Cell Biol Toxicol (2010) 26:509–526
DOI 10.1007/s10565-010-9160-8

Antioxidant enzyme activity and lipid peroxidation in the blood of rats co-treated with vanadium (V^{+5}) and chromium (Cr^{+3})

Agnieszka Ścibior · Halina Zaporowska · Agnieszka Wolińska · Jarosław Ostrowski

Received: 6 October 2009 / Accepted: 15 March 2010 / Published online: 31 March 2010

Abstract Selected biochemical parameters were studied in the blood of outbred, male Wistar rats which daily received to drink deionized water (Group I, control) or solutions of: sodium metavanadate (SMV; 0.100 mg V/mL)—Group II; chromium chloride (CC; 0.004 mg Cr/mL)—Group III; and SMV-CC (0.100 mg V and 0.004 mg Cr/mL)—Group IV for a 12-week period. The diet and fluid intake, body weight gain, and food efficiency ratio (FER) diminished significantly in the rats of Groups II and IV, compared with Groups I and III. The plasma total antioxidant status (TAS) as well as the MDA and the L-ascorbic acid level in the erythrocytes (RBCs) remained unchanged in all the groups, whereas the plasma L-ascorbic acid concentration decreased markedly in Group II, compared with Group III. The activities of Cu,Zn-superoxide dismutase (Cu,Zn-SOD), catalase (CAT), cellular glutathione peroxidase (cGSH-Px), and glutathione reductase (GR) in RBCs remained unaltered in all the treated rats. However, the activity of glutathione *S*-transferase (GST) and the content of reduced glutathione (GSH) in RBCs decreased and increased, respectively, in Groups II, III, and IV, compared with Group I. A vanadium–chromium interaction which affected the GST activity was also found. To summarize, SMV and CC administered separately or in combination in drinking water for 12 weeks did not alter either lipid peroxidation (LPO) or the activities of Cu,Zn-SOD, CAT, cGSH-Px, and GR, which allows a conclusion that both metals in the doses ingested did not reveal their pro-oxidant potential on RBCs.

Keywords Antioxidants · Erythrocytes · Lipid peroxidation · Rats · Trivalent chromium · Total antioxidant status · Vanadate

A. Ścibior (✉) · H. Zaporowska
Department of Cell Biology, Institute of Environmental Protection, John Paul II Catholic University of Lublin, Kraśnicka Ave 102,
20-718 Lublin, Poland
e-mail: cellbiol@kul.lublin.pl

A. Wolińska
Department of Biochemistry and Environmental Chemistry, Institute of Biotechnology,
Catholic University of Lublin,
Kraśnicka Ave 102,
20-718 Lublin, Poland
e-mail: awolin@kul.lublin.pl

J. Ostrowski
Voivodship Inspectorate of Environmental Protection,
Obywatelska Ave 13,
20-092 Lublin, Poland

Abbreviations

AAS	atomic absorption spectrometry
ABTS	2,2′-azino-bis-3-ethyl-benzothiazoline-6-sulfonic acid
$ABTS^{\bullet+}$	ABTS radical cation
AMV	ammonium metavanadate
BMOV	bis(maltolato)oxovanadium(IV)
CAT	catalase

CC	chromium chloride
CDNB	1-chloro-2,4-dinitrobenzene
cGSH-Px	cellular glutathione peroxidase
CNS	central nervous system
Cr	chromium
Cr^{2+}	divalent chromium
Cr^{3+}	trivalent chromium
Cr^{5+}	pentavalent chromium
Cr^{6+}	hexavalent chromium
$CrCl_3$	chromium chloride
CrPic	chromium picolinate
Cu,Zn-SOD	Cu, Zn-superoxide dismutase
DMH	dimethylhydrazine
DNA	deoxyribonucleic acid
FER	food efficiency ratio
GF-AAS	graphite furnace atomic absorption spectrometry
GR	glutathione reductase
GSH	reduced glutathione
GSH-Px	glutathione peroxidase
GST	glutathione *S*-transferase
γ-GCS	gamma-glutamylcysteine synthetase
Hb	hemoglobin
HbA1C	glycated hemoglobin
H_2O_2	hydrogen peroxide
$HO^{\bullet}$	hydroxyl radical
ICP-AES	inductively coupled plasma-atomic emission spectrometry
K_2EDTA	bipotassium ethylene-diaminetetraacetic acid
LOOH	lipid hydroperoxides
LPO	lipid peroxidation
MDA	malondialdehyde
NaCl	sodium chloride
NADH	reduced nicotinamide adenine dinucleotide
$NaVO_3$	sodium metavanadate
NH_4VO_3	ammonium metavanadate
NPY	neuropeptide Y
$O_2^{\bullet-}$	superoxide radical
PBS	buffered solution of physiological salt
RBCs	red blood cells
ROS	reactive oxygen species
SH	sulfhydryl group
SMV	sodium metavanadate
SMV-CC	sodium metavanadate and chromium chloride
SOD	superoxide dismutase
TAS	total antioxidant status
TBARS	thiobarbituric acid reactive substances
V	vanadium
V^{5+}	pentavalent vanadium
VO^{2+}	vanadyl

Introduction

It is well known that enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT), and enzymes involved in glutathione metabolism, like glutathione peroxidase (GSH-Px) and glutathione reductase (GR; Matés 2000; Urso and Clarkson 2003), as well as non-enzymatic antioxidants, such as reduced glutathione (GSH), vitamin E, β-carotene, and flavonoids, play an important role in the defense of the organism against reactive oxygen species (ROS) and free radical attacks (Meister 1992; Lindsay and Astley 2002; Bliska et al. 2007). They act as a coordinated system, where deficiencies in one component may affect the efficiency of the other one (Evans and Halliwell 2001).

Vanadium (V) is an element with complex chemistry and it can exist in many oxidation states (from −1 to +5), which enables the element to function as an electron transfer catalyst in a wide variety of reactions (Badmaev et al. 1999). Since it is a transitional metal having redox properties, it may enter redox processes and produce free radicals (Byczkowski and Kulkarni 1998), in this way inducing lipid peroxidation (LPO) in both in vitro (Donaldson and LaBella 1983; Younes et al. 1991; Younes and Strubelt 1991) and in vivo conditions (Elfant and Keen 1987; Sheriff 1991; Thompson and McNeill 1993; Russanov et al. 1994) and disturbing, thereby, the pro-antioxidative balance in the organism. V may also decrease the activities of some antioxidative enzymes in erythrocytes (RBCs; Soussi et al. 2006), in the liver (Oster et al. 1993; Saxena et al. 1993; Russanov et al. 1994), kidneys (Russanov et al. 1994), and testes (Chandra et al. 2007) of rats. It may diminish the concentrations of GSH and/or L-ascorbic acid in blood and/or internal organs (especially in liver and/or kidneys) of these animals (Chakraborty et al. 1977; Elfant and Keen 1987; Saxena et al. 1993; Zaporowska 1994; Zaporowska et al. 1997) and act as a mitogen, tumor promoter and co-carcinogen. On the other hand, V compounds may prevent chemical carcinogenesis and

act as potential antimetastatic agents, which suggests their use in treatment of cancer in humans (Papaioannou et al. 2004; Laizé et al. 2010). An osteogenic role of V and, thereby, its possible therapeutic application in osteoporosis has been described, too (Facchini et al. 2006; Laizé et al. 2010). Due to its antioxidant action, vanadate may also act as a scavenger of superoxide radicals ($O_2^{\bullet-}$; Matsubara et al. 1995). Its antioxidant properties have been mentioned, too, with respect to 1,2-dimethylhydrazine (DMH)-induced colon cancer in male rats (Kanna et al. 2004). The chemopreventive effects of V, as the authors reported, were accompanied by changes in the activities of some antioxidant enzymes, normalization of which might be caused by the antioxidant properties of the metal interacting with free radicals. Thus, V may behave as an antioxidant or as a pro-oxidant, depending on experimental conditions, the dose and form of its species as well as cell type (Matsubara et al. 1995; Badmaev et al. 1999; Aureliano and Crans 2009). Its "biphasic" effect—essentiality at low concentrations and toxicity at high concentrations is well known and has been presented in the literature (Chatterjee and Bishayee 1998).

As far as the antioxidant properties of trivalent chromium (Cr^{+3}) are concerned, it has been reported that Cr^{3+} as chromium polynicotinate led to reduction in the hepatic and renal LPO in rats with hypertension (Preuss et al. 1998); as chromium picolinate (CrPic) it produced a decrease in LPO in the serum in cold-stressed Japanese quails (Sahin et al. 2003b), in laying hens and in broiler chickens reared at a low (Sahin et al. 2002; Onderci et al. 2003) and a high ambient temperature (Sahin et al. 2003a), respectively, and in hepatic LPO in spontaneously hypertensive rats (Preuss et al. 1997). Supplementation with chromium (Cr), as chromium pidolate or chromium yeast has also been shown to (a) result in a decrease in the plasma thiobarbituric acid reactive substances (TBARS) level in Tunisian adult subjects with type 2 diabetes mellitus (Anderson et al. 2001) and (b) be an effective treatment strategy to minimize increased oxidative stress in type 2 diabetes mellitus patients whose glycated hemoglobin (HbA1C) level was >8.5% (Cheng et al. 2004), respectively. Chromium chloride ($CrCl_3$) has been reported, too, to be able to reduce V toxicity in rats (Curran 1954), chicks (Hill and Matrone 1970; Hafez and Kratzer 1976; Cupo and Donaldson 1987), and in laying hens (Jensen and Maurice 1980). Some of the antagonistic effects between those metals included alterations in growth, mortality, oxidative phosphorylation, lipogenesis and cholesterol, and fatty acid synthesis.

