sciendo

DOI: 10.2478/eces-2023-0043 ECOL CHEM ENG S. 2023;30(4):471-488

Sylwia TERPIŁOWSKA[1*], Katarzyna RAFIŃSKA[2], Adrian GOŁĘBIOWSKI[2]
Tomasz KOWALKOWSKI[2] and Bogusław BUSZEWSKI[2,3]

THE INFLUENCE OF SELECTED TRANSITION ELEMENTS ON CELL METABOLISM

Abstract: The elements are present in the environment. Moreover, they are used in pharmacy and the production of new materials used in medical applications. They are often as environmental pollutants. They can accumulate in organisms and induce toxic effects on the cellular level. HepG2, L_{929} and Caco-2 cell lines were exposed to known concentrations of chromium chloride, iron chloride, nickel chloride, molybdenum trioxide and cobalt chloride (200 or 1000 μ M used alone and in combinations). Concentrations of chromium, iron, nickel, molybdenum and cobalt in the cell lysate and the culture medium were determined by ICP-MS. Moreover, sodium, potassium, calcium and magnesium concentrations were also measured. What is more, cells were observed under light and scanning electron microscope. The dose-dependent increase in the concentration of chromium, iron, nickel, molybdenum and cobalt in all cell lines after incubation with elements was observed. Potassium concentration decreases while sodium calcium and magnesium increase after incubation of cells with of mentioned elements. The incubation of cells with microelements induces cell morphology changes. The presented study shows the crucial role of tested microelements in the induction of cell death as a result of an imbalance of sodium, potassium, calcium and magnesium concentration inside the cell.

Keywords: chromium, iron, nickel, molybdenum, cobalt, cell metabolism, ICP-MS, SEM, biomaterials

Introduction

The elements present in the environment can be divided into macro- and microelements. Macronutrients are present in water, soil, air and organisms at high levels. To this group belongs Na, K, Ca, P, Mg, and S. Water is rich in those elements. The concentration of macroelements in water depends on the concentration of elements in soil and urbanisation processes. The concentration of elements in the soil depends on the sorption properties, acidity, and oxidation-reduction potential of the soil. Microelements are present in environmental sources and living organisms in trace or irregular concentrations. To this group belongs Mn, Mo, Ni, Co, Cu, Fe, Zn, I, and Se. An element belonging to both groups can accumulate in living organisms [1]. They get into the bodies of animals and

[1] Jan Kochanowski University of Kielce, Collegium Medicum, Department of Surgical Medicine with the Laboratory of Medical Genetics, al. IX Wieków Kielc 19A, 25-317 Kielce, Poland, phone +48 41 349 69 78, fax +48 41 349 69 16, e-mail: sylter1@wp.pl, ORCID 0000-0002-2988-9443
[2] Department of Environmental Chemistry and Bioanalytics, Faculty of Chemistry, Nicolaus Copernicus University, ul. J. Gagarina 7, 87-100 Toruń, Poland, ORCID: KR 0000-0002-3358-7317, AG 0000-0001-9321-286X, KT 0000-0001-8866-0246, BB 0000-0002-5482-7500
[3] Professor Jan Czochralski Kuyavian-Pomeranian Scientific Technological Centre, ul. I. Krasińskiego 4, 87-100 Toruń, Poland
[*]Corresponding author: sylter1@wp.pl

people through the respiratory tract, through the skin and to the greatest extent through the digestive tract. They get together with the diet, but also in the form of vitamin and mineral preparations, dietary supplements or energy drinks, whose supply has recently increased significantly.

Elements are used in pharmacy and the production of new materials used in medical applications. Biomaterials are materials that have been designed to interface with biological systems, for the treatment, augmentation, or replacement of biological functions. In many cases, elements used to design new materials may be released to surrounding cells or body fluids. Metallic particles can be accumulated or deposited in tissues or can be transported with blood. Ions released from implants lead to local inflammation or systemic manifestations in various organ systems. Elements enter cells and interact with cell organelles or biomolecules or affect the concentration of elements inside and outside the cell. The biomaterials used in long-term permanent implants are based on cobalt (cobalt-based alloys), cobalt-chromium-molybdenum (Co-Cr, Co-Cr-Mo), and cobalt-chromium-tungsten-nickel (Co-Cr-W-Ni) [2].

A biomaterial placed in the body induces local and systemic interactions. A few seconds after implantation, the biomaterial is covered by proteins and peptides. Platelets, leukocytes, bacteria and fungi then attach to it. Together, they form a biofilm on the surface of the implant. Such interactions are possible due to the charges of the proteins and thus they can interact with the surface of the biomaterial. Moreover, the material can release toxic substances, such as metal particles, when exposed to body fluids. This can disrupt normal healing through the formation of a connective tissue capsule, or deformation of the surgical scar. It can cause local and generalised inflammatory reactions, e.g. allergic reactions, vascular thrombosis and embolisation. This is usually caused by the transfer of decomposed biomaterial via the blood or lymph to distant organs, such as the lungs or brain. It has also been shown that compounds released from the implant can have genotoxic, mutagenic and oncogenic effects. Cytotoxicity of the compounds towards surrounding tissues, activation of immune system cells: neutrophils, macrophages, elements of the complement system, and increased secretion of cytokines or antibodies are observed. This situation has been observed most frequently with metallic biomaterials [3].

Chromium, iron, molybdenum, nickel and cobalt are trace elements, whose balance is essential for proper cell functions. They play a very important role in cell metabolism. Also, it has been proved, that trace elements can be toxic. However, the metabolism of these elements is still unknown. Moreover, investigations about trace elements absorption are conducted on animals. The absorption of elements on the cellular level has not been calculated. That is why this is the first report about the absorption of trace elements used alone and in combinations.

The safety and adequate daily intake for Cr(III) is 50 μg/day - 200 μg/day in adults. Cr(III) absorption ranges from 2 to 0.5 per cent [4]. However, in the available literature, there are no reports about chromium bioaccumulation inside normal and cancer cells. Cr(III) enter the cells via phagocytosis, diffusion or endocytosis [5]. Moreover, trivalent chromium may be transported inside the cell via transferrin receptor [6]. Chromium(III) is involved in the regulation of insulin, glucose, protein, and fat metabolism [7].

Iron is transported into cells by endocytosis or via transferrin receptor 1 (TfR1) and transferrin receptor 2 (TfR2). TfR1 plays an important role in iron transport inside the cell. It has been proved that TfR1 has a 30-fold higher affinity for iron binding than TfR2. Moreover, the number of TfR is higher in cancer cells when compared with normal cells.

It may conclude that cancer cells retain more iron than normal cells to grow and proliferate. Ion can enter the cell also, by divalent cation importers, such as DMT1 and ZIP14 (Zrt-Irt-like protein 14) [8, 9]. Iron is crucial for the synthesis of haemoglobin and myoglobin. What is more, it is involved in transporting oxygen and electrons during cell respiration [10]. Iron is required in DNA biosynthesis as a cofactor of ribonucleotide reductase, ATP generation as a cofactor of many enzymes in citric acid cycles and electron transporter chain. That is why cells must maintain a sufficient concentration of iron [9].

Molybdenum belongs to the group of transition metals. Molybdenum mechanisms of transport in eukaryotic cells are still unknown [11]. It has been identified two types of transporters: MOT1 and MOT2. MOT1 has been identified in plants and green algae. MOT2 has been found in algae and animals including humans [12]. Molybdenum is a component of enzymes which are essential for life [13]. These enzymes are called molybdoenzymes. Sulfite oxidase catalyses the transformation of sulfite to sulfate. This reaction is involved in the metabolism of sulfur-containing amino acids. Xanthine oxidase catalyses the oxidation of xanthine to uric acid. Xanthine oxidase plays a role in the catabolism of purines and the breakdown of nucleotides to form uric acid. Aldehyde oxidase catalyses reactions of hydroxylation of many molecules. Both mentioned enzymes play a role in the metabolism of drugs and toxins. The oxo-transferase activity of these enzymes is connected with the occurrence of oxo-groups in enzymes [14].

Nickel is an essential microelement for plants and animal species (e.g. rats and chick). However, it is not essential in humans. Nickel is widely distributed in the environment. Humans are exposed to nickel through food, water and air. Nickel compounds are transferred into cells in different ways. First, by Divalent Metal Transporter 1 (DMT1). This is a protein expressed in many tissues. It is also involved in iron transport inside the cells. However, DMT1 is also involved in nickel transport [15]. Other research shows that nickel enters the cell by phagocytosis and/or micropinocytosis [16].

