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In Vitro and *In Vivo* Effects of Magnesium on the Lysosomal System

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Original article

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Magnesium is a macroelement with a wide range of biological effects, necessary for the proper functioning of cells. This macroelement shows antioxidant activity, therefore it is a rather effective agent in the prevention and treatment of numerous diseases, including cancer. However, the mechanisms of the therapeutic properties of this element, especially its potential antitumor activities, have not yet been fully explained. One of the more important elements of the current anticancer strategy may be the modulating effect of compounds on the lysosomal system. Therefore, the aim of our research was to evaluate the range of action of magnesium sulphate ($MgSO_4$) on the activity of the lysosomal compartment of *in vitro* and *in vivo* cells. The CHO-K1 normal cell line was used in the *in vitro* studies. The cells were incubated in a medium with the addition of $MgSO_4$ at the following concentrations: 25 μM, 125 μM and 250 μM for 24 and 48 h. The *in vivo* studies were performed on 2-month-old albino male Wistar rats treated with $MgSO_4$ at a concentration of 0.06 mg Mg/ml for 12 and 18 weeks. It was shown that the exposure of CHO-K1 cells and rat liver cells to $MgSO_4$ induced significant ultrastructural, morphological and biochemical changes. The reported *in vivo* and *in vitro* changes, such as an increased number of autophagic vacuoles, primary lysosomes, autophagolysosomes, growth of cytoplasmic vacuolization and an increase in the activity of lysosomal hydrolases, indicate that $MgSO_4$ activates the lysosomal system. The range of the reported changes in relation to the lysosomal system, both *in vitro* and *in vivo*, was dependent on the concentration and duration of action of $MgSO_4$.

Key words: Magnesium sulphate, CHO-K1 cells, hepatocytes, vacuolization, lysosomal system.

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Magnesium (Mg^{2+}) is one of the most important macroelements, responsible for the proper course of physiological reactions of the organism (ROMANI 2011; GRÖBER *et al.* 2015; SCHWALFENBERG & GENUIS 2017). In the human body this element occurs in the form of an intracellular divalent cation (WOLF & TRAPANI 2008; PARIKH & WEBB 2012). The wide spectrum of biological activity of magnesium determines its important role in the prevention and treatment of many diseases. The role of magnesium seems important in the regulation of neurological disorders (LONG & ROMANI 2015), insulin metabolism (HANS *et al.* 2002; BARBAGALLO & DOMINGUEZ 2015), ossification process (BELLUCI *et al.* 2011; CASTIGLIONI *et al.* 2013), cardiovascular disorders as well as blood pressure (ZHANG *et al.* 2012; DAI *et al.* 2013). The body's demand for magnesium depends on the sex, age, physiological activity, and the recommended daily intake of this element is determined in the range of 420 mg Mg^{2+} for men and 320 mg Mg^{2+} for women (DE BAAIJ *et al.* 2015). According to literature data, standard rat food should contain about 0.5-1g magnesium/kg diet (LAURANT *et al.* 2000; MARTIN *et al.* 2008; BERTINATO *et al.* 2014).

Nutritionists increasingly are paying attention to the problem of inadequate supply of magnesium in the diet. According to the literature, the cause of primary magnesium deficiencies may be the loss of these valuable ions during the technological processing of food products and the adverse effects of environmental factors (DE BAAIJ *et al.* 2015). A low level of magnesium in the body may be closely associated with the development of chronic and inflammatory disorders (GRÖBER *et al.* 2015). The influence of magnesium in the formation and therapy of cancer seems to be especially important. Literature data indicate that magnesium may play a significant role in cancer processes, influencing, for example, pathways of angiogenesis and cancer metastasis (CASTIGLIONI & MAIER 2011; WOLF & TRAPANI 2012). ANASTASSOPOULOU and THEOPHANIDES (2002) point to the complex and multi-faceted relationship between intracellular magnesium concentrations and the process of cancer formation.

At this point, the biological mechanisms underlying the therapeutic properties of magnesium have not been fully understood. Therefore, this element and its compounds are still the subject of extensive experimental and clinical research conducted on a variety of cellular systems. It seems particularly important to understand the scope of impact of magnesium on cellular processes directly related to the stages of carcinogenesis. It is currently assumed that one of the most important elements of the anticancer strategy may be the lysosomal system and the intracellular degradation process associated with it (AREDIA *et al.* 2012; HELGASON *et al.* 2013). The available literature still lacks detailed data on the effect of magnesium and its compounds on the lysosomal system. Therefore, the aim of our *in vitro* and *in vivo* studies was to determine the range of influence of an exemplary magnesium compound on the morphological, ultrastructural and biochemical changes of CHO-K1 cells lines and rat hepatocytes, with particular consideration of the lysosomal compartment.