On the other hand, Cr^{+3} is well known to be capable of producing free radicals from hydrogen peroxide (H_2O_2) and lipid hydroperoxides (LOOH; Shi et al. 1993). Its reduction to divalent chromium (Cr^{+2}) by some biological reductants such as L-cysteine and reduced nicotinamide adenine dinucleotide (NADH) and its reaction with H_2O_2 to yield hydroxyl radicals ($HO^{\bullet}$) have been reported, too (Ozawa and Hanaki 1990). This element, likewise V (Keller et al. 1988), may be involved in redox cycling with ROS production, which clearly shows that Cr^{+3} may be one of the biologically most active oxidation states. It may maintain redox activity in vivo through Cr^{3+}/pentavalent chromium (Cr^{5+}) or Cr^{3+}/Cr^{2+} redox pairs, thus re-activating the radical cascade (Bal and Kasprzak 2002). So, Cr^{3+} may enter redox processes and induce oxidative stress due to its redox properties. Moreover, Cr^{3+} may accumulate in animals and humans (Stearns et al. 1995) and be genotoxic (Błasiak and Kowalik 2000; Codd et al. 2001; O'Brien et al. 2003), likewise V, (Owusu-Yaw et al. 1990; Bonnefont-Rousselot 2004; Leopardi et al. 2005). Its interactions with deoxyribonucleic acid (DNA) have been presented in the literature (Codd et al. 2001; Blankert et al. 2003; Levina and Lay 2005). The study of Kirpnick-Sobol et al. (2006) revealed that Cr^{3+} (as $CrCl_3$) is a more potent inducer of DNA deletions than hexavalent chromium (Cr^{6+}) and that nutritional supplement Cr^{3+} increases DNA damage in vitro and in vivo, when ingested with drinking water. $CrCl_3$ has also been demonstrated in the study of Lushchak et al. (2009) to induce stronger oxidative stress than Cr^{6+} at the same concentration. Besides $CrCl_3$, CrPic and chromium nicotinate have also been shown to increase oxidative stress, the former more than the latter, and to cause DNA damage (Bagchi et al. 1995, 2002; Hepburn et al. 2003; Vincent 2003; Whittaker et al. 2005).

As it is well known, several Cr^{3+} salts such as chloride, picolinate and nicotinate, likewise V salts, are used as common and available dietary supplements, especially popular among athletes, who take them for a long time (Gerrard et al. 1993; Anderson 1998). On the one hand, V and Cr dietary supplementation has been postulated to facilitate weight loss, a decrease in serum cholesterol and an increase in muscle mass (Lefavi et al. 1993; Anderson 1998;

Barceloux 1999; Lukaski 1999; Lamson and Plaza 2002; Vincent 2003); on the other hand, prolonged consumption of high-doses of Cr^{3+} and/or V supplements has been reported to give rise to deleterious effects, too (Vincent 2003; Talbott and Hughes 2007). Kirpnick-Sobol et al. (2006) paid attention to the fact that even small amounts of absorbed Cr^{3+} are potentially dangerous. This observation, as the authors stressed, has important implications for public health due to the widespread and unregulated consumption of Cr^{3+}-containing dietary supplements that are designed for efficient absorption. Stearns et al. (1995) have also indicated that Cr^{3+} supplementation might have possible adverse biological effects on the organism due to its accumulation in various tissues and that long-term use of Cr dietary supplements, especially in excessive doses and for extended periods, should be approached with caution. The safety of Cr^{3+}, as Bagchi and co-investigators (2002) emphasize, is largely dependent on the ligand; therefore, adequate clinical studies should be warranted to demonstrate the safety and efficacy of Cr^{3+} for human consumption. CrPic, for example, the most popular and widely marketed trivalent chromium supplement, has been observed to produce an increase in LPO in liver and kidney as well as a decrease in the activity and concentration of some antioxidants in these two tissues in rats (Bagchi et al. 2002). Some of V nutritional supplements are also known to be a very important source of its exposure because they may provide a high-dose of this element (Barceloux 1999; Chemical Information Review Document 2008). Leopardi et al. (2005) stress that in consideration of V genotoxic properties and its ubiquitous occurrence in water and diet items, assessment of the risk posed by the intake of low doses of this metal is an important public health issue.

Therefore, the study of Cr^{3+} and V toxicity is still necessary and further examinations are required because the description of their possible side effects may be helpful in establishing the optimal dose of those elements for pharmacological use. V and Cr^{3+} are well known to display an insulin-mimetic action (Anderson 2000; Crans 2000; Anderson et al. 2001; Thompson and Orvig 2004), and there are trials to use them as therapeutic agents in the therapy of diabetes in humans. V, besides its insulin-mimetic properties, has been reported to prevent and/or treat pancreatic B-cell lesions induced by the streptozotocin treatment (Mongold et al. 1990). Thus, studies of their pro-oxidant potential and other toxicological aspects examined under different experimental conditions are reasonable and still needed. It is essential to realize that although a lot has already been written about V and Cr^{3+} with respect to their toxicity and physiological activities, not all aspects of their action in the organism have been sufficiently recognized and explained until now; especially, there is lack of information regarding changes in the antioxidant defense system caused by precise V and/or Cr^{3+} doses, ingested orally during a specific period of time. It is clear that the effects of those elements on the antioxidant system, and more generally on living organisms, depend not only on the form in which they are administered, but also on the dose ingested and time of exposure.

Therefore, taking into account the above data and the fact that there has been increased interest in V and Cr^{3+} supplements, we wanted to answer some questions: (1) about the extent to which both metals administered separately and in combination in drinking water at the concentrations used will influence the diet and fluid intake, body weight gain, antioxidative enzyme activities, malondialdehyde (MDA) level and L-ascorbic acid concentration, (2) whether they will reveal their pro-oxidant action on RBCs at the doses consumed, (3) whether they will interact to affect the above-mentioned parameters, and (4) whether the changes, if they occur, will result only from independent action of V and/or Cr. It is important to notice that until now the mutual interactions between V and Cr^{3+} have not been fully studied; therefore, we decided to explore this subject. Examination of interactions between V and trivalent Cr is important because it may have medical significance. The knowledge about their effects, in the future, might be helpful in the therapy in people who are intoxicated with V^{+5} and Cr^{3+} environmentally and/or in individuals who consume pharmaceutical preparations containing the two elements separately or together for a long time. To the best of our knowledge, this is the first study of the antioxidant defense system in erythrocytes under co-administration of V^{5+} and Cr^{3+}. We chose erythrocytes because they are not only well equipped with antioxidative enzymes (SOD, CAT, cGSH-Px, GST) which scavenge free radicals and remove peroxides (Siems et al. 2000; Tsantes et al. 2006) as well as with GSH and L-ascorbic acid which are

involved in detoxification of vanadium and in formation of Cr^{3+} and VO^{2+} (vanadyl) complexes (Hojo and Satomi 1991; Yamamoto 1995; Baran 1998; Karan et al. 2005), but also because they provide antioxidant protection to other tissues and organs (Siems et al. 2000). The measurement of the above-mentioned antioxidants in those cells has been considered essential and useful in evaluating possible oxidative stress caused by V^{5+} and/or Cr^{3+} administration in drinking water.

Materials and methods

Reagents

Kits for GSH, cGSH-Px (EC 1.11.1.9), GR (EC 1.6.4.2), GST (EC 2.5.1.18), CAT (EC 1.11.1.6), and Cu,Zn-SOD (EC 1.15.1.1) were obtained from OXIS International Inc., Portland, USA. Kit for Total Antioxidant Status (TAS) was obtained from Calbiochem, San Diego, CA, USA. L-ascorbic acid and nitric acid (65%, Suprapure) were obtained from Sigma Chemicals, St. Louis, USA and from Merck, Darmstadt, Germany, respectively. Buffered solution of physiological salt (PBS) was obtained from Serum and Vaccine Factory (BIOMED, Lublin, Poland). Ultra-pure water assigned for atomic absorption spectrometry (AAS) was received from water purification system Elix 10 (Millipore Corporation, Billerica, MA, USA). All the chemicals were of the highest quality available. Deionized water was used throughout.

Animals and diet

Outbred 2-month old, albino male Wistar rats obtained from Brwinów/Warsaw (Poland) and weighing approximately 241 g (mean) at the beginning of the experiment were used. All the rats were individually housed in stainless steel cages (one rat per cage) and kept in standard laboratory conditions at constant temperature (20–21°C), relative air humidity (55± 10%) and standard light–dark cycles (12 h/12 h). The experimental protocol was approved by the First Local Ethical Committee for Animal Studies in Lublin (registration number 48/2007). All the animals were randomly divided into four groups (14 animals per each group). Group I (control) received daily deionized water to drink from ARIES deionizer (Resin Tech., Inc., USA) over a 12-week period. The other three treatment groups received deionized water, to which V or/and Cr was added; Group II—water solution of sodium metavanadate—$NaVO_3$ (SMV; Sigma, St. Louis, MO, USA) at a concentration of 0.100 mg V/mL (1.96 mM; pH 6.48), Group III—water solution of chromium chloride hexahydrate—$CrCl_3 \cdot 6H_2O$ (CC; Sigma, St. Louis, MO, USA) at a concentration of 0.004 mg Cr/mL (0.077 mM; pH 4.94), Group IV—water solution of SMV-CC at a concentration of 0.100 mg V and 0.004 mg Cr/mL (1.96 and 0.077 mM; pH 6.66) also over the period of 12 weeks. The mean start weight of rats in Groups I, II, III, and IV was about 240, 239, 238, and 245 g, respectively. V and Cr concentrations in drinking water were chosen on the basis of our previous studies (Zaporowska and Ścibior 1998; Terpiłowska et al. 2004; Ścibior 2005) and studies of other authors (Russanov et al. 1994; Bataineh et al. 1997). The V and Cr intake was calculated on the basis of the amount of SMV, CC, and SMV-CC solutions consumed by the rats. All the animals were fed with the same standard granulated rodent laboratory chow (Labofeed B; Fodder and Concentrate Factory, Kcynia, Poland), having the shape of pellets 12 mm in diameter, with the following ingredients per kilogram of Labofeed B (Table 1).

Diet, fluids and deionized water were offered ad libitum and their consumption was monitored daily over the experimental period. Daily intake of fluids and water was measured with a measuring cylinder and the remaining diet plus additional spillage were weighed.