Cobalt is a trace element widespread in the environment. Cobalt is a part of vitamin B12 [17]. The mechanisms of transport of cobalt are unknown. It has been proposed, that proteins Nramp1 (also known as *Slc11a1*) and Nramp2 (also known as *Dmt1*, *Dct1*, *Slc11a2*) are responsible for cobalt transport inside the cells. Moreover, Nramp1 can be involved in the transport of Fe(II) or Mn(II). Both proteins are specified divalent-metal transporters dependent on acidic pH, temperature, and time occurring down the proton gradient [18].

As can be seen, many microelements share ion transporters, e.g. chromium and iron, cobalt with iron and nickel with iron. That is why the aim of this study is the investigate the effect of exposure of chromium chloride, iron chloride, nickel chloride, molybdenum trioxide and cobalt chloride, used alone and in combinations with concentrations of these microelements in cells and remained in a cell culture medium. Also, the influence of supplementation mentioned microelements on the concentration of Ca, Mg, Na, K and Zn were studied. The next step of studying the changes in cell morphology, observed under light and electron scanning microscopy was conducted.

Materials and methods

ICP-MS analysis

HepG2 Cell Line, human hepatocyte carcinoma cells and L_{929} Cell Line from mouse, mouse C3H/An connective tissue cells and Caco-2 cell lines were obtained from the

European Collection of Authenticated Cell Cultures (Sigma Aldrich, USA). The experiments were conducted according to Figure 1. The cells were maintained in 75-cm^2 flasks in Dulbecco's minimal essential medium (DMEM) supplemented with 10 % (v/v) fetal bovine serum, 2 mmol/L L-glutamine (Biological Industries, Israel) and Antibiotic Antimycotic Solution (1 mL per 100 mL of cell culture medium) 100 U/mL penicillin G, and 100 µg/mL streptomycin (Sigma Aldrich, USA). The culture medium was replaced with a fresh medium every 2-3 d. After being nearly confluent, the cells were washed with phosphate-buffered saline (PBS, Sigma Aldrich, USA) to remove any unattached cells. The attached cells were harvested using 0.25 % trypsin, EDTA (Biological Industries, Israel). The cells were seeded in 25-cm^2 flasks at approximately $2 \cdot 10^5$ cells/mL density. The cells were used for further studies and were exposed to chromium chloride ($CrCl_3 \cdot 6H_2O$, Acros Organics, Belgium), iron chloride ($FeCl_3 \cdot 6H_2O$, Acros Organics, Belgium), nickel chloride ($NiCl_2 \cdot 6H_2O$, Acros Organics, Belgium), molybdenum trioxide (MoO_3, Acros Organics, Belgium) and cobalt chloride ($CoCl_2$, Sigma Aldrich, USA) at the concentrations of 200 µM or 1000 µM used alone and in combinations (Cr/Fe/Ni/Mo/Co 200 µM or Cr/Fe/Ni/Mo/Co 1000 µM) in the culture medium and incubated for 24 h. Next, the medium was collected. At the same time cells were washed with phosphate-buffered saline (PBS). The cells were harvested using 1 mL of 0.25 % trypsin, EDTA. The cells were suspended in 3 mL of fresh medium and the number of cells was determined with trypan blue (Sigma Aldrich, USA) and next centrifuged at 100 · g for 5 minutes. The cell pellet was seeded in 5 mL of 65 % nitric acid (Suprapure, Merck KGaA, Darmstadt, Germany) and incubated for 40 minutes at 40 °C. After the incubation period samples were filled up to 5 mL of 1 % nitric acid. Concentrations of chromium, iron, nickel, molybdenum and cobalt in the cell lysate and culture medium were determined by ICP-MS (ICPMS 2030, Shimadzu, Japan). Concentrations of the abovementioned microelements were calculated per 10^5 cells. Moreover, sodium, potassium, calcium and magnesium concentrations were also measured and calculated per 10^5 cells.

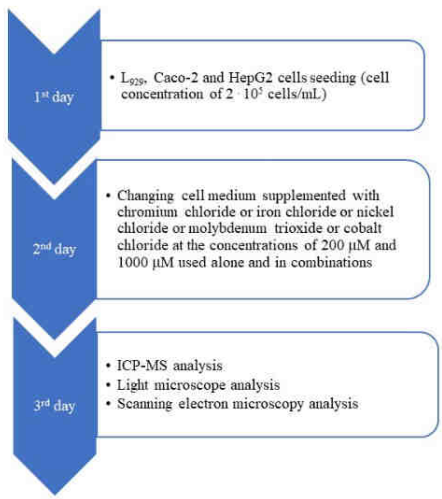


Fig. 1. Roadmap of cell culture experiments

Microscopic imaging analyses

Light microscopy analysis

The cells were seeded at approximately $2 \cdot 10^5$ cells/mL density used for further studies and were exposed to known concentrations of chromium chloride, iron chloride, nickel chloride, molybdenum trioxide and cobalt chloride (200 μM or 1000 μM used alone and in combinations) in the culture medium and incubated for 24 h. Next cells were observed under inverted Observer D1 Zeiss microscopy.

SEM analysis

The cells were seeded at approximately $2 \cdot 10^5$ cells/mL density used for further studies and were exposed to known concentrations of chromium chloride, iron chloride, nickel chloride, molybdenum trioxide and cobalt chloride (200 μM or 1000 μM used alone and in combinations) in the culture medium and incubated for 24 h. Next, the medium was removed. At the same time cells were washed with phosphate-buffered saline (PBS). Adherent cells were fixed in 2.5 % glutaraldehyde in PBS for 30 min and washed 3 times for 5 minutes in PBS. Next, cells were washed 2 times for 2 min with dH_2O, dehydrated in 95 % EtOH for 2 min followed by 4 times for 5 min in 100 % EtOH. In the next step, cells were dehydrated 2 times for 10 min with 100 % HMDS at room temperature. Slides with cells were fixed on a holder with carbon tape and coated with Au within an SC7620 Mini Sputter Coater (Quorum Technologies, Lewes, UK). The cells were examined using a Quanta 3D FEG scanning electron microscope/focused ion beam (SEM/FIB).

Statistical analysis of the data

The results were expressed as mean ± *SD* (for all experiments it was lower than 10 %) and the data were analysed using one-way analysis of variance (ANOVA) with Tukey's multi-comparison post-test using the Statistica programme. In all cases, $p < 0.05$ was considered significant.

The Tukey criterion (*T*) was defined as:

$$T = q_{\alpha\,(c,n-c)} \sqrt{\frac{MSE}{n_i}} \tag{1}$$

where $q_{\alpha\,(c,n-c)}$ is Studentised range distribution, based on *c* and *n-c* df, *c* - number of treatments, *n* - total sample size, *MSE* - mean square error (form ANOVA table), n_i - sample size of the treatment group with the smallest number of observations.

When the differences in mean values between the two groups were higher than the *T* value, the difference was significant.

Results

First, we have determined the number of cells after incubation with chromium chloride, iron chloride, nickel chloride, molybdenum trioxide and cobalt chloride and their combinations in L_{929}, HepG2 and Caco-2 cells. In all cell lines, the number of cells decreases after incubation with microelements used alone in tested concentrations. The number of HepG2 cells was higher when compared with L_{929} cells, except the cells

incubated with nickel chloride in both concentrations, cobalt chloride and molybdenum trioxide at a concentration of 200 μM. Moreover, the highest number of cells was observed in the case of Caco-2 cells. Mentioned above results are presented in Table 1.

