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Evaluation of lipid peroxidation and the level of some elements in rat erythrocytes during separate and combined vanadium and magnesium administration

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ABSTRACT

The impact of vanadium (V) and magnesium (Mg) as sodium metavanadate (SMV, 0.125 mg V/ml) and magnesium sulfate (MS, 0.06 mg Mg/ml) on lipid peroxidation (LPO) and selected elements in the rat erythrocytes (RBCs) was investigated. Relationships between some indices determined in RBC were also studied. SMV alone (Group II) elevated the malondialdehyde level (MDA_{RBC}) (by 95% and 60%), compared with the control (Group I) and MS-supplemented rats (Group III), respectively, reduced the concentration of Cu_{RBC} (by 23.5%), in comparison with Group I, but did not change the levels of Na_{RBC}, K_{RBC}, and Ca_{RBC}, whereas MS alone (Group III) only reduced the Cu_{RBC} concentration (by 22%), compared with Group I. The SMV + MS combination (Group IV) reduced and elevated the Cu_{RBC} (by 24%) and Ca_{RBC} (by 111%) concentrations, respectively, in comparison with Groups I and III, and these changes were induced by the V-Mg antagonistic and synergistic interaction, respectively. The combined SMV + MS effect also enhanced the MDA_{RBC} level, compared with Groups I (by 79%) and III (by 47%) and slightly limited its concentration, compared with Group II, which, in turn, resulted from the distinct trend toward the V-Mg antagonistic interaction. We can conclude that V (as SMV) is able to stimulate LPO in rat RBCs and that V-Mg interactive effects are involved in changes in Cu_{RBC}, Ca_{RBC}, and MDA_{RBC}. Further studies are needed to elucidate the exact mechanisms of the V-Mg antagonistic/synergistic interactions and to provide insight into the biochemical mechanisms of changes in rats suffering from anemia [1], characterized by a disrupted antioxidant barrier in RBCs [2] and an intensified free radical process in these cells.

1. Introduction

Erythrocytes (RBCs) are part of the complicated multifunctional system [3]. They contain only a cytosol and plasma membrane, which is made up of various areas and plays a crucial role in maintenance of ion homeostasis essential for generating ion gradient, regulating cell volume, uptake of nutrients, and many other physiological activities [4–8]. RBCs also represent a major component of the antioxidant capacity of the blood through enzymes, the glutathione system, and low-molecular-weight antioxidants [9]. As they are the most adaptable and highly specialized cells in the body and due to their short life span, RBCs can constitute a vital indicator of health [4,10]. In addition, the vulnerability of those cells to peroxidation makes them a good biological membrane model for analyzing oxidative stress (OS) and lipid

Abbreviations: A, antagonistic; AMV, ammonium metavanadate; CAT, catalase; CCS, copper metallocharperone; CRMs, certified reference materials; DL (LOD), limit of detection; DMT-1, divalent metal transporter 1; GR, glutathione reductase; GPx, glutathione peroxidase; GSH, reduced glutathione; GST, glutathione transferase; Hb, hemoglobin; Ht, hematocrit index; IMgA, independent magnesium action; IVA, independent vanadium action; LPO, lipid peroxidation; MCH, mean corpuscular Hb; MCV, mean corpuscular volume; MDA, malondialdehyde; MS, magnesium sulfate; OS, oxidative stress; RBC, erythrocytes; RBC count, erythrocyte count; RDW, red cell distribution width; ROS, reactive oxygen species; S, synergistic; SMV, sodium metavanadate; SOD, superoxide dismutase; TRPC6, transient receptor potential cation channel 6; $T_{(-)}$, trend toward a negative correlation; $T_{(+)}$, trend toward a positive correlation; TtA, trend toward antagonistic interaction; TtS, trend toward synergistic interaction

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peroxidation (LPO) [11]. All this taken together makes these cells a valuable model to evaluate the toxicity of various xenobiotics [11].

Elements are well known to play an important role in many life-supporting processes. Mg, for example, is involved in several essential physiological, biochemical, and cellular actions [5]. It is transported across the cell membrane through channels, which are predominantly involved in Mg accumulation, and exchangers, which essentially mediate Mg extrusion [12]. It has been demonstrated that Mg is required for modulation of the activities of the Na^+/K^+- and Ca^{2+}-ATPases, Na^+-K^+-Cl^- and K^+-Cl^- cotransport as well as Ca and K channels [5,13–15]. This element may also (a) alter the deoxygenated state of Hb, (b) stabilize a damaged RBC membrane as well as (c) affect cell dehydration, RBC survival, and fluidity of the RBC membrane. The improvement of anemia during Mg supplementation and an increase of morphological abnormalities of RBCs during its deficiency have also been reported [16–23]. In turn, vanadium (V), which (a) in the form of vanadate (V^{5+}, VO_3^-) crosses the RBC membrane rapidly through anion channels and undergoes bioreduction to tetravalent vanadyl (V^{4+}, VO^{2+}) inside cells [4,24,25] and (b) whose concentration in RBC is determined by the balance between the efflux and influx rates [26], can (a) inhibit the activity of RBC membrane ion-transport Na^+/K^+-, Ca^{2+}-, and Ca^{2+}/Mg^{2+}-ATPases [25–27]; (b) change the shape of intact RBCs [25], and (c) alter the structure and properties of the RBC membrane [28]. The influence of V on LPO [29,30] and on the level/activity of some antioxidants [2,31] in rats' RBCs and on some OS markers in different rat soft tissues [32–37] as well as the impact of Mg on LPO in certain rats' soft tissues [38–42] and the effect of Mg on this free radical process in human RBCs [43,44] have also been reported. However, nothing is known about the influence of simultaneous administration of both elements on the level of LPO, which is a well-established mechanism for estimating cellular injury, in rat RBCs. Moreover, there are no available reports on the character of the interactions between V (which has strong pro-oxidant activity) and Mg (which may protect against OS) with reference to indices examined in rat RBC and their mutual relationships. Similarly, there are no data about the effect of simultaneous V and Mg treatment on the level of elements in rat RBC. An aspect worth to be highlighted is that exploration of the role of any element is pointless if it is not carried out in a comprehensive way and in conjunction with analysis of the levels of different micro- and macroelements, as only a combination thereof is vital in crossing the protective barriers of the organism. Undisputed is the fact that a correct level of elements and their relative proportions in cells/tissues determine the health of the organism.