Biochemical analyses

Blood samples were taken from the jugular vein following anesthesia with pentobarbital (30 mg/kg body weight; intraperitonealy) into plastic tubes with bipotassium ethylene-diaminetetraacetic acid (K_2EDTA) as an anticoagulant. The plasma was separated by centrifugation of whole blood (5 min, 1,500×*g*, 4°C) and used for determination of L-ascorbic acid concentration and TAS. The L-ascorbic acid content in the plasma was determined by Kyaw's method (Kyaw 1978) and the results were expressed as micromoles per liter (μmol/L). As far as TAS is concerned, in this method 2,2′-azino-bis-3-ethyl-benzothiazoline-6-sulfonic acid (ABTS) is incubated with metmyoglobin

Table 1 Diet composition for animals used in the experiment

Ingredients	Content (per 1 kg of Labofeed B)
Total protein	170.0 g
Digestible protein	155.0 g
Starch	314.8 g
Crude lipid	35.0 g
Crude fiber	70.0 g
Aminoacids	43.5 g
Total Ca	10.0 g
Total P	7.5 g
Total Na	2.2 g
Mg	2.5 g
K	9.0 g
Cl	1.2 g
Vitamin A	5.172 mg
Vitamin D_3	0.037 mg
Nicotinic acid	30.000 mg
Pantothenic acid	21.000 mg
Folic acid	12.000 mg
V[a]	0.44 mg
Cr[a]	2.12 mg

[a] The V and Cr concentrations in the Labofeed B laboratory chow were determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES). The content of other minerals and vitamins (per kg of Labofeed B) was: Mn (100.0 mg), Zn (100.0 mg), Fe (250.0 mg), Cu (20.0 mg), J (0.8 mg), Se (0.8 mg), Co (1.8 mg); vitamin E (70.0 mg), K_3 (2.4 mg), C (trace), B1 (15.0 mg), B2 (12.0 mg), B6 (15.0 mg), B12 (0.06 mg)

(a peroxidase) and H_2O_2 to produce the radical cation ($ABTS^{\bullet+}$), which has a relatively stable blue-green color and can be monitored by reading the absorbance at 600 nm. A 20-μl sample was used in the analysis and the results were expressed as millimoles per liter (mmol/L).

The L-ascorbic acid concentration in RBCs, which were washed three times in ice-cold 0.9% sodium chloride (NaCl), was also determined by Kyaw's method (Kyaw 1978) and the results were expressed as micromoles per liter (μmol/L). The MDA level in these cells was determined as TBARS using Stocks and Dormandy's method (Stocks and Dormandy 1971), modified by Gilbert et al. (1984) and it was calculated using the molar extinction coefficient 1.56×10^5 M^{-1} cm^{-1}. The results were expressed as nanomoles per milligram of hemoglobin (nmol/mg Hb). The GSH concentration in RBCs was determined according to the Bioxytech® GSH-420 assay procedure and the results were expressed as micromoles per liter (μmol/L). For determination of Cu,Zn-SOD, CAT, cGSH-Px, GR, and GST activities, washed RBCs were resuspended in four packed-cell volumes of ice-cold deionized water. For determination of Cu,Zn-SOD activity which was measured in the aqueous phase, erythrocyte lysates were treated with a cold extraction reagent (absolute ethanol/chloroform 62.5/37.5 *v*/*v*) in order to remove Hb; while measuring CAT activity, the erythrocyte lysates were diluted with a sample diluent, and the results for both enzymes were expressed in units per milligram of hemoglobin (U/mg Hb). To measure the activity of cGSH-Px, GR and GST (determined using 1-chloro-2,4-dinitrobenzene (CDNB) as substrate), the erythrocyte lysates were diluted with buffer—pH 7.6 or with a diluent—pH 7.5 or with a sample diluent, respectively, and the results were expressed as milliunits per milligram of hemoglobin (mU/mg Hb).

Hb concentration

The Hb concentration in the erythrocyte lysates was measured by the cyanmethemoglobin method using Alpha Diagnostics reagents (Warsaw, Poland) in order to determine the specific activity of Cu,Zn-SOD, CAT, cGSH-Px, GR, and GST.

Determination of V and Cr in the blood

The V and Cr concentrations in the chow and in the plasma were determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES) using the Liberty II AX plasma emission spectrometer (VARIAN) with the Ultrasonic Nebulizer (U-5000 AT^+, CETAC), whereas V and Cr concentrations in RBCs were determined by Graphite Furnace Atomic Absorption Spectrometry (GF-AAS) using the Z-8200 HITACHI atomic absorption spectrophotometer after microwave digestion. Before measurement, all the samples were diluted with ultra-pure water. An internal quality control was employed to ensure that the measurement process was reliable. The V and Cr concentrations in the chow were expressed as milligrams per kilogram (mg/kg); whereas in the plasma and in RBCs they were expressed as micrograms per milliliter (μg/mL).

Statistical analysis

The data were processed with Statistica version 12.0 PL for Windows. Normal distribution was tested by the Shapiro–Wilk's test. Homogeneity of variances was analyzed employing Levene's test. Since there were no significant deviations from the assumptions of the ANOVA, the two-way analysis of variance was used (two-way ANOVA; factors of vanadium and chromium, test *F*). If the two-way ANOVA indicated a significant V-treatment effect, Cr-treatment effect or V × Cr interaction, subsequent post-hoc comparisons between the individual groups were performed using Tukey's multiple range test when the variances were homogeneous or the Dunnett's T3 test when the variances were not homogeneous. Student's '*t*' test was applied for detection of significant differences in the concentrations of V in the plasma and RBCs only between Group II and IV (the two-way ANOVA was not performed in this case). Student's '*t*' test was also applied for comparison of the consumed V doses between Group II and IV and Cr doses between Group III and IV. The differences between the Groups were considered to be significant at $P<0.05$. All the results were expressed as means±SEM.

Results

During the 12 weeks of the experimental period, no distinctive differences in physical appearance and in motor behavior were observed in most of the rats treated with SMV and/or CC, compared with Group I. Four rats intoxicated with SMV alone, nine treated with CC alone and five exposed to SMV and CC in combination had transient diarrhea, whose main cause was probably the intake of V and/or Cr solutions at the concentrations used. More details of the diarrhea are presented in Table 2.

The two-way ANOVA revealed a significant main effect of V on the diet and fluid intake, body weight gain and food efficiency ratio (FER); and, as the results of those ANOVA analysis indicated, the changes in the above-mentioned parameters in the rats co-treated with SMV and CC only resulted from independent action of V (Table 3). Post-hoc comparisons demonstrated that the administration of SMV alone (Group II) or in combination with CC (Group IV) produced a decrease in the diet and fluid intake as well as in body weight gain and FER, compared with Group I (diet intake was lower by: 14.6%, $P<0.001$ and 11.5%, $P<0.01$; fluid intake by: 31%, $P<0.001$ and 24.7%, $P<0.001$; body weight gain by: 25%, $P<0.01$ and 23%, $P<0.05$; FER by: 16%, $P<0.05$ and 17%, $P<0.05$, respectively) and compared with Group III (diet intake was lower by: 12.5%, $P<0.01$ and 9.5%, $P<0.05$; fluid intake by: 29%, $P<0.001$ and 22.7%, $P<0.001$; body weight gain by: 29.8%, $P<0.001$ and 28%, $P<0.01$; FER by: 22.2%, $P<0.01$ and 23%, $P<0.001$, respectively; Table 3).

The rats from the particular experimental groups consumed with drinking water about 9 mg V/kg b.w./24 h (0.18 mmol V/kg b.w./24 h; Group II), 0.47 mg Cr/kg b.w./24 h (0.010 mmol Cr/kg b.w./24 h; Group III), 9.8 mg V (0.19 mmol) and 0.39 mg (0.007 mmol) Cr/kg b.w./24 h (Group IV; Table 4). The rats in Group IV ingested about 5% of V more, compared with the animals in Group II, but this difference was not statistically significant. However, the dose of Cr ingested by the rats in Group IV was lower (by 17%, $P<0.001$), compared with that ingested by the animals in Group III (Table 4).

V concentrations in RBCs and in the plasma in Group I and III were below the detection limit (Table 5). The exposure to SMV alone or in combination with CC resulted in a significant increase in V concentration in the plasma and RBCs, compared with Group I and III; but no significant changes in its content between the rats in Group II and IV were demonstrated (Table 5). Despite the fact that the two-way ANOVA of Cr concentration in RBCs yielded a significant main effect of Cr, post-hoc comparisons did not demonstrate any marked changes in this parameter in all the treated Groups of rats; although a visible tendency to an increase in Cr content in RBCs was observed. The concentration of Cr in the plasma also slightly increased but no significant changes were demonstrated, too (Table 5).

A significant main effect of Cr on L-ascorbic acid concentration in the plasma was revealed by the two-way ANOVA and according to those ANOVA analysis the changes in the content of this antioxidant in the plasma of rats co-treated with SMV and CC were a consequence of independent action of Cr (Table 6). Post-hoc comparisons demonstrated that in the rats treated with CC alone, the plasma L-ascorbic acid concentration was higher (by 54%, $P<0.05$), as compared to the animals in Group II. However, no

Table 2 The animals in which diarrhea occurred during a 12-week experimental period

Groups	Number of animals in the Groups (n)	Number of animals with diarrhea (n)		Weeks																													
				I			II			III				IV				V		VII			VIII		IX			X		XI			
				2	3	4	2	3	5	1	2	3	6	2	3	4	6	2	7	1	2	3	2	3	1	2	3	1	5	1	2		
Control	14	-																															
SMV	14	4	1	a																													
			1																a												a	a	
			1																		a						a						
			1												a																		
CC	14	9	1		a												a		a											a	a		
			1											a																			
			1																a														
			1									a	a			a		a	a														
			1					a															a	a									
			1	a				a																									
			1																						a	a							
			1																	a	a												
			1							a					a																		
SMV-CC	14	5	1				a															a										
			1								a																						
			1	a		a			a																								
			1			a																											
			1					a																									

Control the control rats received deionized water to drink; the other groups of rats were exposed to sodium metavanadate (*SMV*; 0.100 mg V/mL) and/or chromium chloride (*CC*; 0.004 mg Cr/mL) solutions for 12 weeks. In week VI and XII no marked gastrointestinal discomfort was observed: *1*,*2*,*3*,*4*,*5*,*6*,*7*—days of the week in which diarrhea in some of the rats from particular groups occurred: *4*, *9*, *5*—the total of the rats with diarrhea in the experimental groups.

