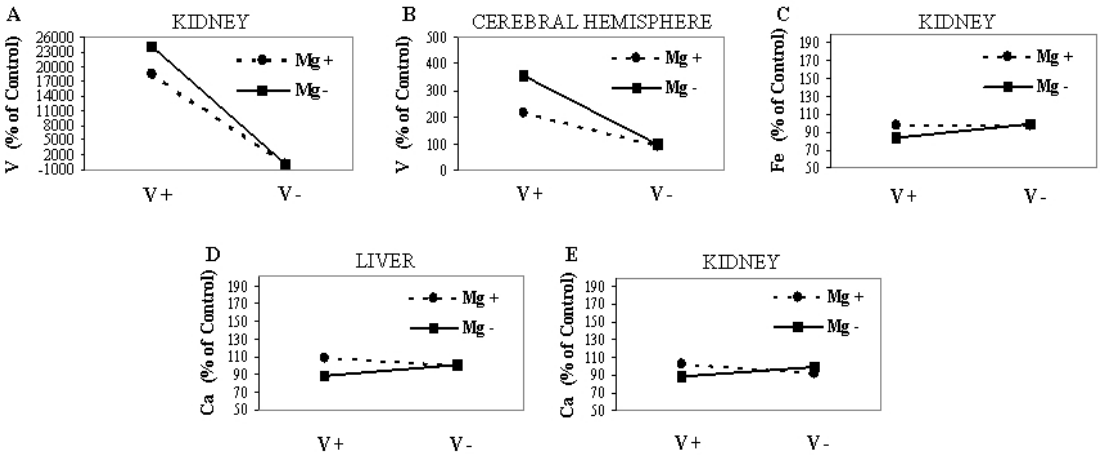


Figure 3

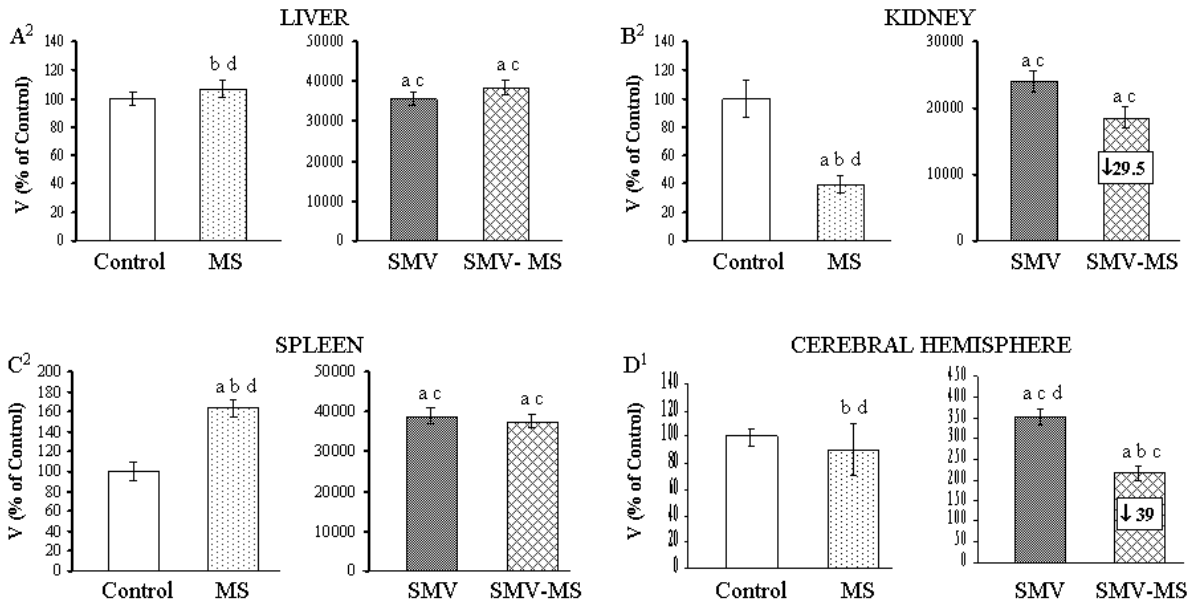


Figure 4