The magnesium sulphate selected for this study is a model inorganic magnesium compound commonly used in both *in vivo* (ESHRAGHI *et al.* 2015; ASGHARI *et al.* 2017; LI *et al.* 2017) and *in vitro* experimental studies (FARRUGGIA *et al.* 2014; XIA *et al.* 2016), as well as in clinical trials (TAHERI *et al.* 2015). The concentrations of $MgSO_4$ analyzed in the study were selected based on verification of published data related to *in vivo* (ŚCIBIOR *et al.,* 2006, 2009, 2012, 2013; ŚCIBIOR and ZAPOROWSKA 2010) and *in vitro* studies (KOMORI *et al.* 1999).

Materials and Methods

Studied compound

Magnesium sulphate ($MgSO_4$) was used for our research. Its purity was 99.9%, according to the manufacturer (Sigma-Aldrich, St. Louis, MO, USA).

In vitro studies

In vitro studies were performed on CHO-K1 cells obtained from the Department of Radiobiology and Immunology of the Jan Kochanowski University in Kielce. The cells were grown in plastic culture dishes (Nunc, Poland), in DMEM medium (Dulbecco's Modified Eagle Medium, PAA Laboratories GmbH, UK) containing L-glutamine and 4.5 g/l (25 mM) glucose. In addition, the medium was enriched with 10% inactivated fetal bovine serum (FBS, PAA Laboratories GmbH, UK) and a mixture of antibiotics: penicillin (10.000 U/ml), streptomycin (10 mg/ml) and amphotericin (25 μg/ml) (Antibiotic-Antimycotic, Gibco). The cell culture was kept at 37°C in a humidified atmosphere with 5% CO_2 in a DirectHeat incubator (Thermo Scientific, Waltham, MA, USA). Before each experiment, the cells were passaged using 0.2% trypsin (Sigma-Aldrich, St. Louis, MO, USA) and were grown for the next 24 hours. Cell density was evaluated in a "Countess" apparatus (Automated Cell Counter, Invitrogen, UK) using 0.2% trypan blue solution (Sigma-Aldrich, St. Louis, MO, USA).

The $MgSO_4$ was dissolved in sterile, deionized water, just before performing the experiment, therefore preparing a stock solution with a 50 mM starting concentration. Then, the solution was added to the culture medium to obtain the desired final concentrations. In the cell cultures of experimental groups, the medium was changed to a medium with the addition of $MgSO_4$ to obtain appropriate concentrations (25, 125, 250 μM). The control culture (not exposed to $MgSO_4$) was grown simultaneously with the cell culture of the experimental groups. The control and experimental cells (with the addition of $MgSO_4$) were incubated for 24 and 48 h.

In vivo studies

In vivo studies were performed on 2-month-old albino male Wistar rats with average initial body weight of about 187 g. The outbred animals came from the Animal Breeding Center, Brwinów. The animals were kept under constant veterinary supervision. Throughout the experimental period,

the animals were individually kept in stainless cages, in a ventilated room with a naturally regulated light to dark ratio (LD 12:12), with a controlled temperature (20-21^{o}C) and relative humidity (55±5%). The rats were fed a standard, granulated rodent feed, providing a properly balanced diet (Labofeed B; Fodder and Concentrate Factory, Kcynia, Poland). The animals were randomly divided into four experimental groups. Groups I and II (control animals) (K/12 and K/18) received deionized water for 12 and 18 weeks, respectively (Aries, Resin Tech., Inc., USA). The animals from experimental groups III ($MgSO_4$/12) and IV ($MgSO_4$/18) received, as a drinking fluid, magnesium sulphate ($MgSO_4$) at a concentration of 0.06 mg Mg/ml dissolved in deionized water. The deionized water was administered according to published data (ŚCIBIOR *et al.* 2009, 2010, 2012; ESHRAGHI *et al.* 2015; ZHANG *et al.* 2015). The Mg content in water was checked by atomic absorption spectrometry (AAS) (ŚCIBIOR *et al.* 2012). Fluid intake as well as the general conditions of the rats in the experimental groups were monitored daily. After 12 and 18 weeks of the experiment, the animals were killed. Liver segments from the caudate lobe were taken for biochemical, ultrastructural and histological studies.