Table 1
Number of cells after chromium chloride, iron chloride, nickel chloride, molybdenum trioxide and cobalt chloride treatment in L_{929}, HepG2 and Caco-2 cells

Concentration [μM]	L_{929} [cells/mL]	HepG2 [cells/mL]	CaCo-2 [cells/mL]
Control cells	$4.4 \cdot 10^5$	$5 \cdot 10^5$	$6.4 \cdot 10^5$
Cr200	$2.5 \cdot 10^5$	$3 \cdot 10^5$	$4 \cdot 10^5$
Cr1000	$1.7 \cdot 10^5$	$2 \cdot 10^5$	$3.5 \cdot 10^5$
Fe200	$2.3 \cdot 10^5$	$3 \cdot 10^5$	$4.7 \cdot 10^5$
Fe1000	$1.9 \cdot 10^5$	$2 \cdot 10^5$	$3.5 \cdot 10^5$
Ni200	$2.0 \cdot 10^5$	$1.9 \cdot 10^5$	$3 \cdot 10^5$
Ni1000	$1.6 \cdot 10^5$	$1.0 \cdot 10^5$	$2.5 \cdot 10^5$
Mo200	$1.2 \cdot 10^5$	$1.3 \cdot 10^5$	$2.4 \cdot 10^5$
Mo1000	$0.7 \cdot 10^5$	$0.9 \cdot 10^5$	$2 \cdot 10^5$
Co200	$1.3 \cdot 10^5$	$1.1 \cdot 10^5$	$2.1 \cdot 10^5$
Co1000	$0.3 \cdot 10^5$	$0.5 \cdot 10^5$	$1.1 \cdot 10^5$
Cr200+Fe1000	$2.9 \cdot 10^5$	$3.2 \cdot 10^5$	$4.5 \cdot 10^5$
Cr200+Mo1000	$1.0 \cdot 10^5$	$1.2 \cdot 10^5$	$2.5 \cdot 10^5$
Cr1000+Fe200	$0.8 \cdot 10^5$	$1.0 \cdot 10^5$	$3 \cdot 10^5$
Cr1000+Mo200	$1.9 \cdot 10^5$	$2.4 \cdot 10^5$	$4.1 \cdot 10^5$
Cr200+Ni1000	$1.9 \cdot 10^5$	$2.2 \cdot 10^5$	$1.3 \cdot 10^5$
Cr200+Co1000	$0.9 \cdot 10^5$	$1.4 \cdot 10^5$	$1.4 \cdot 10^5$
Cr1000+Ni200	$1.2 \cdot 10^5$	$1.4 \cdot 10^5$	$0.6 \cdot 10^5$
Cr1000+Co200	$1.4 \cdot 10^5$	$1.6 \cdot 10^5$	$2.5 \cdot 10^5$

The simultaneous incubation of cells with chromium chloride at a concentration of 200 μM plus iron chloride at a concentration of 1000 μM statistically decreases the number of cells when compared with control cells, while increases the number of cells when compared with cells incubated with chromium chloride and iron chloride alone. The simultaneous incubation of cells with chromium chloride at a concentration of 1000 μM plus iron chloride at a concentration of 200 μM statistically decreases the number of cells when compared with control cells and cells incubated with chromium chloride and iron chloride alone. The number of cells incubated with chromium chloride at a concentration of 200 μM plus molybdenum trioxide, nickel chloride or cobalt chloride at a concentration of 1000 μM decreases the number of cells when compared with control cells and cells incubated with chromium chloride at a concentration of 200 μM, while increases when compared with cells incubated with molybdenum trioxide, nickel chloride or cobalt chloride at a concentration of 1000 μM. In cells simultaneously treated with 1000 μM of chromium chloride and 200 μM of nickel chloride, a decrease in the number of cells was observed, when compared to control cells and cells incubated with these microelements alone. The simultaneous incubation of cells with 1000 μM chromium chloride and 200 μM molybdenum trioxide statistically decreases the number of cells compared to control cells, while increases the number of cells compared to chromium chloride and molybdenum trioxide used alone. The number of cells incubated with chromium chloride at a concentration of 1000 μM plus cobalt chloride at a concentration of 200 μM decreases when compared with control cells and cells incubated with chromium

chloride at a concentration of 1000 μM, while increases when compared with cells incubated with cobalt chloride at a concentration of 200 μM. The same observations were made in all three cell lines.

ICP-MS analyses

To investigate the uptake by cells chromium, iron, molybdenum, nickel and cobalt were used alone and in combinations, levels of mentioned above microelements were measured in cells and culture medium.

In all three types of untreated cells, the iron level was detectable. The highest concentration was observed in fibroblasts. Chromium, molybdenum and cobalt were detectable only in Caco-2 cells. Whereas nickel was undetectable in all used cell lines (Table 2).

In cells treated with chromium chloride, iron chloride, molybdenum trioxide, nickel chloride and cobalt chloride used alone, in all cell lines, the dose-related increase of microelements concentration inside the cells was observed (Table 2). The simultaneous incubation of cells with chromium chloride at a concentration of 200 μM plus iron chloride at a concentration of 1000 μM statistically increases chromium concentration, while decreases iron concentration. The same results were observed in all used cell lines (Table 2). The simultaneous incubation of cells with chromium chloride at a concentration of 1000 μM plus iron chloride at a concentration of 200 μM statistically increases chromium and iron concentration in all three types of cell lines (Table 2). The simultaneous incubation of L_{929} and HepG2 cells with chromium chloride at a concentration of 200 μM plus molybdenum trioxide at a concentration of 1000 μM statistically increases chromium concentration, while decreases molybdenum concentration (Table 2). However, in the case of Caco-2 cells, simultaneous incubation of the abovementioned elements statistically increase chromium and molybdenum concentration was observed (Table 2). In a cell treated with chromium chloride at a concentration of 1000 μM and molybdenum trioxide at a concentration of 200 μM the increase in molybdenum concentration, while a decrease in chromium concentration was observed in all three cell lines (Table 2). All used cells treated with chromium chloride at a concentration of 200 μM and nickel chloride at a concentration of 1000 μM show an increase in chromium concentration while a decrease in nickel concentration (Table 2). In L_{929} and Caco-2 cells incubated with chromium chloride at a concentration of 1000 μM and nickel chloride at a concentration of 200 μM, an increase in chromium and nickel concentration was observed. However, a decreased concentration of both elements was observed in HepG2 cells incubated with the same mix of microelements (Table 2). In L_{929} and HepG2 cells incubated with chromium chloride at a concentration of 200 μM and cobalt chloride at a concentration of 1000 μM a decrease in chromium and cobalt concentration was observed. However, in the Caco-2 cell incubated with the same mixture of microelements an increase in chromium and cobalt concentration was observed. The same results were observed in the case of a mixture of chromium chloride at a concentration of 1000 μM and cobalt chloride at a concentration of 200 μM. The decrease in chromium and cobalt concentration was observed in all cell lines (Table 2).

The levels of microelements in cells that remained in the cell culture medium are presented in Table 3.

Table 2
Cellular accumulation of microelements in L_{929}, HepG2 and Caco-2 cells supplemented with chromium chloride, iron chloride, nickel chloride, molybdenum trioxide and cobalt chloride used alone and in combinations

Concentration [μM]	Cellular microelements content [nM/10^5 cells]														
	L_{929}					HepG2					Caco-2				
	Cr	Fe	Ni	Mo	Co	Cr	Fe	Ni	Mo	Co	Cr	Fe	Ni	Mo	Co
Control cells	0	0.835	0	0	0	0	0.324	0	0	0	0.009	0.1	0	0.005	0.008
Cr200	0.405*	-	-	-	-	0.343*	-	-	-	-	0.338*	-	-	-	-
Cr1000	3.797*	-	-	-	-	2.650*	-	-	-	-	1.799*	-	-	-	-
Fe200	-	1.242*	-	-	-	-	2.258*	-	-	-	-	0.434*	-	-	-
Fe1000	-	8.840*	-	-	-	-	13.951*	-	-	-	-	2.018*	-	-	-
Ni200	-	-	0.425*	-	-	-	-	1.913*	-	-	-	-	0.189*	-	-
Ni1000	-	-	2.188*	-	-	-	-	4.135*	-	-	-	-	1.483*	-	-
Mo200	-	-	-	0.257*	-	-	-	-	0.177*	-	-	-	-	0.426*	-
Mo1000	-	-	-	1.179*	-	-	-	-	3.454*	-	-	-	-	2.398*	-
Co200	-	-	-	-	3.634*	-	-	-	-	6.362*	-	-	-	-	0.719*
Co1000	-	-	-	-	72.952*	-	-	-	-	28.284*	-	-	-	-	6.491*
Cr200 +Fe1000	0.807*1	5.603*2	-	-	-	0.353*1	1.918*2	-	-	-	0.411*1	2.002*2	-	-	-
Cr200 +Mo1000	0.612*1	-	-	1.066*2	-	0.610*1		-	1.872*2	-	0.475*,1		-	4.500*2	-
Cr1000 +Fe200	7.632*1	4.199*2	-	-	-	4.399*1	2.467*2	-		-	1.848*1	2.355*2	-		-
Cr1000 +Mo200	3.384*1	-	-	0.262*2	-	1.462*1	-	-	0.212*2		1.234*1	-	-	0.443*2	
Cr200 +Ni1000	0.443*1	-	0.232*2	-	-	0.415*1	-	2.415*2	-	-	1.080*1	-	1.464*2	-	-
Cr200 +Co1000	0.212*1	-	-	-	10.195 *2	0.227*1	-	-	-	20.723 *2	1.152*1	-	-	-	19.836 *2
Cr1000 +Ni200	5.996*1	-	1.736*2	-	-	2.601*1	-	1.293*2	-	-	10.276 *1	-	0.770*2	-	-
Cr1000 +Co200	9.001*1	-	-	-	4.431*2	3.125*1	-	-	-	7.660*2	5.199*1	-	-	-	0.822*2