Given the sensitivity of RBC to oxidative damage, the pro-oxidant properties of V (the concentration of which in the environment is still growing) [45], the antioxidant potential of Mg [39], and the fact that LPO can alter the RBC membrane and lead to abnormal cell morphology and hemolysis [11], we undertook the investigations to expand the knowledge of the influence of V (administered as sodium metavanadate, SMV) and Mg (as magnesium sulfate, MS) (a) on the level of a highly reactive LPO marker - malondialdehyde (MDA) which is able to impair a variety of the membrane-related functions by cross-linking RBC phospholipids and proteins leading to diminished survival and death [46] and (b) on the concentration of selected minerals in rat RBC, with special emphasis on the consequence of possible V-Mg interactive effects. It is well known that interactions between metals can change the final effect of the action of one of the elements simultaneously introduced into the organism and can affect the cell metabolism and function of organs and, consequently, affect the whole organism. Therefore, the interactions are still an important issue in toxicology.

2. Materials and methods

2.1. Reagents and chemicals

$NaVO_3$ and $MgSO_4$ were obtained from Sigma Chemical (St. Louis, USA). Sodium azide (NaN_3) and 2-thiobarbituric acid (TBA, $C_4H_4N_2O_2S$) were purchased from Sigma-Aldrich (St. Louis, USA). Hydrogen peroxide (H_2O_2), sodium arsenite ($NaAsO_2$), sodium hydroxide (NaOH), and trichloroacetic acid (TCA, CCl_3COOH) were purchased from POCH (Gliwice, Poland). The physiological buffered saline (PBS with Mg^{2+} and Ca^{2+}, 137 mM NaCl, 2.7 mM KCl, 8.1 mM $Na_2HPO_4x12H_2O$, 1.5 mM KH_2PO_4, pH = 7.4) and nitric acid (HNO3, 65% suprapure) were acquired from Serum and Vaccine Factory (BIOMED, Lublin, Poland) and from Merck (Darmstadt, Germany), respectively. Heparin (5000 j. m/ml) was obtained from Warsaw Pharmaceutical Companies (Polfa, Warsaw, Poland). Stocks of Cu and Ca were purchased from InorganicVentures (Christiansburg, USA) and Spectracer (UK), respectively, whereas the multielement standard solution for Na and K was acquired from Sigma-Aldrich (St. Louis, USA). All the other chemicals and reagents used were of analytical grade or better and commercially available.

2.2. Instrumentation

A Speedwave Four microwave digestion system (Berghof, Germany)[1] equipped with a temperature and pressure sensor in each vessel and a SpectrAA Z2000 TANDEM atomic absorption spectrometer (Hitachi, Japan)[1] equipped with a Zeeman background corrector were used for digestion of rat RBC and determination of Cu, Ca, Na, and K in these cells, respectively. An ultra-pure water system (HLP Spring 5 R system, Hydrolab, Gdańsk, Poland)[1] was used to obtain ultra-pure water for biochemical analyses. A Universal 32 centrifuge (Hettich, Germany) and a UV-Vis BioMate spectrophotometer were used to prepare biological material for analyses and for determination of the MDA level in rat RBC, respectively.

2.3. Animals and experimental protocol

The experiment was carried out on male outbred albino Wistar rats, which were acclimated for one week before treatment. The number of animals was 40 or 56 individuals (10 or 14 rats/each Group). During the whole 12-week experiment, all the animals were housed individually in stainless cages (1 rat/cage) in a room under controlled laboratory conditions (temperature: 20–21 °C, humidity: 55 ± 5%, and controlled 12 h light:12 h dark cycles). The animals had *ad libitum* access to fresh deionized water, fluids, and a standard granulated rodent chow (Labofeed B obtained from Fodder and Concentrate Factory, Kcynia, Poland). Four groups were established. Group I (Control, mean initial body weight: 213 g) received deionized water to drink. Group II (mean initial body weight: 212 g) received a water solution of $NaVO_3$ (SMV) at a concentration of 0.125 mg V/ml (pH 7.18). Group III (mean initial body weight: 214 g) received $MgSO_4$ (MS) at a concentration of 0.06 mg Mg/ml (pH 5.73). Group IV (mean initial body weight: 218 g) received SMV-MS at the same concentration of V and Mg as in Group II and Group III, respectively (pH 7.10). The intake of food, fluids, and water was monitored every day, whereas the body weight was checked once a week. The animals were also observed in order to assess their health and well-being. More details of the experiment were provided by us previously [1]. All animal experimental procedures were approved by the 1 s t Local Ethical Committee for Animals Studies in Lublin.

2.4. Preparation of RBC for biochemical analyses

The whole blood collected into plastic tubes with heparin as an

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