The experiment was carried out in three independent repetitions with four individuals each. Since the age of animals and all conditions in the replications were identical, the results were pooled and analyzed together, hence the number of subjects per group was 12. The permission to carry out the experiment was obtained from the 1st Local Ethical Committee for Animal Studies in Lublin (No. 48/2007).

Ultrastructural studies using transmission electron microscopy

In vitro and *in vivo* cells were fixed in 3% glutaraldehyde (Serva, Heidelberg, Germany), contrasted with 2% OsO_4 (SPI Supplies, West Chester, USA), dehydrated in a graded series of ethyl alcohol (Chempur, Poland), overexposed using propylene oxide (Sigma-Aldrich, St. Louis, MO, USA) and then immersed in epoxy resin Epon 812 (Serva, Heidelberg, Germany) according to the modified method of MARZELLA and GLAUMANN (1980a). Ultrathin slices were obtained with the use of diamond knives in a Leica EM UC7 ultramicrotome (Leica Microsystems, Wetzlar, Germany). The sections were contrasted with uranyl acetate and lead citrate (SPI Supplies, West Chester, USA). Analysis of ultrastructural changes was performed using a transmission electron microscope Tecnai G2 Spirit (FEI Company, Oregon, USA). Photographic documentation was made using TIA microscope software (TEM Imaging & Analysis 3,2 SP6).

Morphological studies using optical microscopy

The liver sections *in vivo* studies were fixed in a mixture of 70% ethanol (Chempur, Poland), acetic acid (Chempur, Poland) and formaldehyde (Poch, Poland). Then, the tissues were dehydrated in ethyl alcohol and passed through intermediate fluids using methyl benzoate (POCH, Poland). The liver sections were put into liquid paraffin (Chempur, Poland), and then immersed in paraffin blocks according to the modified methodology described by ZAWISTOWSKI (1970). Paraffin sections of 5 μm thickness were cut on a HM 360 CN rotary microtome (Thermo Fisher Scientific, USA). In turn, the CHO-K1 cells in the *in vitro* studies were cultured on coverslips and were fixed in methyl alcohol (Chempur, Poland) and hydrated. *In vitro* and *in vivo* cells were stained with the H&E technique, using a hematoxylin solution ("Aqua-Med" ZPAM - KOLASA sp.j., Poland) and a 1% eosin solution (POCH, Poland). In the final stage, the preparations were dehydrated and were closed using Histokitt (GmbH & Co KG, Sondheim/Rhön, Germany). The analysis of the morphological image of the tested cells was carried out using a Eclipse 80i optical microscope (Nicon, Poland), and photographs were taken using Nikon Nis Elements software.

Evaluation of cell vacuolization range

In the morphological analysis of *in vitro* cells, the number of cells with visible cytoplasmic vacuolization was determined. A total of 120 000 cells were analyzed (N = 5000 cells/concentration/time of incubation, with control groups).

Analysis of biochemical indicators

Liver fragments and CHO-K1 cell lines for the biochemical analysis were suspended in 0.25 M saccharose. The tissues were homogenized in a Potter S with a Teflon piston homogenizer (Sartorius, Germany). The lysis of CHO-K1 cells was performed using a triple cycle of freezing at -86^{o}C and defrosting at room temperature. The *in vitro* and *in vivo* cell homogenates obtained this way were then centrifuged for 10 min at 700 x g in a Sorvall RC 6 Plus (Thermo Scientific, Waltham, MA, USA) and MPW 351/R (MPW Med. Instruments, Poland) centrifuge according to the method of MARZELLA and GLAUMANN (1980b). In the obtained fractions of *in vitro* and *in vivo* cells, the activity of selected lysosomal hydrolases was

determined using the specific substrates of Sigma-Aldrich (St. Louis, MO, USA). The activity of cathepsin D/L (Cath D, EC 3.4.23.5; Cath L, EC 3.4.22.15) was determined according to the method of LANGNER (1973), β-D-glucuronidase (β-Gr, EC 3.2.1.31) and β-N-acetylhexosaminidase (HeX, EC 3.2.1.30) with the BARRETT method (1972). The activity of the studied enzymes was calculated based on the total protein content, which was determined by LOWRY's method, as modified by KIRSCHKE and WIEDERANDERS (1984). The absorbency of enzymes and proteins was read spectrophotometrically (UV-VIS Evolution 220, Termo Scientific, USA). The activity of enzymes was expressed in μmol/mg protein/h.