*$p < 0.05$, the significance of difference compared with control in all concentrations in both cell lines; [1]$p < 0.05$, the significance of difference compared with chromium chloride/iron chloride/nickel chloride/molybdenum trioxide/cobalt chloride at a concentration of 200 μM; [2]$p < 0.05$, the significance of difference compared with chromium chloride/iron chloride/nickel chloride/molybdenum trioxide/cobalt chloride at a concentration of 1000 μM

The concentration of sodium, potassium, magnesium and calcium was also investigated. Potassium concentration decreased in all used cell lines when compared with control cells incubated with all microelements used alone (Table 4). In all cell lines incubated with chromium chloride and iron chloride in both combinations (Cr200+Fe1000 or Cr1000+Fe200), potassium concentrations increase when compared to cells incubated mentioned above microelements used alone. Potassium concentrations in all cell lines incubated with chromium chloride and molybdenum trioxide (Cr200+Mo1000 or Cr1000+Mo200) increase when compared with cells incubated with chromium chloride alone (at concentrations 200 μM or 1000 mM) while decrease when compared with cells incubated with molybdenum trioxide alone (at concentrations 200 μM or 1000 μM). Potassium concentrations in all cell lines incubated with chromium chloride and nickel chloride simultaneously decrease when compared with cells incubated with nickel chloride alone (200 μM and 1000 μM) and chromium chloride at a concentration of 1000 mM, while

increase when compared with cells incubated with chromium chloride at a concentration of 200 μM used alone. Potassium concentration in cells incubated with chromium chloride and cobalt chloride increases when compared with cells incubated with chromium (at concentrations 200 μM or 1000 μM) used alone and with cells incubated with cobalt at a concentration of 200 μM, while decreased when cells were incubated with cobalt at a concentration of 1000 μM. The same observations were made in all used cell lines.

Table 3

Residual microelements content in cell culture L_{929}, HepG2 and Caco-2 medium supplemented with chromium chloride, iron chloride, nickel chloride, molybdenum trioxide and cobalt chloride used alone and in combinations

Concentration [μM]	**Residual microelements content in cell culture medium [μM]**														
	L_{929}					**HepG2**					**Caco-2**				
	Cr	**Fe**	**Ni**	**Mo**	**Co**	**Cr**	**Fe**	**Ni**	**Mo**	**Co**	**Cr**	**Fe**	**Ni**	**Mo**	**Co**
Control cells	0	0,765	0	0	0	0	0	0	0	0	0.032	0.098	0.023	0.012	0.011
Cr200	0,578*	-	-	-	-	1.029*	-	-	-	-	5.197*	-	-	-	-
Cr1000	5,026*	-	-	-	-	5.179*	-	-	-	-	25,721*	-	-	-	-
Fe200	-	0.854*	-	-	-	-	1.065*	-	-	-	-	3.629*	-	-	-
Fe1000	-	4.958*	-	-	-	-	5.002*	-	-	-	-	4.938*	-	-	-
Ni200	-	-	1.013*	-	-	-	-	0.806*	-	-	-	-	43.300*	-	-
Ni1000	-	-	4.569*	-	-	-	-	4.536*	-	-	-	-	249.5*	-	-
Mo200	-	-	-	0.844*	-	-	-	-	1.002*	-	-	-	-	6.295*	-
Mo1000	-	-	-	4.782*	-	-	-	-	4.991*	-	-	-	-	32.474*	-
Co200	-	-	-	-	0.904*	-	-	-	-	0.904*	-	-	-	-	7.411*
Co1000	-	-	-	-	4.506*	-	-	-	-	4.929*	-	-	-	-	35.00*
Cr200 +Fe1000	0.855*1	3.734*2		-	-	0.954*1	4.692*2	-	-	-	1.447*1	11.827 *2	-	-	-
Cr200 +Mo1000	0.948*1	-	-	4.016 *1,2	-	0.823*1	-	-	4.493*2	-	4.894*1	-	-	17.596 *2	-
Cr1000 +Fe200	4.314*1	0.884*2	-	-	-	4.538*1	1.016*2	-	-	-	15.00*1	2.103*2	-	-	-
Cr1000 +Mo200	4.471*1	-	-	0.791 *1,2	-	4.577*1	-	-	1.030*2	-	26.971 *1	-	-	4.105*2	-
Cr200 +Ni1000	1.005*1	-	0.428*2	-		0.898*1	-	3.944*2	-	-	5.260*1	-	17.708 *2	-	-
Cr200 +Co1000	0.872*1	-	-	-	3.605*2	0.800*1	-	-	-	4.903*2	3.237*1	-	-	-	19.294 *2
Cr1000 +Ni200	4.750*1	-	0.833*2	-	-	4.154*1	-	0.924*2	-	-	26.971 *1	-	3.529*2	-	-
Cr1000 +Co200	4.285*1	-	-	-	0.809*2	4.048*1	-	-	-	4.013*2	20.513 *1	-	-	-	4.398*2

*$p < 0.05$, the significance of difference compared with control in all concentrations in both cell lines; [1]$p < 0.05$, the significance of difference compared with chromium chloride/iron chloride/nickel chloride/molybdenum trioxide/cobalt chloride at a concentration of 200 μM; [2]$p < 0.05$, the significance of difference compared with chromium chloride/iron chloride/nickel chloride/molybdenum trioxide/cobalt chloride at a concentration of 1000 μM

Sodium concentration in all used cell lines incubated with chromium chloride, iron chloride, molybdenum trioxide, nickel chloride and cobalt chloride used alone increases when compared with control cells. However, in cells incubated with the above-mentioned microelements in all used combinations, the decrease in sodium concentrations was

observed when compared with the control cells and cells incubated with microelements used alone (Table 4).

Magnesium and calcium concentrations increase in cells incubated with chromium chloride, iron chloride, molybdenum trioxide, nickel chloride and cobalt chloride used alone when compared with control cells. Moreover, magnesium and calcium concentrations increased in cells incubated with the above-mentioned microelements used in all combinations when compared with control cells and cells incubated with microelements used alone (Table 4).