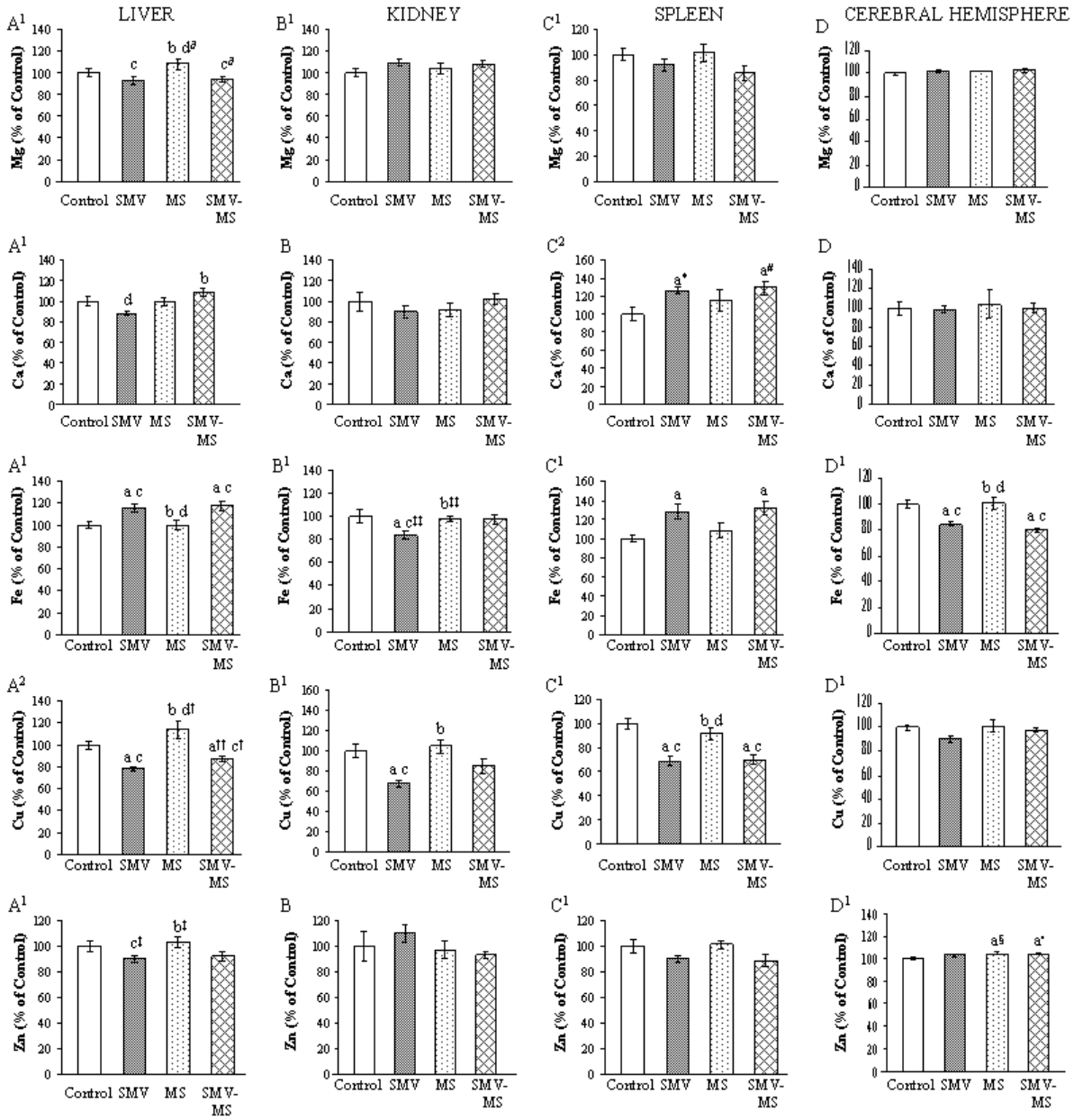
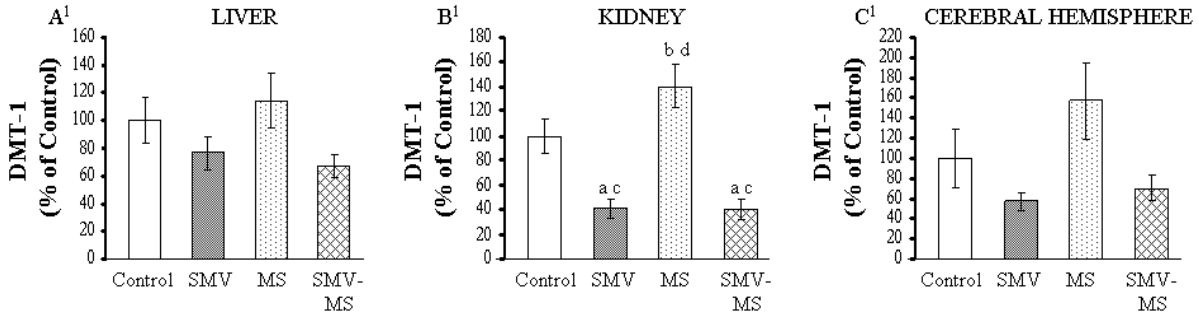


Figure 5



Scheme 1