[a] Diarrhea observed in a particular individual animal in the treated group

Table 3 Intake of diet and water/fluids as well as body weight gain and food efficiency ratio (FER) in the tested animal groups

Groups	Diet [g/rat/24 h]	Water/fluids [mL/rat/24 h]	Body weight gain [g/12 weeks]	FER
I. Control	26.40±0.78b	44.62±1.08b	213.36±11.98b	0.100±0.004b
II. SMV	22.53±0.43a	30.87±0.89a	160.32±10.59a	0.084±0.004a
III. CC	25.74±0.66b	43.49±0.95b	228.50±11.78b	0.108±0.002b
IV. SMV-CC	23.37±0.55a	33.60±1.15a	164.40±10.09a	0.083±0.003a
Main effect of V[a]	F=24.395; P=0.000	F=128.393; P=0.000	F=27.667; P=0.000	F=26.946; P=0.000
Main effect of Cr[a]	NS	NS	NS	NS
Interactive effect of V and Cr[a]	NS	NS	NS	NS

Control the control rats received deionized water to drink; the other groups of rats were exposed to sodium metavanadate (*SMV*; 0.100 mg V/mL) and/or chromium chloride (*CC*; 0.004 mg Cr/mL) solutions for 12 weeks. The results were expressed as mean ± SEM of 14 animals. The means for the intake of the diet [g], fluids [mL] and deionized water [mL] were calculated from week 1 to 12 (84 values). The gain in body weight was calculated using the formula: body weight gain [g/12 weeks]=mean end weight−mean start weight. Values followed by different letters (a, b) indicate significant differences between the groups at the indicated level $P<0.05$ (ANOVA/Tukey's test)

FER food efficiency ratio: body weight gain (g)/food consumed by the rats in the experimental period

[a] Two-way analysis of variance (ANOVA; test F; $P<0.05$). *NS* no significant effect

significant changes were found in the values of MDA and L-ascorbic acid in RBCs and in the plasma TAS level in all the Groups of the rats studied (Table 6).

The two-way ANOVA did not reveal any significant main effect of V and/or Cr on the activities of Cu-Zn-SOD, CAT, cGSH-Px, and GR in RBCs but it indicated a significant effect of Cr on the GSH concentration in RBCs as well as a significant effect of Cr and V×Cr interaction on the GST activity in RBCs (Tables 7 and 8). Post-hoc comparisons demonstrated that the administration of CC alone or in combination with SMV resulted in an increase in GSH content (by 59%, $P<0.05$ and by 53.6%, $P<0.01$, respectively) and led to a decrease in the activity of GST (by 56.7%, $P<0.001$ and by 40%, $P<0.01$), compared with Group I. The administration of SMV alone also caused an increase in GSH level (by 28%, $P>0.05$) and produced a decrease in GST activity (by 30%, $P<0.05$), in comparison with Group I (Table 8). As the two-way ANOVA showed, the changes in GSH concentration in the rats following SMV and CC co-treatment resulted from independent action of Cr; however, the changes in GST activity in the same Group of rats were a consequence of independent effect of Cr and they also resulted from V–Cr interaction (Table 8).

Discussion

We studied the changes induced in certain blood biochemical parameters upon administration to rats

Table 4 Vanadium and chromium doses consumed by the rats with drinking water during 12 weeks

Groups	Number of animals	Vanadium (mg/kg b.w./24 h) (mmol/kg b.w./24 h)		Chromium (mg/kg b.w./24 h) (mmol/kg b.w./24 h)	
I. Control	14	–	–	–	–
II. SMV	14	9.33±0.36a	0.18±0.007a	–	–
III. CC	14	–	–	0.47±0.01	0.010±0.0002
IV. SMV-CC	14	9.80±0.27a	0.19±0.005a	0.39±0.01[a]	0.007±0.0002[a]

Control the control rats received deionized water to drink; the other groups of rats were exposed to sodium metavanadate (*SMV*; 0.100 mg V/mL) and/or chromium chloride (*CC*; 0.004 mg Cr/mL) solutions for 12 weeks. The results were expressed as mean ± SEM of 14 animals. Identical letters (a) did not indicate significant differences between Groups II and IV (t test; $P<0.05$)

[a] Value is significantly different in comparison with Group III (t test; $P<0.05$)

Table 5 Concentrations of vanadium and chromium in the erythrocytes (RBCs) and plasma in the tested animal groups

Groups	Vanadium [μg/mL]		Chromium [μg/mL]	
	RBCs	Plasma	RBCs	Plasma
I. Control	–[a]	–[a]	0.281±0.02a	0.020±0.005
II. SMV	0.101±0.01b	0.518±0.06b	0.292±0.04a	0.022±0.007
III. CC	–[a]	–[a]	0.576±0.17a	0.048±0.004
IV. SMV-CC	0.106±0.04b	0.459±0.04b	0.460±0.03a	0.026±0.014
Main effect of V[b]	–	–	NS	NS
Main effect of Cr[b]			F=5.149; P=0.044	NS
Interactive effect of V and Cr[b]			NS	NS

Control the control rats received deionized water to drink; the other groups of rats were exposed to sodium metavanadate (*SMV*; 0.100 mg V/mL) and/or chromium chloride (*CC*; 0.004 mg Cr/mL) solutions for 12 weeks. The results were expressed as mean ± SEM of 14 animals. Identical letters (b) did not indicate significant differences between Group II and IV at the indicated level $P<0.05$ (t test). Identical letters (a) did not indicate significant differences between the groups at the indicated level $P<0.05$ (ANOVA/Tukey's test)

[a] Below the detection limit

[b] Two-way analysis of variance (ANOVA; test F; $P<0.05$). *NS* no significant effect. Since the concentrations of V in the plasma and RBCs of the rats in Group I and III were below the detection limit, Student's 't' test was applied for detection of significant differences in these parameters only between Group II and IV. Therefore, the two-way ANOVA was not performed in this case

SMV and CC separately and in combination in drinking water. Although it was difficult to compare our results with the studies of other researchers, because of different V and Cr compounds used, modes of their administration, concentrations and length of exposure, we decided to present some of them taking into account the above-mentioned differences to compare toxicological effects of the two different metals, especially in the sense of their some toxic effects, which will partially be presented in the further part of the discussion.

As it was here demonstrated, V at the dose of 9 mg V/kg b.w./24 h produced a significant fall in the diet and fluid intake, body weight gain and FER (Table 3). The decreased diet and/or fluid intake and/or body weight gain in rats after administration of vanadium compound solutions as vanadate or vanadyl was also observed in our previous studies (Zaporowska

Table 6 The level of malondialdehyde (MDA) in the erythrocytes (RBCs) and the concentration of L-ascorbic acid in the plasma and erythrocytes as well as the total antioxidant status (TAS) in the plasma in the tested animal groups

Groups	MDA L-ascorbic acid [nmol/mg Hb] [μmol/L] RBCs	L-Ascorbic acid [μmol/L] RBCs	L-Ascorbic acid [μmol/L] Plasma	TAS [mmol/L] Plasma
I. Control	0.175±0.02	255.90±52.33	105.97±9.33a,b	0.900±0.03
II. SMV	0.186±0.03	178.10±28.91	85.48±7.72a	0.767±0.08
III. CC	0.244±0.05	190.58±29.39	131.59±7.78b	0.906±0.03
IV. SMV-CC	0.217±0.03	203.64±31.61	107.64±14.65a,b	0.918±0.05
Main effect of V[a]	NS	NS	NS	NS
Main effect of Cr[a]	NS	NS	F=4.518; P=0.043	NS
Interactive effect of V and Cr[a]	NS	NS	NS	NS

Control the control rats received deionized water to drink; the other groups of rats were exposed to sodium metavanadate (*SMV*; 0.100 mg V/mL) and/or chromium chloride (*CC*; 0.004 mg Cr/mL) solutions for 12 weeks. The results were expressed as mean ± SEM of fourteen animals. Values followed by different letters (a, b) indicate significant differences between the groups at the indicated level $P<0.05$ (ANOVA/Tukey's test)

[a] Two-way analysis of variance (ANOVA; test F; $P<0.05$). *NS*, no significant effect

Table 7 The activity of Cu, Zn-superoxide dismutase (Cu, Zn-SOD) and catalase (CAT) in the erythrocytes (RBCs) in the tested animal groups

Groups	Cu, Zn-SOD [U/mg Hb]	CAT [U/mg Hb]
I. Control	5.39±0.36	580.32±35.81
II. SMV	4.73±0.46	597.91±36.08
III. CC	4.59±0.51	646.37±43.08
IV. SMV-CC	5.32±0.40	601.28±22.85
Main effect of V[a]	NS	NS
Main effect of Cr[a]	NS	NS
Interactive effect of V and Cr[a]	NS	NS

Control the control rats received deionized water to drink; the other groups of rats were exposed to sodium metavanadate (*SMV*; 0.100 mg V/mL) and/or chromium chloride (*CC*; 0.004 mg Cr/mL) solutions for 12 weeks. The results were expressed as mean ± SEM of fourteen animals

[a] Two-way analysis of variance (ANOVA; test *F*; $P<0.05$). *NS*, no significant effect.

et al. 1997; Ścibior and Zaporowska 1998; Zaporowska and Ścibior 1999; Zaporowska et al. 1999; Ścibior and Zaporowska 2002; Ścibior 2005) and in the studies of other authors (Meyerovitch et al. 1989, Thompson and McNeill 1993, Thompson et al. 1993, Russanov et al. 1994, Dai et al. 1995, Poggioli et al. 2001, Thompson et al. 2002, Mohamad et al. 2004, Kordowiak et al. 2005, Dąbroś et al. 2006). However, other investigators did not demonstrate any significant changes in the diet and fluid intake (Bishayee and Chatterjee 1995; De Tata et al. 2000; Poggioli et al. 2001) or in body weight gain (Dai et al. 1995; Thompson et al. 2002) after treating rats with vanadate or vanadyl in drinking water. A reduction in diet intake and/or body weight gain after administration of vanadium was also shown in other animals: in laying hens (Ousterhout and Berg 1981), sheep (Hansard et al. 1982), chicks (Cupo and Donaldson 1987), and in male mice (Llobet et al. 1993).