Table 4

Cellular accumulation of potassium, sodium, magnesium and calcium in L_{929}, HepG2 and CaCo-2 cells supplemented with chromium chloride, iron chloride, nickel chloride, molybdenum trioxide and cobalt chloride used alone and in combinations

Concentration [μM]	Cellular K content [μM/10^5 cells]			Cellular Na content [μM/10^5 cells]			Cellular Ca content [μM/10^5 cells]			Cellular Mg content [μM/10^5 cells]		
	L_{929}	HepG2	Caco-2	L_{929}	HepG2	Caco-2	L_{929}	HepG2	Caco-2	L_{929}	HepG2	Caco-2
Control cells	0.561	0.720	0.546	0.096	0.014	0.196	0.001	0.001	0.001	0.013	0.001	0.013
Cr200	0.020*	0.031*	0.188*	0.106*	0.017*	0.204*	0.003*	0.002*	0.189*	0.015*	0.011*	2.050*
Cr1000	0.034*	0.044*	0.226*	0.109*	0.021*	0.239*	0.003*	0.003*	0.194*	0.017*	0.017*	2.364*
Fe200	0.033*	0.049*	0.163*	0.105*	0.009*	0.200*	0.002*	0.002*	0.139*	0.018*	0.008*	1.678*
Fe1000	0.046*	0.048*	0.224*	0.110*	0.013*	0.239*	0.004*	0.004*	0.195*	0.014*	0.016*	2.350*
Ni200	0.031*	0.054*	0.214*	0.105*	0.014*	0.227*	0.004*	0.005*	0,167*	0.016*	0.024*	2.258*
Ni1000	0.035*	0.382*	0.352*	0.106*	0.030*	0.263*	0.005*	0.009*	0.276*	0.022*	0.046*	2.870*
Mo200	0.081*	0.199*	0.346*	0.111*	0.016*	0.357*	0.006*	0.007*	0.283*	0.026*	0.033*	3.698*
Mo1000	0.111*	0.315*	0.351*	0.114*	0.018*	0.370*	0.014*	0.005*	0.278*	0.057*	0.024*	3.800*
Co200	0.046*	0.286*	0.342*	0.108*	0.020*	0.410*	0.007*	0.008*	0.373*	0.030*	0.037*	3.988*
Co1000	0.213*	0.438*	0.485*	0.021*	0.031*	0.533*	0.030*	0.016*	0.567*	0.103*	0.068*	6.409*
Cr200 +Fe1000	0.625*1,2	0.080*1,2	0.245*1,2	0.004*1,2	0.006*1,2	0.102*1,2	0.005*1,2	0.007*1,2	0.199*1,2	0.018*1,2	0.018*1,2	2.352*1,2
Cr200 +Mo1000	0.057*1,2	0.076*1,2	0.267*1,2	0.006*1,2	0.011*1,2	0.156*1,2	0.027*1,2	0.008*1,2	0.310*1,2	0.071*1,2	0.044*1,2	2.890*1,2
Cr1000 +Fe200	0.602*1,2	0.884*1,2	0.361*1,2	0.014*1,2	0.007*1,2	0.168*1,2	0.011*1,2	0.008*1,2	0.226*1,2	0.043*1,2	0.034*1,2	2.615*1,2
Cr1000 +Mo200	0.059*1,2	0.121*1,2	0.254*1,2	0.008*1,2	0.009*1,2	0.138*1,2	0.009*1,2	0.009*1,2	0.291*1,2	0.028*1,2	0.042*1,2	2.941*1,2
Cr200 +Ni1000	0.031*1,2	0.195*1,2	0.281*1,2	0.004*1,2	0.011*1,2	0.100*1,2	0.009*1,2	0.016*1,2	0.300*1,2	0.027*1,2	0.057*1,2	5.462*1,2
Cr200 +Co1000	0.158*1,2	0.239*1,2	0.232*1,2	0.013*1,2	0.012*1,2	0.157*1,2	0.040*1,2	0.024*1,2	0.676*1,2	0.140*1,2	0.090*1,2	4.100*1,2
Cr1000 +Ni200	0.028v	0.021*1,2	0.131*1,2	0.007*1,2	0.013*1,2	0.116*1,2	0.009*1,2	0.028*1,2	0.293*1,2	0.037*1,2	0.028*1,2	3.001*1,2
Cr1000 +Co200	0.253*1,2	0.289*1,2	0.460*1,2	0.018*1,2	0.012*1,2	0.058*1,2	0.044*1,2	0.017*1,2	0.406*1,2	0.048*1,2	0.040*1,2	4.003*1,2

*$p < 0.05$, the significance of difference compared with control in all concentrations in both cell lines; [1]$p < 0.05$, the significance of difference compared with chromium chloride/iron chloride/nickel chloride/molybdenum trioxide/cobalt chloride at a concentration of 200 μM; [2]$p < 0.05$, the significance of difference compared with chromium chloride/iron chloride/nickel chloride/molybdenum trioxide/cobalt chloride at a concentration of 1000 μM

Microscopic analyses

All studied types of cells were observed under an inverted light microscope and scanning electron microscopy (SEM). Control cells showed normal morphology.

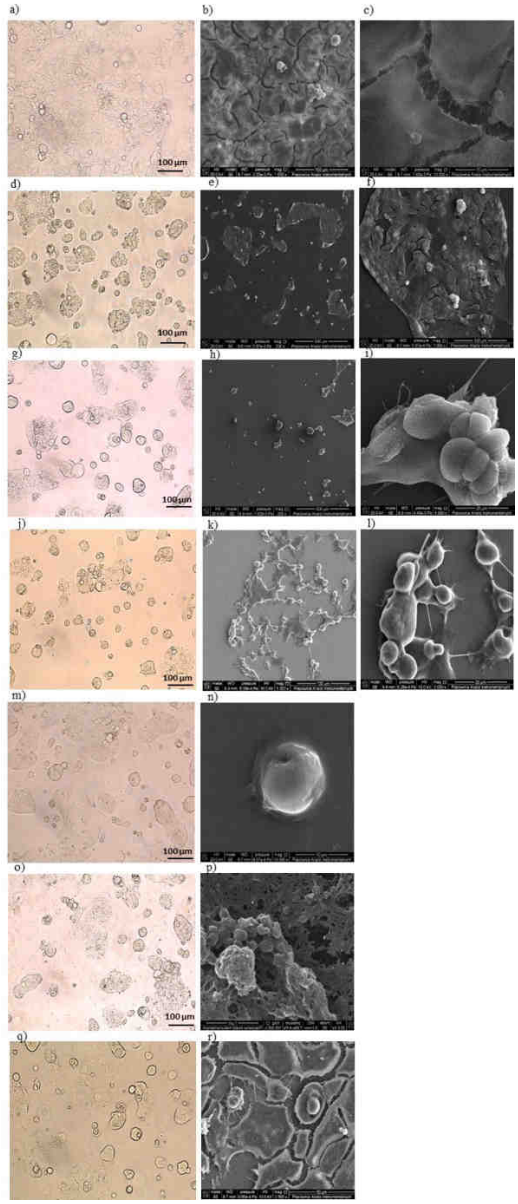


Fig. 2. Microscopic analyses of Caco-2 cells morphology: a) control cells (light microscope), b) and c) control cells (SEM); d), e) and f) cells incubated with nickel chloride at a concentration of 1000 µM; g), h) and i) cells incubated with cobalt chloride at a concentration of 200 µM; j), k) and l) cells incubated with chromium chloride at a concentration of 1000 µM and nickel chloride at a concentration of 200 µM; m) and n) cells incubated with iron chloride at a concentration of 1000 µM; o) and p) cells incubated with chromium chloride at a concentration of 200 µM and iron chloride at a concentration of 1000 µM; q) and r) cells incubated with chromium chloride at a concentration of 1000 µM and molybdenum trioxide at a concentration of 200 µM

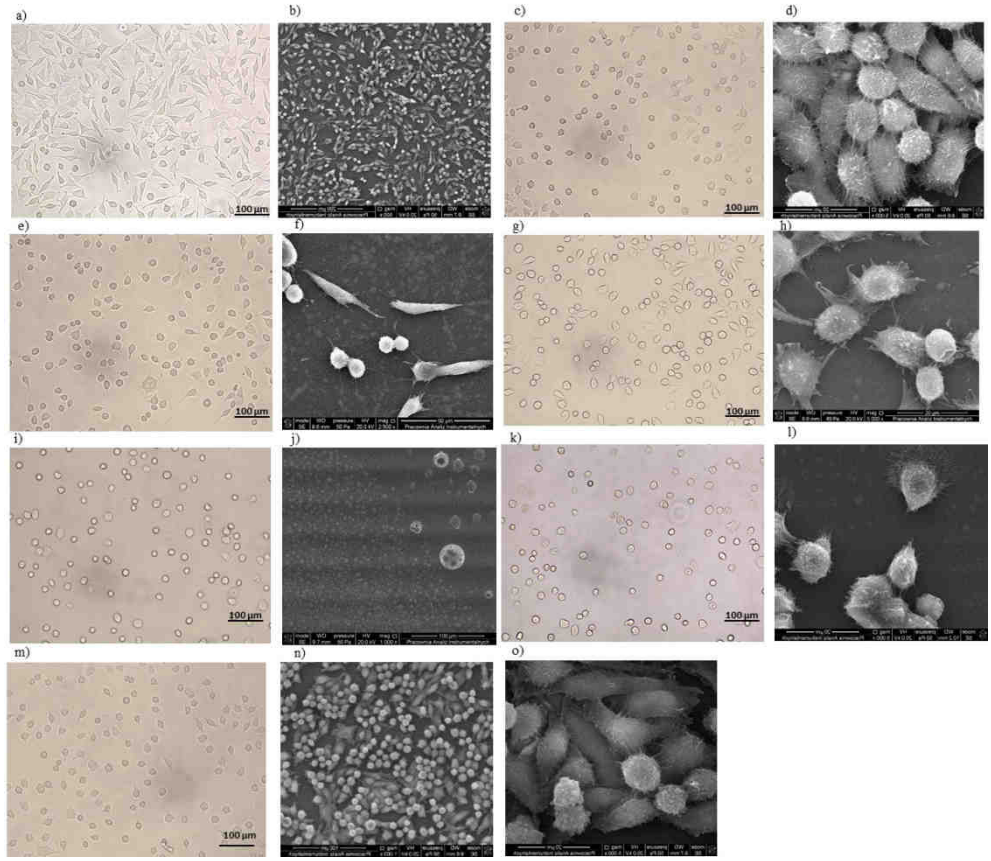


Fig. 3. Microscopic analyses of L_{929} cells morphology: a) control cells (light microscope), b) control cells (SEM); c) and d) cells incubated with iron chloride at a concentration of 200 µM; e) and f) cells incubated with nickel chloride at a concentration of 200 mM; g) and h) cells incubated with molybdenum trioxide at a concentration of 200 µM; i) and j) cells incubated with cobalt chloride at a concentration of 200 µM; k) and l) cells incubated with chromium chloride at a concentration of 200 µM and cobalt chloride at a concentration of 1000 µM; m), n) and o) cells incubated with chromium chloride at a concentration of 200 µM

The SEM images show that the absorptive surface of Caco-2 cells is covered with densely packed microvilli, indicating the formation of a well-differentiated brush border structure (Fig. 2a, b and c). The L_{929} cells were well-spread and flattened, and they overlapped each other, covering the coverslip (Fig. 3a and b). The HepG2 cells were also well-spread, covering the coverslip (Fig. 4a, b and c).

Incubation of cells with microelements used alone and in combinations induces cell morphology changes. The dose-response changes in cell morphology were observed. In Caco-2 cells incubated with microelements, the following changes were observed: decreased number of cells (Fig. 2g, h, d, e and f), cells rounded up with loosened cell junctions (Fig. 2j, k, l, q and r), cells with gap junctions shrank and showed decreased surface microvilli, cells showed a round apoptotic-like morphology (Fig. 2i, m, n, o and p).

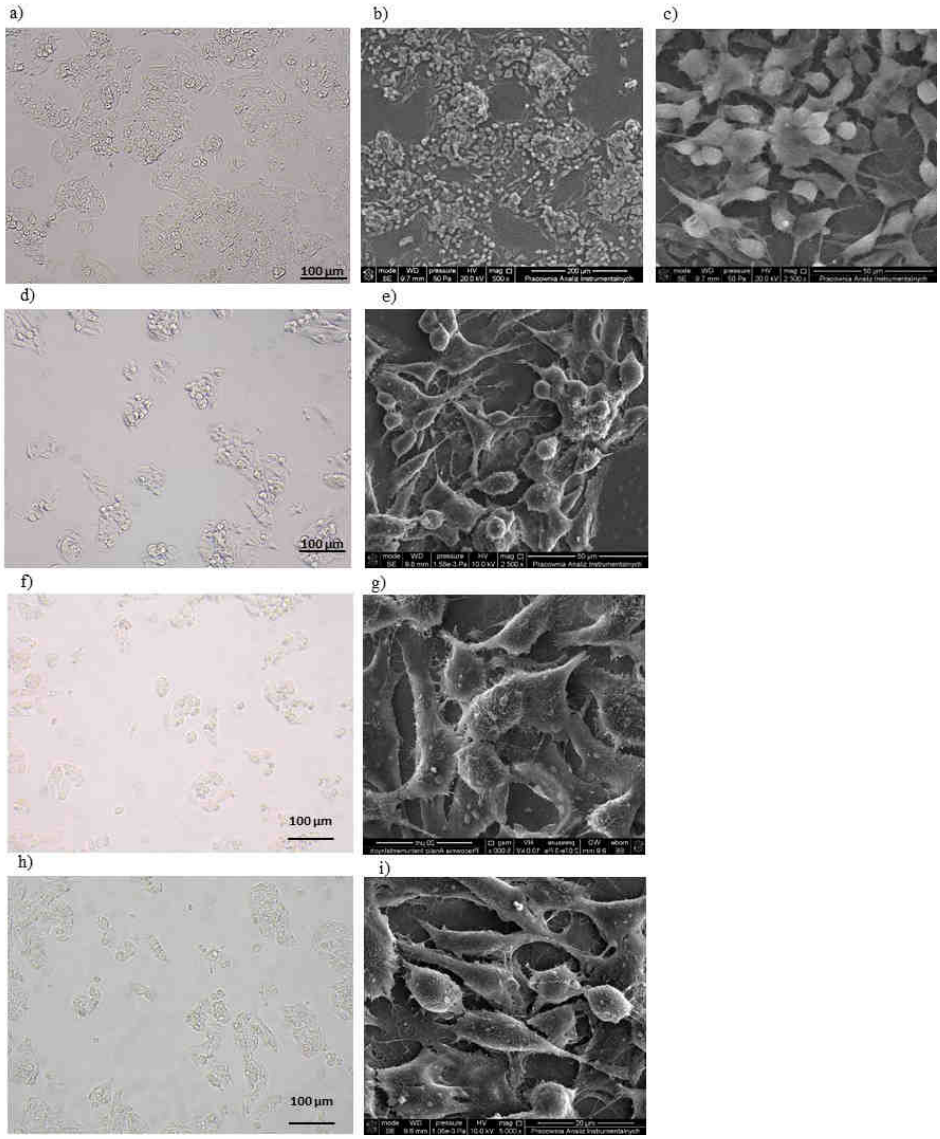


Fig. 4. Microscopic analyses of HepG2 cells morphology: a) control cells (light microscope), b) and c) control cells (SEM); d) and e) cells incubated with cobalt chloride at a concentration of 200 µM; f) and g) cells incubated with cobalt chloride at a concentration of 1000 µM; h) and i) cells incubated with chromium chloride at a concentration of 200 µM and cobalt chloride at a concentration of 1000 µM

L_{929} cells incubated with microelements used alone and in combinations show cells with changed morphology, e.g. with apoptotic blebs (Fig. 3c, d, m, n and o) cells with changes in the shape of the cell (Fig. 3e and f), a rounded morphology of the cells, with

disruption of cell membranes (Fig. 3g, h, i and j), detached cells (Fig. 2k and l), also, many globular clusters were visible on the cells surface (Fig. 2h).

HepG2 cells incubated with microelements used alone and in combinations show cells with pores formed in the cell membrane and the cell membranes became unintegral as a result of the falling of some globular clusters (Fig. 4d and e). Which were characteristic only in HepG2 cells. Moreover, a decrease in cell number and cells with irregular morphological structures were observed (Fig. 4f, g, h and i).

Discussion

Biometals are present in the environment and used in many medical applications. They are used as supplements to the diet and in orthopaedic implants due to their mechanical properties. However, inside the body biomaterials composed of elements can be corrosive. The released elements from biomaterials can induce local changes in cell metabolism or can be transported by the blood to other regions. That is why we decided to investigate the concentration of chromium, iron, molybdenum, nickel, cobalt, sodium, potassium, calcium and magnesium in various cell lines.