Reduced fluid intake after SMV treatment, as it was shown (Table 3), might be caused by an altered taste of vanadate solution which might lead to development of rats' aversion to drink, and in consequence, cause its decreased consumption. Heyliger et al. (1985) suggested that vanadate ingestion may induce water retention, which is subsequently responsible for the diminished water intake. Mongold et al. (1990) paid attention to a hypodipsic effect of the vanadium salts. Thus, this phenomenon still remains to be explained. The addition of $CrCl_3$ (CC) into SMV solution (Group IV) did not improve the SMV-CC taste and did not elevate its consumption. The animals drank significantly less SMV-CC solution compared with the control rats and with those drinking CC alone (Group III). It is important to mention that this decrease, as the two-way ANOVA indicated, was only a consequence of independent action of V. On the other hand, the administration of SMV and CC in combination did not result in further reduction in fluid intake, which allows

Table 8 The concentration of reduced glutathione (GSH) and activity of cellular glutathione peroxidase (cGSH-Px), glutathione reductase (GR) and glutathione transferase (GST) in the erythrocytes (RBCs) in the tested animal groups

Groups	GSH [μmol/L]	cGSH-Px [mU/mg Hb]	GR [mU/mg Hb]	GST [mU/mg Hb]
I. Control	79.06±6.72a	97.03±5.76	2.03±0.19	0.30±0.02b
II. SMV	101.41±13.53a,b	84.49±4.47	2.43±0.09	0.21±0.02a
III. CC	125.78±13.50b	91.87±9.01	2.46±0.08	0.13±0.01a
IV. SMV-CC	121.48±7.11b	100.12±7.10	2.98±0.34	0.18±0.01a
Main effect of V[a]	NS	NS	NS	NS
Main effect of Cr[a]	$F=9.652$; $P=0.003$	NS	NS	$F=20.288$;$P=0.000$
Interactive effect of V and Cr[a]	NS	NS	NS	$F=11.518$;$P=0.002$

Control the control rats received deionized water to drink; the other groups of rats were exposed to sodium metavanadate (*SMV*; 0.100 mg V/mL) and/or chromium chloride (*CC*; 0.004 mg Cr/mL) solutions for 12 weeks. The results were expressed as mean ± SEM of 14 animals. Values followed by different letters (a, b) indicate significant differences between the groups at the indicated level $P<0.05$ (ANOVA/Tukey's test). Values followed by different letters (a, b) indicate significant differences between the groups at the indicated level $P<0.05$ (ANOVA/Dunnett's T3 test)

[a] Two-way analysis of variance (ANOVA; test *F*; $P<0.05$). *NS*, no significant effect

us to claim that the rats simultaneously treated with SMV and CC did not develop stronger aversion to drink (Table 3). It is also worth noting that the animals in Group IV ingested a significantly lower Cr dose in comparison with that consumed by the rats treated with CC alone (Table 4) and, as a result, Cr concentration in the plasma and RBCs in these rats was lower (but not significantly) than in the animals of Group III (Table 5).

As far as the inhibitory effect of orally (via drinking water) administered $NaVO_3$ to male Wistar rats on diet intake is concerned, Meyerovitch et al. (1989) suggest that this effect may result from direct action of the ion in the central nervous system (CNS) involving stimulation of local glucose uptake. Thus, as the authors stress, vanadate is capable of blocking diet intake by a specific effect in the CNS mentioned above. This local effect, which leads to a rise in intracellular glucose, transduces a signal that can suppress diet intake and, consequently, body weight gain. Other authors reported that decreased appetite produced by vanadium administered in drinking water may be linked to decreased neuropeptide Y (NPY) levels in the hypothalamus (Wang et al. 2001), which was observed in the Zucker fatty rats, characterized by hyperinsulinemia, high NPY levels, and hyperphagia. However, this mechanism cannot be directly related to our experiment because, among others, a different V compound—bis(maltolato)oxovanadium (IV) (BMOV) and a different animal model were used by the mentioned authors. In contrast, the studies of Malabu et al. (1994) demonstrated an increase (higher than in the control) in the hypothalamic NPY concentration in the non-diabetic and diabetic rats treated by gavage $NaVO_3$. The difference in the results obtained by Wang et al. (2001) and by Malabu et al. (1994), first of all, was due to the different animal model used and to the application of different experimental conditions.

Cr, at the dose of 0.47 mg Cr/kg b.w./24 h, ingested by rats via drinking water during 12 weeks, did not produce any significant changes in the diet and fluid intake and in body weight gain (Table 3), which is in agreement with our previous studies, in which the level of the consumed Cr dose during only 6 weeks was very similar to that presented above (Ścibior 2003; 2005). Unchanged diet intake was also shown in pigs (Mooney and Cromwell 1997), in broiler chicks (Kalaycioglu et al. 1999), and in laying hens (Piva et al. 2003) when $CrCl_3$ was added into the diet. More data about the changes in body weight gain in rats and also in other animals after Cr^{+3} administration were presented and discussed in our previous paper (Ścibior 2005).

The decreased body weight gain in the rats co-treated with SMV and CC (Table 3), which (as the two-way ANOVA indicated) was only a consequence of action of V alone, was also shown by other authors in chicks fed with a diet supplemented with ammonium metavanadate (NH_4VO_3) and chromium acetate (Wright 1968; Nielsen et al. 1980), or with NH_4VO_3 and $CrCl_3$ (Hill and Matrone 1970; Hafez and Kratzer 1976) or with $NaVO_3$ and $CrCl_3$ (Cupo and Donaldson 1987).

The unchanged L-ascorbic acid concentration in RBCs between the control and SMV-only treated rats shown in the present experiment (Table 6) was also observed in our previous studies after ammonium metavanadate (AMV) intoxication (Zaporowska et al. 1993, 1997). The plasma L-ascorbic acid content did not change either; however, in our earlier studies, its significant decrease in the plasma following vanadate treatment in drinking water was found (Zaporowska et al. 1997; Ścibior and Zaporowska 1998; Ścibior 1999). This is not surprising, because previously we used a different V compound and the period of its administration was shorter (4 weeks). The dose of V ingested was also higher (10–11 mg V/kg b.w./24 h) than that consumed by the rats in this study.

L-ascorbic acid, a potent antioxidant (Lukaski 2004), is an endogenous compound for rats, which can synthesize it in the liver (Meister 1992). By causing a decrease in its concentration, which could be due to its inhibited biosynthesis, enhanced catabolism and/or increased utilization under some experimental conditions, as it was demonstrated in our previous experiment and in the studies of other researchers (Chakraborty et al. 1977), V influences all the metabolic processes, in which this non-enzymatic antioxidant performs regulatory functions. This is very important because L-ascorbic acid in high concentrations acts as an antioxidant (Niki 1991; Halliwell 1996), but in low concentrations acts as a pro-oxidant (Song et al. 1999). V and L-ascorbic acid have been reported to act synergistically in some conditions, too (Sakurai et al. 2004). L-ascorbic acid has also been demonstrated to form complexes with many metals (Kleszczewska 2001), and it has often been considered one of the possible natural reducing agents of vanadate

to vanadyl (VO^{2+}), which is then complexed with the acid (Baran 1998). Attention needs to be paid to the fact that during vanadate reduction mechanism in the presence of ascorbic acid generation of free radicals with strong oxidation potential has been postulated (Ding et al. 1994) and that some complexes of VO^{2+} with this acid may cause DNA nicks, LPO, cytotoxicity as well as depletion of the level of GSH and changed activity of GSH-Px, GR and GST. Cr^{3+} complexes with ascorbic acid may also cause DNA nicks, cytotoxicity and produce an increase in LPO, but they do not show any effect on the GSH level and on the activity of the above-mentioned enzymes (Sreedhara et al. 1997).

The administration of CC alone (Group III) or in combination with SMV (Group IV) resulted in a significant increase in the GSH concentration in RBCs, which was about 59% and 53.6% higher, respectively, than in Group I (Control), whereas in Group II (SMV-only intoxicated rats) it was elevated by 28% (Table 8). GSH is known to play an important role in detoxification processes and in protecting cells against oxidative stress (Cnubben et al. 2001). Its concentration in RBCs may reflect GSH status in other tissues. The increased GSH concentration might be, at least in part, a consequence of elevated GR activity (by 19% in Group II, by 21% in Group III, and by 46% in Group IV); on the other hand, it might be associated with an increase in the erythrocyte gamma-glutamylcysteine synthetase (γ-GCS) activity, an enzyme which together with glutathione synthetase participates in the synthesis of GSH de novo from three amino acids such as glycine, cysteine, and glutamic acid (Wang and Ballatori 1998). Furthermore, the significantly decreased GST activity in Groups II, III, and IV (by 30, 56 and 40%, respectively) might also be, at least partially, responsible for the enhancement of GSH content, which in this case might indicate its decreased use. We cannot exclude a reduction in its export, either. Thus, the above-mentioned effects could take place together, and although the mechanism responsible for the observed increase in the erythrocyte GSH level is not fully clear at present and further studies are necessary to confirm our assumptions, we may claim that the increase probably resulted from an additive and/or an adaptive response to SMV and/or CC exposure. It is important to stress that in our previous studies, in which we observed slightly decreased (Zaporowska et al. 1993) or significantly lowered (Ścibior 1999) GSH concentration after vanadate treatment in rats, different experimental conditions were used and this antioxidant was assessed in whole blood, not in RBCs.

The unchanged level of MDA, which is considered to be one of decomposing product of LOOH (Niki 2009), observed in RBCs after SMV treatment (Group II, Table 6) is at odds with our previous study, in which a significant increase in its content was demonstrated (Ścibior 1999). Like in the case of the plasma L-ascorbic acid concentration, these discrepancies might be due to large differences between V doses consumed, V compounds used, and the time of their administration.

Vanadate is well known to be transported into RBCs through anion channels and reduced to vanadyl (Baran 1998) which is potentially very damaging because it can autoxidize a thus produced superoxide anion radical and vanadate, which may subsequently react with this radical to yield reactive intermediates responsible for initiation of LPO (Byczkowski and Kulkarni 1998). Moreover, vanadyl may react with H_2O_2 and produce reactive $HO^{\bullet}$ in a Fenton-type reaction (Byczkowski and Kulkarni 1998). However, the results of our study showed that V (as SMV), at the dose of 9 mg V/kg b.w./24 h, ingested by rats in drinking water during 12 weeks, did not have a pro-oxidant effect on RBCs, which was reflected in the unchanged L-ascorbic acid concentration and MDA level as well as Cu,Zn-SOD, CAT, cGSH-Px, and GR activities in these cells (Tables 6, 7, and 8). The unaltered SOD activity in RBCs after treating the rats vanadate in drinking water was also demonstrated in our previous study (Ścibior 1999) and in the studies of Sekar et al. (1990), Oster et al. (1993) and Russanov et al. (1994); whereas the unchanged erythrocyte CAT activity after vanadate intoxication was shown by Sekar et al. (1990), Zaporowska et al. (1993) and by Russanov et al. (1994). The unaltered erythrocyte cGSH-Px activity found in the present experiment was also demonstrated in the studies of Russanov et al. (1994) and in our earlier study (Ścibior 1999), but contradictory results showing increased activity (Tas et al. 2006) have been reported, too. These conflicting results might be due to the different experimental conditions used by the authors (Tas et al. 2006). The lack of significant changes in the plasma TAS value after SMV

treatment (Table 6) remains consistent with the results from our previous experiment (Ścibior 2005).

The treatment of rats with CC alone (Group III) did not change the level of MDA (Table 6) and the activities of Cu,Zn-SOD, CAT and cGSH-Px in RBCs (Tables 7 and 8), which allows us to conclude that $CrCl_3$, (0.47 mg Cr/kg b.w./24 h) did not act as a pro-oxidant at the dose ingested. However, its administration separately (Group III) or together with SMV (Group IV) caused a significant decrease in GST activity (by 56% and 40%, respectively, Table 8). Since in the available literature we did not find any information about the decrease in GST activity in RBCs of rats after V^{+5} and/or Cr^{+3} administration in drinking water, it is difficult to discuss our results. Our in vivo studies are the first, to the best of our knowledge, which show this effect. However, the decreased GST activity in blood was described in lead-exposed workers by Hunaiti et al. (1995), who postulated direct or indirect inhibition of GST activity and suggested that this reduction might be caused by depletion of GSH or binding of lead to sulfhydryl (SH) groups. A decrease in GST activity was also demonstrated by Hunaiti and Sound (2000) during incubation of human whole blood containing some lead compounds. Reddy et al. (1981) also observed inhibition of GST by lead and by other metals, too, such as cadmium and mercury in in vitro studies on calf liver homogenates, and the authors suggested that these metals inhibit GST activity by direct binding with an essential group(s) of the protein which promotes the catalytic function.

It is difficult to explain why in all the treated Groups of rats the GST activity, an enzyme which catalyzes the conjugation of GSH to a wide variety of endogenous toxic electrophilic compounds (Townsend et al. 2003), significantly decreased in RBCs, instead of increasing, when, paradoxically, GSH concentration in these Groups of animals increased. Cr^{+3} is well known to have affinity for the histidyl, methionyl, and cysteinyl groups of proteins as well as for such sulfur-type ligands as GSH (El-Shahawi 1995; Baran 1998; O'Brien et al. 2003). Thus, Cr^{+3} may bind tightly to many types of proteins and GSH to form stable complexes, some of which may damage the structure and function of proteins (Yamamoto 1995). The reaction of V^{+5} with SH-containing molecules is also well known to result in the reduction of vanadium and oxidation of thiol-containing molecules (Ballistreri et al. 2000; Çakir and Biçer 2004). Therefore, we may suppose that inhibition of GST activity in our experimental model might result from a direct interaction of V and/or Cr with the SH group of cysteine, which led to its modification and consequently diminished its activity. On the other hand, we did not exclude the possibility that V and/or Cr might produce some other factors leading to site-specific inactivation of GST. Therefore, our speculations need to be elucidated in further investigations to find the correct mechanism by which both elements administered separately or in combination decrease the erythrocyte GST activity, because the present results cannot explain this issue at this stage of studies. Recently, vanadium-binding GST has been identified from the vanadium-rich ascidian (*Ascidia sydneiensis samea*; Yoshinaga et al. 2006), which seems to have the ability to bind vanadium ions and seems to be able to act as a dual function protein as vanadium-specific metallochaperone and as a GST enzyme; this may suggest a new role of this enzyme in vanadium homeostasis and detoxification (Yoshinaga et al. 2007). However, as the authors indicated, further studies are needed to confirm this hypothesis.

To summarize, the results of this study showed that V (as $NaVO_3$, SMV) and Cr (as $CrCl_3$, CC) administered to rats separately (at the doses ingested, 9 mg V and 0.47 mg Cr/kg b.w./24 h) or in combination (9.8 mg V and 0.39 mg Cr/kg b.w./24 h) in drinking water for 12 weeks did not demonstrate their pro-oxidant potential on RBCs, which was confirmed by the unaltered MDA level and L-ascorbic acid concentration as well as Cu,Zn-SOD, CAT, cGSH-Px, and GR activities in these cells; however, a significantly decreased diet and fluid intake as well as body weight gain and FER were observed in the rats after the treatment with SMV alone or together with CC. The present experiment also indicated that both the elements applied separately or in conjunction elevated the GSH concentration, whose rise could probably be an adaptive response to exposure to the two metals, and decreased GST activity in RBCs. An important finding in the study is that interactions between V and Cr were involved in the changes in GST activity at co-treatment with both elements at the doses mentioned. However, more studies are needed to interpret correctly the V and Cr interactive effect on the activity of that enzyme and to elucidate the mechanism of their

action at the molecular level, which cannot be explained thoroughly at the moment. Finally, we may conclude that in spite of the visible changes in some of the parameters examined, V and Cr at the doses ingested were not able to induce oxidative stress in our experimental conditions.

Acknowledgments We would like to thank Anna Bagniuk (M.Sc.), Voivodship Inspectorate of Environmental Protection (Lublin, Poland) for determining the V and Cr concentrations in selected blood samples of rats.

References

Anderson RA. Effects of chromium on body composition and weight loss. Nutr Rev. 1998;56:266–70.

Anderson RA. Chromium in the prevention and control of diabetes. Diabete Metab. 2000;26:22–7.

Anderson RA, Roussel AM, Zouari N, Mahjoub S, Matheau JM, Kerkeni A. Potential antioxidant effects of zinc and chromium supplementation in people with type 2 diabetes mellitus. J Am Coll Nutr. 2001;20:212–8.

Aureliano M, Crans DC. Decavanadate ($V_{10}O_{28}^{6-}$) and oxovanadates: oxometalates with many biological activities. J Inorg Chem. 2009;99:979–85.

Badmaev V, Prakash S, Majeed M. Vanadium: a review of its potential role in the fight against diabetes. J Altern Complement Med. 1999;5:273–91.

Bagchi D, Hassoun EA, Bagchi M, Stohs SJ. Chromium-induced excretion of urinary lipid metabolites, DNA damage, nitric oxide production, and generation of reactive oxygen species in Sprague–Dawley rats. Comp Biochem Physiol. 1995;110C:177–87.

Bagchi D, Stohs SJ, Downs BW, Bagchi M, Preuss HG. Cytotoxicity and oxidative mechanisms of different forms of chromium. Toxicology. 2002;180:5–22.

Bal W, Kasprzak KS. Induction of oxidative DNA damage by carcinogenic metals. Toxicol Lett. 2002;127:55–62.

Ballistreri FP, Barbuzzi EGM, Tomaselli GA, Toscano RM. Insulin mimesis of vanadium derivatives. Oxidation of cysteine by V(V) oxo diperoxo complexes. J Inog Biochem. 2000;80:173–6.

Baran EJ. Vanadium detoxification. In: Nriagu JO, editor. Vanadium in the environment part II: health effects. New York: Wiley; 1998. p. 317–45.

Barceloux DG. Vanadium. J Toxicol Clin Toxicol. 1999;37:265–78.

Bataineh H, al-Hamood MH, Elbetieha A, Bani Hani I. Effect of long-term ingestion of chromium compounds on aggresion, sex behavior and fertility in adult male rat. Drug Chem Toxicol. 1997;20:133–49.

Bishayee A, Chatterjee M. Time course effects of vanadium supplement on cytosolic reduced glutathione level and glutathione *S*-transferase activity. Biol Trace Elem Res. 1995;48:275–85.

Blankert SA, Coryell VH, Picard BT, Wolf KK, Lomas RE, Stearns DM. Characterization of nonmutagenic Cr(III)-DNA interactions. Chem Res Toxicol. 2003;16:847–54.

Błasiak J, Kowalik J. A comparison of the in vitro genotoxicity of tri- and hexavalent chromium. Mut Res. 2000;469:135–45.

Bliska A, Kryczyk A, Włodek L. The different aspects of the biological role of glutathione. Post Hig Med Dośw. 2007;61:438–53.

Bonnefont-Rousselot D. The role of antioxidant micronutrients in the prevention of diabetic complications. Treat Endocrinol. 2004;3:41–52.

Byczkowski JZ, Kulkarni AP. Oxidative stress and pro-oxidant biological effects of vanadium. In: Nriagu JO, editor. Vanadium in the environment part II: health effects. New York: Wiley; 1998. p. 235–64.

Çakır S, Biçer E. Redox behaviour of cysteine in the presence of ammonium trioxovanadate(V). Bioelectrochem. 2004;64:1–6.

Chakraborty D, Bhattacharyya A, Majumdar K, Chatterjee GC. Effects of chronic vanadium pentoxide administration on L-ascorbic acid metabolism in rats: influence of L-ascorbic acid supplementation. Internat J Vit Nutr Res. 1977;47:81–7.

Chandra AK, Ghosh R, Chatterjee A, Sarkar M. Effects of vanadate on male rat reproductive tract histology, oxidative stress markers and androgenic enzyme activities. J Inorg Biochem. 2007;101:944–56.

Chatterjee M, Bishayee A. Vanadium- a new tool for cancer prevention. In: Nriagu JO, editor. Vanadium in the environment part II: health effects. New York: Wiley; 1998. p. 347–90.

Chemical Information Review Document for Oral Exposure to Tetravalent and Pentavalent Vanadium Compounds. National Toxicology Program, National Institute of Environmental Health Sciences, National Institutes of Health, U.S. Department of Health and Human Services, Research Triangle Park, NC, January 2008

Cheng HH, Lai MH, Hou WC, Huang CL. Antioxidant effects of chromium supplementation with type 2 diabetes mellitus and euglycemic subjects. J Agric Food Chem. 2004;52:1385–9.

Cnubben NHP, Rietjens IMCM, Wortelboer H, van Zanden J, van Bladeren PJ. The interplay of glutathione-related processes in antioxidant defense. Environ Toxicol Pharmacol. 2001;10:141–52.

Codd R, Dillon CT, Levina A, Lay PA. Studies on the genotoxicity of chromium: from the test tube to the cell. Coord Chem Rev. 2001;216–217:537–82.

Crans DC. Chemistry and insulin-like properties of vanadium (IV) and vanadium (V) compounds. J Inorg Biochem. 2000;80:123–31.

Cupo MA, Donaldson WE. Chromium and vanadium effects on glucose metabolism and lipid synthesis in the chick. Poult Sci. 1987;66:120–6.

Curran GL. Effect of certain transition group elements on hepatic synthesis of cholesterol in the rat. J Biol Chem. 1954;210:765–70.

Dąbroś W, Goc A, Turyna B, Kordowiak AM. Sodium metavanadate affected control and streptozotocin-diabetic rat liver Golgi complexes. Pol J Pathol. 2006;57:91–7.

Dai S, Vera E, McNeill JH. Lack of haematological effect of oral vanadium treatment in rats. Pharmacol Toxicol. 1995;76:263–8.