In the first step, we investigated the concentration of chromium, iron, nickel, molybdenum and cobalt in cells treated with the above-mentioned microelements. A statistically significant dose-related increase in the concentration of chromium, iron, nickel, molybdenum and cobalt was observed. The same results were observed in all used cell lines. The obtained results confirm the transport of microelements inside the cells. The mechanism of these transports is still unknown. However, the absorption of chromium ranges from 0.0016 per mille to 0.003 per mille in all cell types. The lowest absorption was observed in the case of CaCo-2 cells. This is the first report indicating chromium absorption by cells. Most studies were performed with the use of animals and humans. The absorption of iron ranges from 0.002 per mille to 0.01 per mille in all cell types. The highest iron absorption was observed in HepG2 cells when compared with L_{929} and CaCo-2 cells. The absorption of nickel ranges from 0.0009 per mille to 0.005 per mille cells in all cell types. The higher absorption was observed in HepG2 cells. The absorption of molybdenum ranges from 0.0008 to 0.003. The absorption of cobalt ranges from 0.003 to 0.072. The highest absorption of cobalt was observed in L_{929} and HepG2 cells. The differences in absorption between cell lines depend on the presence of ion transporters on the cell membranes and the different metabolisms of used cell types. Moreover, elements form complexes in cell culture media, especially in the presence of serum. In the presence of fetal bovine serum, elements interact with albumin, transferrin, and immunoglobulins (Ig). Newly formed complexes cannot be transported into the cells. This can be the reason for the low absorption of elements [19].

As mentioned above, we observed a dose-dependent increase in concentrations of chromium, iron, nickel, molybdenum and cobalt after those elements' treatment when compared with control cells. Those observations confirm our earlier investigations concerning the biological effects of the above-mentioned microelements. The increase in concentrations of microelements correlates with the increase of reactive oxygen species concentrations (ROS) and a decrease in antioxidant activity such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) [20]. These observations and observations conducted by other authors show oxidative stress as a crucial factor for changes in cell metabolism after element treatment. Other authors observed apoptosis in

cells treated with microelements. Also in previous studies, apoptosis has been observed. Cells treated with chromium, iron, nickel and molybdenum show characteristics for apoptosis. Giant, multinuclear cells with vacuolisation and membrane blebbing were observed [21]. Moreover, cytotoxicity, loss of Mitochondrial Transmembrane Potential (MTP) and genotoxicity were observed [21, 22]. All these changes are induced by the direct interaction of microelements with biomolecules, such as DNA, lipids, and proteins. It has been proved, that Cr(III) compounds can bind directly to DNA in vitro, forming Cr-DNA adducts and DNA-DNA crosslinks [23]. Moreover, the metal-induced ROS production induces changes in cell metabolism leading to cell death [20].

Apoptosis can be induced also as a result of an imbalance of the concentration of other elements inside the cells. In all cell lines the increase in sodium concentration, while the decrease in potassium concentration was observed after treatment with elements. In normal conditions, the resting potential of the cell membrane is established by the concentration gradient of sodium and potassium ions across the cell membrane. This gradient is maintained by the activity of leak channels and the Na^+/K^+ pump, a protein located in the cell membrane. The pump transports three sodium ions outside and two potassium ions inside the cell, with the use of ATP [24]. The research conducted by Yurinskaya et al. show that the imbalance of activity of Na^+/K^+ pump induces membrane shrinkage, which leads to apoptosis [25]. In our study, the imbalance of sodium and potassium concentrations in cells treated with elements used alone and in combinations was observed. It leads to the destruction of the cell membrane. The destruction of cell membrane we also observed in our earlier study in LDH release assay [20]. What is more, the imbalance of concentration of other elements leads to the induction of cell death. One of these microelements is calcium. Inside the cells, Ca^{2+} ions are present in the cytoplasm, mitochondria, lysosomes and nucleus However, the highest concentration of calcium ions can be observed in the endoplasmic reticulum. Ca^{2+} ions can be transported inside the cells by many channels located in the plasma membrane, e.g. transient receptor potential channels (TRPC), store-operated Ca^{2+} entry (SOCE) channels, and voltage-gated Ca^{2+} channels (VGCC) [26].

Receptor-operated Ca^{2+} channels and purinergic P2 receptors are responsible for cytosolic calcium ions influx. Whereas an increase in Ca^{2+} concentration may be also due to calcium release from cell organelles, mainly from the endoplasmic reticulum. The inositol 1,4,5-triphosphate receptors (IP3Rs) situated in the ER are responsible for this transport. As mentioned above, mitochondria play a crucial role in calcium ions' homeostasis. They may act as Ca^{2+} buffers. Calcium ions are transported inside mitochondria by mitochondrial calcium uniporter (MCU). Moreover, calcium may be accumulated in mitochondria in the form of amorphous calcium phosphate. However, it has been proved that Ca^{2+} and ROS create a self-perpetuating cascade that can culminate in mitochondrial Ca^{2+} overload and perturbed cell functions. Further, in the conditions of oxidative stress, Na^+/Ca^{2+} exchangers, function in a reverse mode promoting calcium overload in cytoplasm [27].

The interaction of mitochondria and the endoplasmic reticulum plays a crucial mechanism in the regulation of calcium ion concentration. Disruption of receptor function leads to an overload of calcium ions in the cytoplasm. The results of calcium ion concentration after chromium, iron, molybdenum, cobalt and nickel presented in this study confirm our earlier observations regarding the induction of apoptosis. Calcium ions are also involved in the cell cycle regulation. Calcium is involved in G1/S, G2/M and metaphase-to-anaphase transitions. Moreover, calcium is required in microtubule polymerisation and depolymerisation. The decrease of Ca^{2+} concentration is required for

microtubule depolymerisation. That is why the calcium concentration increase disturbs the spindle apparatus's formation during the cell cycle. The disorders of spindle apparatus formation can be identified in micronuclei formation assay. The previous study shows that chromium, iron, nickel and molybdenum induce micronuclei formation [21]. What is more, changes in the polarisation of the cell membrane lead to an influx of calcium ions and cytosolic overload of this element. It can induce a disruption in proliferation, induction of cell death and changes in shape and migration [28]. In the presented studies we observed in SEM observations changes in the shape of all cell lines as a result of disruption of cytoskeleton formation.

Another crucial element for cell metabolism is magnesium. It is the fourth most common element in the human body. On the cellular level, magnesium plays a crucial role as a cofactor of more than 300 enzymatic reactions involved in nucleic acid synthesis and energy metabolism. Magnesium ions are also involved in hormone receptor binding, gating of calcium channels, transmembrane ion flux, and interaction of cytoskeleton elements [29]. It has been proved, that overload of cells with magnesium ions induces apoptosis, mainly by increasing chromatin condensation, decreasing the transcription factor NFkB expression and disturbing cytoskeleton protein function [29]. High concentrations of magnesium induce G2/M cell cycle arrest consequently leading to inhibition of cell proliferation. What is more magnesium ions decreased the expression of Bcl-2 and increased the expression of Bax protein and enhanced the release of cytochrome from mitochondria. Magnesium ions up-regulate caspase3/9 and induce expression of ER-resident chaperones, such as CHOP, calnexin and GRP78 (glucose-regulated protein), which are markers of apoptosis promoted by the endoplasmic reticulum [30]. In our study, the increase of magnesium concentration in all three cell lines after chromium, iron, nickel, molybdenum and cobalt treatment, was used alone and simultaneously. Moreover, our earlier investigations show the induction of apoptosis after chromium, iron, nickel and molybdenum are used alone and in combination treatment. Apoptosis has been induced by oxidative stress and a decrease in antioxidant activity. In the light of results presented in this paper, it can be concluded, that apoptosis may be induced also by the imbalance of concentration of magnesium and depends on mitochondrial and RE disruption. Induction of apoptosis was confirmed in microscopic observations. In all cell lines cells with apoptotic morphology were observed, i.e. rounded cells with membrane blebbing and cytoplasm shrinkage. Also, cells with loosened cell junctions were observed.

Conclusion

Elements are used in pharmacy and the production of new materials used in medical applications. However, it has been shown that elements can induce reactive oxygen species production and decrease antioxidant activity. These mechanisms lead to the activation of cell death. The presented study confirms our earlier investigations concerning the induction of apoptosis in cells treated with chromium, iron, nickel, molybdenum and cobalt. The mechanism leading to apoptosis depends on mitochondrial and RE disruption. What is more, the presented study shows the crucial role of these elements in the induction of cell death as a result of an imbalance of sodium, potassium, calcium and magnesium concentration inside the cell.

Acknowledgements

Katarzyna Rafińska is a member of the Toruń Center of Excellence "Towards Personalised Medicine" operating under Excellence Initiative-Research.

We acknowledge Jolanta Wółkiewicz, MSc for professional technical assistance in scanning electron microscopy analysis.

References

[1] Isvoran A, Roman D, Dascalu D, Vlad-Oros B, Ciorsac A, Pitulice L, et al. Human health effects of heavy metal pollution in the cross-border area of Romania and Serbia: A review. Ecol Chem Eng. 2021;28(3):365-88. DOI: 10.2478/eces-2021-0025.

[2] Ude CC, Esdaille CJ, Ogueri KS, Kan H-O, Laurencin SJ, Nair LS et al. The mechanism of metallosis after total hip arthroplasty. Regen Eng Transl Med. 2021;7:247-61. DOI: 10.1007/s40883-021-00222-1.

[3] Terpiłowska S, Siwicki AK. Modern biomaterials, their application and effects on the body. In: Skopińska-Różewska E, Siwicki AK, Zdanowski R, editors. Medical Biology - Selected Issues. Olsztyn: Edycja; 2014.

[4] Lamson DW, Plaza SM. The safety and efficacy of high-dose chromium. Alternative Med Rev. 2002;7(3):218-35. Available from: https://www.researchgate.net/publication/11253854_The_safety_and_efficacy_of_high-dose_chromium.

[5] DesMarias TL, Costa M. Mechanisms of chromium-induced toxicity. Current Opinion Toxicol. 2019;14:1-7. DOI: 10.1016/j.cotox.2019.05.003.

[6] Costa M, Murphy A. Overview of chromium(III) toxicology. Chapter 11. In: Vincent JB, editor. The Nutritional Biochemistry of Chromium(III). Amsterdam: Elsevier BV; 2019. DOI: 10.1016/B978-0-444-64121-2.00011-8.

[7] Zha L-Y, Xu Z-R, Wang M-Q, Gu L-Y. Chromium nanoparticle exhibits higher absorption efficiency than chromium picolinate and chromium chloride in Caco-2 cell monolayers. J Anim Physiol Anim Nutr. 2008;92:131-40. DOI: 10.1111/j.1439-0396.2007.00718.x.

[8] Bystrom LM, Guzman ML, Rivella S. Iron and reactive oxygen species: Friends or foes of cancer cells? Antioxid Redox Signaling. 2014;20(12):1917-24. DOI: 10.1089/ars.2012.501.

[9] Bogdan AR, Miyazawa M, Hashimoto K, Tsuji Y. Regulators of iron homeostasis: New players in metabolism cell death and diseases. Trends Biochem Sci. 2016;41(3):274-86. DOI: 10.1016/j.tibs.2015.11.012.

[10] Staniek H, Wójciak RW, Prokop K, Tubacka M, Krejpcio Z. Combined effect of diversified Fe(III) content in the diet and Cr(III) supplementation on the magnesium status in rats. J Elem. 2018;23(2):569-80. DOI: 10.5601/jelem.2017.22.2.1466.

[11] Mendel RR, Bittner F. Cell biology of molybdenum. Biochim Biophys Acta. 2006;1763:621-35. DOI: 10.1016/j.bbamcr.2006.03.013.

[12] Peng T, Xu Y, Zhang Y. Comparative genomics of molybdenum utilization in prokaryotes and eukaryotes. BMC Genomics. 2018;19:691. DOI: 10.1186/s12864-018-5068-0.

[13] Burzlaff A, Beevers C, Pearce H, Lloyd M, Klipsch K. New studies on the in vitro genotoxicity of sodium molybdate and their impact on the overall assessment of the genotoxicity of molybdenum substances. Regul Toxicol Pharmacol. 2017;86:279-91. DOI: 10.1016/j.yrtph.2017.03.018.

[14] Hussein MA, Guan TS, Haque RA, Khadeer Ahamed MB, Abdul Majid AMS. Synthesis and characterization of thiosemicarbazone to molybdenum(VI)complexes: In vitro DNA binding, cleavage, and antitumor activities. Polyhedron. 2015;85:93-103. DOI: 10.1016/j.poly.2014.02.048.

[15] Mims MP, Prchal JT. Divalent metal transporter 1. Hematology. 2005;10(4):339-45. DOI: 10.1080/10245330500093419.

[16] Di Bucchianico S, Gliga AR, Åkerlund E. Skoglund S, Wallinder IO, Fadeel B, et al. Calcium-dependent cyto- and genotoxicity of nickel metal and nickel oxide nanoparticles in human lung cells. Part Fibre Toxicol. 2018;15:32 DOI: 10.1186/s12989-018-0268-y.

[17] Camara-Martos F, Moreno-Rojas R. Cobalt: Toxicology. In: Caballero B, Finglas PM, Toldrá F, editors. Encyclopedia of Food and Health. Cambridge, MA: Academic Press; 2016. DOI: 10.1016/B978-0-12-384947-2.00176-8.

[18] Forbes JR, Gros P. Iron, manganese, and cobalt transport by Nramp1 (Slc11a1) and Nramp2 (Slc11a2) expressed at the plasma membrane. Blood. 2003;102(5):1884-92. DOI: 10.1182/blood-2003-02-0425.

[19] Tkaczyk C, Huk OL, Mwale F, Antoniou J, Zukor DJ, Petit A et al. Investigation of the binding of Cr(III) complexes to bovine and human serum proteins: A proteomic approach. J Biomed Mater Res. 2010;94A:214-22. DOI: 10.1002/jbm.a.32700.
[20] Terpilowska S, Siwicki AK. Pro- and antioxidant activity of chromium(III), iron(III), molybdenum(III) or nickel(II) and their mixtures. Chem Biol Interact. 2019;298:43-51. DOI: 10.1016/j.cbi.2018.10.028.
[21] Terpilowska S, Siwicki AK. Interactions between chromium(III) and iron(III), molybdenum(III) or nickel(II): cytotoxicity, genotoxicity and mutagenicity studies. Chemosphere. 2018;201:780-9. DOI: 10.1016/j.chemosphere.2018.03.062.
[22] Terpilowska S, Siwicki AK. Cell cycle and transmembrane mitochondrial potential analysis after chromium(III), iron(III), molybdenum(III) or nickel(II) and their mixture treatment. Tox Res. 2019;8(2):188-95. DOI: 10.1039/c8tx00233a.
[23] O'Brien TJ, Ceryak S, Patierno SR. Complexities of chromium carcinogenesis: role of cellular response, repair and recovery mechanisms. Mutat Res. 2003;533:3-36. DOI: 10.1016/j.mrfmmm.2003.09.006.
[24] Xu N. On the concept of resting potential-pumping ratio of the Na^+/K^+ pump and concentration ratios of potassium ions outside and inside the cell to sodium ions inside and outside the cell. J Membr Biol. 2013;246(1):75-90. DOI: 10.1007/s00232-012-9507-6.
[25] Yurinskaya VE, Vereninov IA, Vereninov AA. The balance of Na^+, K^+, and Cl^- unidirectional fluxes in normal and apoptotic U937 cells computed with all main types of cotransporters. Front Cell Dev Biol. 2020;8:591872. DOI: 10.3389/fcell.2020.591872.
[26] Patergnani S, Danese A, Bouhamida E, Aguiari G, Previati M, Pinton P, et al. Various aspects of calcium signaling in the regulation of apoptosis, autophagy, cell proliferation, and cancer. Int J Mol Sci. 2020;21(21):8323. DOI: 10.3390/ijms21218323.
[27] Duvvuri B, Lood C. Mitochondrial calcification. Immunometabolism. 2021;3(1):e210008. DOI: 10.20900/immunometab20210008.
[28] Janssen LJ, Mukherjee S, Ask K. Calcium homeostasis and ionic mechanisms in pulmonary fibroblasts. Am J Respir Cell Mol Biol. 2015;53(2):135-48. DOI: 10.1165/rcmb.2014-0269TR.
[29] Santos JM, Hussain F. Magnesium chloride increases apoptosis and decreases prostate cancer cells migration. Funct Foods Health Dis. 2018;8(1):62-78. DOI: 10.31989/ffhd.v8i1.368.
[30] Bian M, Chen X, Zhang C, Jin H, Wang F, Shao J, et al. Magnesium isoglycyrrhizinate promotes the activated hepatic stellate cells apoptosis via endoplasmic reticulum stress and ameliorates fibrogenesis in vitro and in vivo. BioFactors. 2017;43:836-46. DOI: 10.1002/biof.1390.