De Tata V, Bergamini E, Bombara M, Lupi R, Novelli M, Masiello P. Effects of low-dose $VOSO_4$ on age-related changes in glucose homeostasis in rats. Eur J Pharmacol. 2000;398:169–75.

Ding M, Gannett PM, Rojanasakul Y, Liu K, Shi X. One-electron reduction of vanadate by ascorbate and related free radical generation at physiological pH. J Inorg Biochem. 1994;55:101–12.

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. 1983;12: 119–26.

Elfant M, Keen CL. Sodium vanadate toxicity in adult and developing rats. Biol Trace Elem Res. 1987;14:193–208.

El-Shahawi MS. Chromium(III) complexes of naturally occurring ligands. Spectrochim Acta. 1995;51:161–70.

Evans P, Halliwell B. Micronutrients: oxidant/antioxidant status. Br J Nutr. 2001;85 Suppl 2:67–74.

Facchini DM, Yuen VG, Battell ML, McNeill JH, Grynpas MD. The effects of vanadium treatment on bone in diabetic and non-diabetic rats. Bone. 2006;38:368–77.

Gerrard DF, Fawcett JP, Farquhar SJ. Vanadium use by athletes. N Z Med J. 1993;106:259.

Gilbert NS, Stump DD, Roth EF. A method to correct for errors caused by generation of interfering compounds during erythrocyte lipid peroxidation. Anal Biochem. 1984;137: 282–6.

Hafez YSM, Kratzer FH. The effect of diet on the toxicity of vanadium. Poult Sci. 1976;55:918–22.

Halliwell B. Vitamin C: antioxidant or prooxidant in vivo? Free Radical Res. 1996;25:439–54.

Hansard SL, Ammerman CB, Henry PR, Simpson CF. Vanadium metabolism in sheep. I. Comparative and acute toxicity of vanadium compounds in sheep. J Anim Sci. 1982;55:344–9.

Hepburn DDD, Burney JM, Woski SA, Vincent JB. The nutritional supplement chromium picolinate generates oxidative DNA damage and peroxidized lipids in vivo. Polyhedron. 2003;22:455–63.

Heyliger CE, Tahiliani AG, McNeill JH. Effect of vanadate on elevated blood glucose and depressed cardiac performance of diabetic rats. Science. 1985;227:1474–7.

Hill CH, Matrone G. Chemical parameters in the study of in vivo and in vitro interactions of transition elements. Fed Proc. 1970;29:1474–81.

Hojo Y, Satomi Y. In vivo nephrotoxicity induced in mice by chromium (VI). Biol Trace Elem Res. 1991;31:21–31.

Hunaiti AA, Sound M. Effect of lead concentration on the level of glutathione, glutathione *S*-transferase, reductase and peroxidase in human blood. Sci Total Environ. 2000;248:45–50.

Hunaiti AA, Sound M, Khalil A. Lead concentration and the level of glutathione, glutathione *S*-transferase, reductase and peroxidase in the blood of some occupational workers from Irbid city. Jordan Sci Total Environ. 1995;170:95–100.

Jensen LS, Maurice DV. Dietary chromium and interior egg quality. Poult Sci. 1980;59:341–6.

Kalaycioglu L, Coskun B, Kurtoglu F, Kurtoglu V. Effect of chromium and magnesium supplementation in broiler diets on body weight and some blood parameters. Ind J Anim Sci. 1999;69:832–7.

Kanna PS, Mahendrakumar CB, Indira BN, Srivastawa S, Kalaiselvi K, Elayaraja T, et al. Chemopreventive effects of vanadium toward 1, 2-dimethylhydrazine-induced genotoxicity and preneoplastic lesions in rat colon. Environ Mol Mutagen. 2004;44:113–8.

Karan BZ, Ay AN, Unaleroglu C, Firat T, Ristau T, Jabs W. Structural and magnetic studies on mono and polynuclear chromium ascorbate complexes. Transit Met Chem. 2005;30: 451–9.

Keller RJ, Sharma RP, Grover TA, Piette LH. Vanadium and lipid peroxidation: evidence for involvement of vanadyl and hydroxyl radical. Arch Biochem Biophys. 1988;265:524–33.

Kirpnick-Sobol Z, Reliene R, Schiestl RH. Carcinogenic Cr(VI) and the nutritional supplement Cr(III) induce DANN deletions in yeast and mice. Cancer Res. 2006;66:3480–4.

Kleszczewska E. Biologiczna rola reakcji kwasu L-askorbinowego z metalami. Post Hig Med Dośw. 2001;55:81–94.

Kordowiak AM, Goc A, Drozdowska E, Turyna B, Dąbroś W. Sodium orthovanadate exerts influence on liver Golgi complexes from control and streptozotocin-diabetic rats. J Inorg Biochem. 2005;99:1083–9.

Kyaw A. A simple colorimetric method for ascorbic acid determination in blood plasma. Clin Chim Acta. 1978;86: 153–7.

Laizé V, Tiago DM, Aureliano M, Cancela ML. New insights into mineralogenic effects of vanadate. Cell Mol Life Sci, 2010. doi: 10.1007/s00018-009-0137-9.

Lamson DW, Plaza SM. The safety and efficacy of high-dose chromium. Altern Med Rev. 2002;7:218–35.

Lefavi RG, Wilson GD, Keith RE, Anderson RA, Blessing DL, James CG, et al. Lipid-lowering effect of a dietary chromium(III)-nicotinic acid complex in male athletes. Nutr Res. 1993;13:239–49.

Leopardi P, Villani P, Cordelli E, Siniscalchi E, Veschetti E, Crebelli R. Assessment of the in vivo genotoxicity of vanadate: analysis of micronuclei and DNA damage induced in mice by oral exposure. Toxicol Lett. 2005;158:39–49.

Levina A, Lay PA. Mechanistic studies of relevance to the biological activities of chromium. Coord Chem Rev. 2005;249:281–98.

Lindsay DG, Astley SB. European research on the functional effects of dietary antioxidants—EUROFEDA. Mol Aspects Med. 2002;23:1–38.

Llobet JM, Colomina MT, Sirvent JJ, Domingo JL, Corbella J. Reproductive toxicity evaluation of vanadium in male mice. Toxicology. 1993;80:199–206.

Lukaski HC. Chromium as a supplement. Annu Rev Nutr. 1999;19:279–302.

Lukaski HC. Vitamin and mineral status: effects on physical performance. Nutrition. 2004;20:632–44.

Lushchak OV, Kubrak OL, Lozinsky OV, Storey JM, Storey KB, Lushchak VI. Chromium (III) induces oxidative stress in goldfish liver and kidney. Aquat Toxicol. 2009;93:45–52.

Malabu UH, Dryden S, Mccarthy HD, Kilpatrick A, Williams G. Effects of chronic vanadate administration in the STZ-induced diabetic rat. Diabetes. 1994;43:9–15.

Matés JM. Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. Toxicology. 2000;153:83–104.

Matsubara T, Musat-Marcu S, Misra HP, Dhalla NS. Protective effect of vanadate on oxyradical-induced changes in

isolated perfused heart. Mol Cell Biochem. 1995;153:79–85.

Meister A. On the antioxidant effects of ascorbic acid and glutathione. Biochem Pharmacol. 1992;44:1905–15.

Meyerovitch J, Shechter Y, Amir S. Vanadate stimulates in vivo glucose uptake in brain and arrests food intake and body weight gain in rats. Physiol Behav. 1989;45:1113–6.

Mohamad A, Taha A, Bamezai RNK, Basir SF, Baquer NZ. Lower doses of vanadate in combination with trigonella restore altered carbohydrate metabolism and antioxidant status in alloxan-diabetic rats. Clin Chim Acta. 2004;342: 105–14.

Mongold JJ, Cros GH, Vian L, Tep A, Ramanadham S, Siou G, et al. Toxicological aspects of vanadyl sulphate on diabetic rats: effects on vanadium levels and pancreatic B-cell morphology. Pharmacol Toxicol. 1990;67:192–8.

Mooney KW, Cromwell GL. Efficacy of chromium picolinate and chromium chloride as potential carcass modifiers in swine. J Anim Sci. 1997;75:2662–71.

Nielsen FH, Hunt CD, Uthus EO. Interactions between essential and ultratrace elements. Ann NY Acad Sci. 1980;355:152–64.

Niki E. Vitamin C as an antioxidant. World Rev Nutr Diet. 1991;64:1–30.

Niki E. Lipid peroxidation: physiological levels and dual biological effects. Free Radic Biol Med. 2009;47:469–84.

O' Brien TJ, Ceryak S, Patierno SR. Complexities of chromium carcinogenesis: role of cellular response, repair and recovery mechanisms. Mut Res. 2003;533:3–36.

Onderci M, Sahin N, Sahin K, Kilic N. Antioxidant properties of chromium and zinc: in vivo effects on digestibility, lipid peroxidation, antioxidant vitamins and some minerals under a low ambient temperature. Biol Trace Elem Res. 2003;92:139–49.

Oster MH, Lobet JM, Domingo JB, Keen CL. Vanadium treatment of diabetic Sprague–Dawley rats results in tissue vanadium accumulation and pro-oxidant effects. Toxicology. 1993;83:115–30.

Ousterhout LE, Berg LR. Effects of diet composition on vanadium toxicity in laying hens. Poult Sci. 1981;60:1152–9.

Owusu-Yaw J, Cohen MD, Fernando SY, Wei CI. An assessment of the genotoxicity of vanadium. Toxicol Lett. 1990;50:327–36.

Ozawa T, Hanaki A. Spin-trapping studies on the reactions of Cr (III) with hydrogen peroxide in the presence of biological reductants: is Cr (III) non-toxic? Biochem Int. 1990;22:343–52.

Papaioannou A, Manos M, Karkabounas S, Liasko R, Evangelou AM, Correia I, et al. Solid state and solution studies of a vanadium(III)-L-cysteine compound and demonstration of its antimetastatic, antioxidant and inhibition of neutral endopepridase activities. J Inorg Biochem. 2004;98:959–68.

Piva A, Meola E, Gatta PP, Biagi G, Castellani G, Mordenti AL, et al. The effect of dietary supplementation with trivalent chromium on production performance of laying hens and the chromium content in the yolk. Anim Feed Sci Technol. 2003;106:149–63.

Poggioli R, Arletti R, Bertolini A, Frigeri C, Benelli AA. Behavioral and developmental outcomes of prenatal and postnatal vanadium exposure in the rats. Pharmacol Res. 2001;43:341–7.

Preuss HG, Grojec PL, Lieberman S, Anderson RA. Effects of different chromium compounds on blood pressure and lipid peroxidation in spontaneously hypertensive rats. Clin Nephrol. 1997;47:325–30.

Preuss HG, Jarrell ST, Scheckenbach R, Lieberman S, Anderson RA. Comparative effects of chromium, vanadium and gymnema sylvestre on sugar-induced blood pressure elevations in SHR. J Am Coll Nutr. 1998;17:116–23.

Reddy CC, Scholz RW, Massaro EJ. Cadmium, methylmercury, mercury and lead inhibition of calf liver glutathione *S*-transferase exhibiting selenium-independent glutathione peroxidase activity. Toxicol Appl Pharmacol. 1981;61:460–8.

Russanov E, Zaporowska H, Invancheva E, Kirkova M, Konstantinova S. Lipid peroxidation and antioxidant enzymes in vanadate-treated rats. Comp Biochem Physiol. 1994;107C:415–21.

Sahin K, Sahin N, Kucuk O. Effects of dietary chromium and ascorbic acid supplementation on digestion of nutrients, serum antioxidant status and mineral concentrations in laying hens reared at a low ambient temperature. Biol Trace Elem Res. 2002;87:113–24.

Sahin K, Sahin N, Kucuk O. Effects of chromium and ascorbic acid supplementation on growth, carcass traits, serum metabolites and antioxidant status of broiler chickens reared at a high ambient temperature (32°C). Nutr Res. 2003a;23:225–38.

Sahin N, Sahin K, Onderci M, Ozcelik M, Smith MO. In vivo antioxidant properties of vitamin E and chromium in cold-stressed Japanese qualis. Arch Anim Nutr. 2003b;57:207–15.

Sakurai H, Inohara T, Adachi Y, Kawabe K, Yasui H, Takada J. A new candidate for insulinomimetic vanadium complex: synergism of oxovanadium(IV)porphyrin and sodium ascorbate. Bioorg Med Chem Lett. 2004;14:1093–6.

Saxena AK, Srivastava P, Kale RK, Baquer NZ. Impaired antioxidant status in diabetic rat liver. Effect of vanadate. Biochem Pharmacol. 1993;45:539–42.

Ścibior A, Wybrane parametry układu antyoksydacyjnego szczurów w interakcji wanad-selen i wanad-cynk. (In English: Some selected parameters of the antioxidative system of rats in vanadium-selenium and vanadium-zinc interactions). Doctoral dissertation, UMCS, Lublin, 1999

Ścibior A. Chrom (III)—znaczenie i wybrane interakcje u ssaków. (In English: Chromium (III)—significance and selected interaction in mammals). In: Gworek B, Misiak J, editors. Obieg pierwiastków w przyrodzie. Monografia Tom II, Warszawa: Instytut Ochrony Środowiska; 2003. p. 490–4.

Ścibior A. Some selected blood parameters in rats exposed to vanadium and chromium via drinking water. Trace Elem Electrolytes. 2005;22:40–6.

Ścibior A, Zaporowska H. Peroksydacja lipidów w wątrobie i nerkach szczurów w interakcji wanad-cynk (In English: Lipid peroxidation in liver and kidney of rats in vanadium-zinc interaction). W: Kabata-Pendias A, Szteke B, red. Cynk w środowisku—problemy ekologiczne i metodyczne. Zesz. Nauk. Komit. "Człowiek i Środowisko". Warszawa: PAN; 2002; 33:511–20.

Ścibior A, Zaporowska H. Biochemical blood parameters of rats intoxicated with vanadium or/and selenium in drinking water. Proceedings of 2nd International Conference "Trace

Elements; Effects on Organisms and Environment". Cieszyn 23-26 June 1998, Poland

Sekar N, Kanthasamy A, William S, Balasubramaniyan N, Govindasamy S. Antioxidant effect of vanadate on experimental diabetic rats. Acta Diabetol Lat. 1990;27:285–93.

Sheriff DS. Effect of vanadium on rat liver and testicular glutathione (GSH) and lipid peroxide levels. Reprod Toxicol. 1991;5:513–5.

Shi X, Dalal NS, Kasprzak KS. Generation of free radicals from hydrogen peroxide and lipid hydroperoxides in the presence of Cr (III). Arch Biochem Biophys. 1993;302:294–9.

Siems WG, Sommerburg O, Grune T. Erythrocyte free radical and energy metabolism. Clin Nephrol. 2000;53:S9–17.

Song JH, Shin SH, Ross GM. Prooxidant effects of ascorbate in rat brain slices. J Neurosci Res. 1999;58:328–36.

Soussi A, Croute F, Soleilhavoup JP, Kammoun A, El Feki A. Impact of green tea on oxidative stress induced by ammonium metavanadate exposure in male rats. CR Biologies. 2006;329:775–84.

Sreedhara A, Susa N, Rao CP. Vanadate and chromate reduction by saccharides and L-ascorbic acid: effect of the isolated V(IV) and Cr(III) products on DNA nicking, lipid peroxidation, cytotoxicity and on enzymatic and non-enzymatic antioxidants. Inorg Chim Acta. 1997;263:189–94.

Stearns DM, Belburno JJ, Wetterhahn KE. A prediction of chromium (III) accumulation in humans from chromium dietary supplements. FASEB J. 1995;9:1650–7.

Stocks J, Dormandy TL. The oxidation of human red cell lipids induced by hydrogen peroxide. Br J Haematol. 1971;20:95–111.

Talbott SM, Hughes K. The health professional's guide to dietary supplements. Baltimore: Lippincott Williams & Wilkins; 2007. p. 1–422.

Tas S, Sarandol E, Ziyanok-Ayvalik S, Ocak N, Serdar Z, Dirican M. Vanadyl sulfate treatment improves oxidative stress and increases serum paraoxonase activity in streptozotocin-induced diabetic rats. Nutr Res. 2006;26:670–6.

Terpiłowska S, Zwolak I, Skrzypek H, Zaporowska H. Red blood cell system of rats treated with chromium in drinking water. Pol J Environ Stud. 2004;13:106–9.

Thompson KH, McNeill JH. Effect of vanadyl sulfate feeding on susceptibility to peroxidative change in diabetic rats. Res Commun Chem Pathol Pharmacol. 1993;80:187–200.

Thompson KH, Orvig C. Vanadium compounds in the treatment of diabetes. Met Ions Biol Syst. 2004;41:221–52.

Thompson KH, Leichter J, McNeill JH. Studies of vanadyl sulfate as a glucose-lowering agent in STZ-diabetic rats. Biochem Biophys Res Commun. 1993;197:1549–55.

Thompson KH, Tsukada Y, Xu Z, Battell M, McNeill JH, Orving C. Influence of chelation and oxidation state on vanadium bioavailability and their effects on tissue concentrations of zinc, copper and iron. Biol Trace Elem Res. 2002;86:31–44.

Townsend DM, Tew KD, Tapiero H. The importance of glutathione in human disease. Biomed Pharmacother. 2003;57:145–55.

Tsantes AE, Bonovas S, Travlou A, Sitaras NM. Redox imbalance, macrocytosis and RBC homeostasis. Antioxid Redox Signal. 2006;8:1205–16.

Urso ML, Clarkson PM. Oxidative stress, exercise and antioxidant supplementation. Toxicology. 2003;189:41–54.

Vincent JB. The potential value and toxicity of chromium picolinate as a nutritional supplement, weight loss agent and muscle development agent. Sports Med. 2003;33:213–30.

Wang W, Ballatori N. Endogenous glutathione conjugates: occurrence and biological functions. Pharmacol Rev. 1998;50:335–55.

Wang J, Yuen VG, McNeill JH. Effect of vanadium on insulin sensitivity and appetite. Metabolism. 2001;50:667–73.

Whittaker P, San RHC, Clarke JJ, Seifried HE, Dunkel VC. Mutagenicity of chromium picolinate and its components in Salmonella typhimurium and L5178Y mouse lymphoma cells. Food Chem Toxicol. 2005;43:1619–25.

Wright WR. Metabolic interrelationship between vanadium and chromium. Ph.D. Thesis, North Carolina State University. Raleigh, N.C., 1968

Yamamoto A. Chromium-protein interactions. In: Berthon G, editor. Handbook of metal–ligand interactions in biological fluids: bioinorganic chemistry vol. 1, part II, chapter 1. New York: Marcel Dekker Ltd; 1995. p. 248–53.

Yoshinaga M, Ueki T, Yamaguchi N, Kamino K, Michibata H. Glutathione transferases with vanadium-binding activity isolated from the vanadium-rich ascidian *Ascidia sydneiensis samea*. BBA. 2006;1760:495–503.

Yoshinaga M, Ueki T, Michibata H. Metal binding ability of glutathione transferases conserved between two animal species, the vanadium-rich ascidian *Ascidia sydneiensis samea* and the schistosome *Schistosoma japonicum*. BBA. 2007;1770:1413–8.

Younes M, Strubelt O. Vanadate-induced toxicity towards isolated perfused rat livers: the role of lipid peroxidation. Toxicology. 1991;66:63–74.

Younes M, Kayser E, Strubelt O. Effect of antioxidants on vanadate-induced toxicity towards isolated perfused rat livers. Toxicology. 1991;70:141–9.

Zaporowska H. Effect of vanadium on L-ascorbic acid concentration in rat tissues. Gen Pharmacol. 1994;25:467–70.

Zaporowska H, Ścibior A. Hematological effects of vanadium on living organisms. In: Nriagu JO, editor. Vanadium in the Environment part II: health effects. New York: Wiley; 1998. p. 135–57.

Zaporowska H, Ścibior A. Activity of neutrophilic granulocytes in rats following intoxication with vanadium and zinc. Folia Histochem Cytobiol. 1999;37:113–4.

Zaporowska H, Ścibior A, Słotwińska M. Some selected blood parameters in the rats intoxicated with vanadium and selenium. Pol J Environ Stud. 1997; 6 Supl.: 206–209.

Zaporowska H, Wasilewski W, Słotwińska M. Effect of chronic vanadium administration in drinking water to rats. Biometals. 1993;6:3–10.

Zaporowska H, Ścibior A, Matysiak J. Lipid peroxidation in liver and kidneys of rats intoxicated with vanadium and/or zinc. Acta Polon Toxicol. 1999;7:189–94.