Statistical analysis

Normality of distribution was tested by the Shapiro-Wilk's test, whereas homogeneity of variance was analyzed by Levene's test. The statistical evaluation was performed using the Tukey's post-hoc parametric test (ANOVA) and two-way analysis of variance (two-way ANOVA analysis). The two-way ANOVA analysis was used to show the effect of the time and factor and the time and concentrations. The amount of fluids consumed by the experimental animals was analyzed by Tukey's test compared with the control groups. The number of cells with visible cytoplasmic vacuolization was evaluated using the $\chi 2$ non-parametric test. A significance level of $p<0.05$ was considered as statistically significant. The statistical analysis was performed using IBM SPSS Statistics 24.

Results

Magnesium consumption by the experimental animals

The animals which received a water solution of $MgSO_4$ for 12 and 18 weeks consumed similar amounts of fluids per day ($MgSO_4$/12 – 128.48 ml/kg b.w. and $MgSO_4$/18 – 96.46 ml/kg b.w.) with respect to the control groups (K/12 – 126.92 ml/kg b.w. and K/18 – 96.46 ml/kg b.w.). No statistically significant differences were observed.

Changes in the lysosomal compartment *in vitro* and *in vivo* cells induced by $MgSO_4$

In the analysis of microscopic images, both the *in vitro* (Figs 1C-1H) and the *in vivo* (Figs 2C-2D) cells exposed to $MgSO_4$ revealed clear changes in the lysosomal compartment in relation to cells of the control group (Figs 1A-1B, 2A-2B). In the ultrastructure of liver cells of animals encumbered with $MgSO_4$ for 12 weeks, we found an accumulation of a large number of autophagic vacuoles in the cytoplasm with diverse material intended for degradation visible inside (Fig. 2C). The prolongation of the time of action of $MgSO_4$ to 18 weeks caused an increase in the population of primary and secondary lysosomes, numerously occurring in the region of the bile duct (Fig. 2D). The changes also occurred in the mitochondria and endoplasmic reticulum (Fig. 2D). The changes revealed *in vivo* in the lysosomal compartment of the tested animals were also confirmed by *in vitro* studies. Already after 24 hours of $MgSO_4$ activity, an increased number of autophagic vacuoles (Figs 1C, 1E, 1G) and secondary lysosomes (Fig. 1G) were observed. In addition, in the ultrastructural image of the CHO-K1 cells after 24 hours of incubation with $MgSO_4$ at a concentration of 125 μM, numerous primary lysosomes and changes in the Golgi apparatus were observed (Fig. 1E). These structures were characterized by a pronounced growth with profuse secretory vesicles dispersed in the cytosol (Fig. 1E).

Numerous autophagic vacuoles with diverse structure, size and content were also observed after 48 hours of exposure to $MgSO_4$ at concentrations of 25, 125 and 250 μM (Figs 1D, 1F, 1H).

Evaluation of vacuolization of *in vitro* and *in vivo* cells after exposure to $MgSO_4$

The microscopic evaluation of CHO-K1 cells exposed to the action of $MgSO_4$ at the concentration of 25 μM for 24 and 48 hours, revealed, relative to the control (Figs 3A-3B), an increase in the number of vacuoles in the cytoplasm (Figs 3C-3D). These changes were confirmed by the quantitative analysis, which showed an increase in the number of cells with vacuolization, respectively to 20.4% ($\chi 2 = 1035.04$, $p = 0.0000$) and 21.3% ($\chi 2 = 904.74$, $p = 0.0000$) (Fig. 5). Meanwhile, as a result of increasing the concentrations of $MgSO_4$, an intensification of the vacuolization range was found (Figs 3E-3H). We demonstrated a statistically significant increase in cell vacuolization to 23.6% ($\chi 2 = 1240.38$, $p = 0.0000$) after 24 h of action of $MgSO_4$ at a concentration of 125 μM, and a 31.8% increase ($\chi 2 = 1786.63$, $p = 0.0000$) at a concentration of 250 μM (Fig. 5). Also, increasing the time of incubation of CHO-K1 cells with $MgSO_4$ to 48 hours at concentrations of 125 μM and 250 μM caused a statistically significant increase of the vacuolization range, respectively to 24.6% ($\chi 2 = 1109.86$, $p = 0.0000$) and 30.6% ($\chi 2 = 1501,57$, $p = 0.0000$)

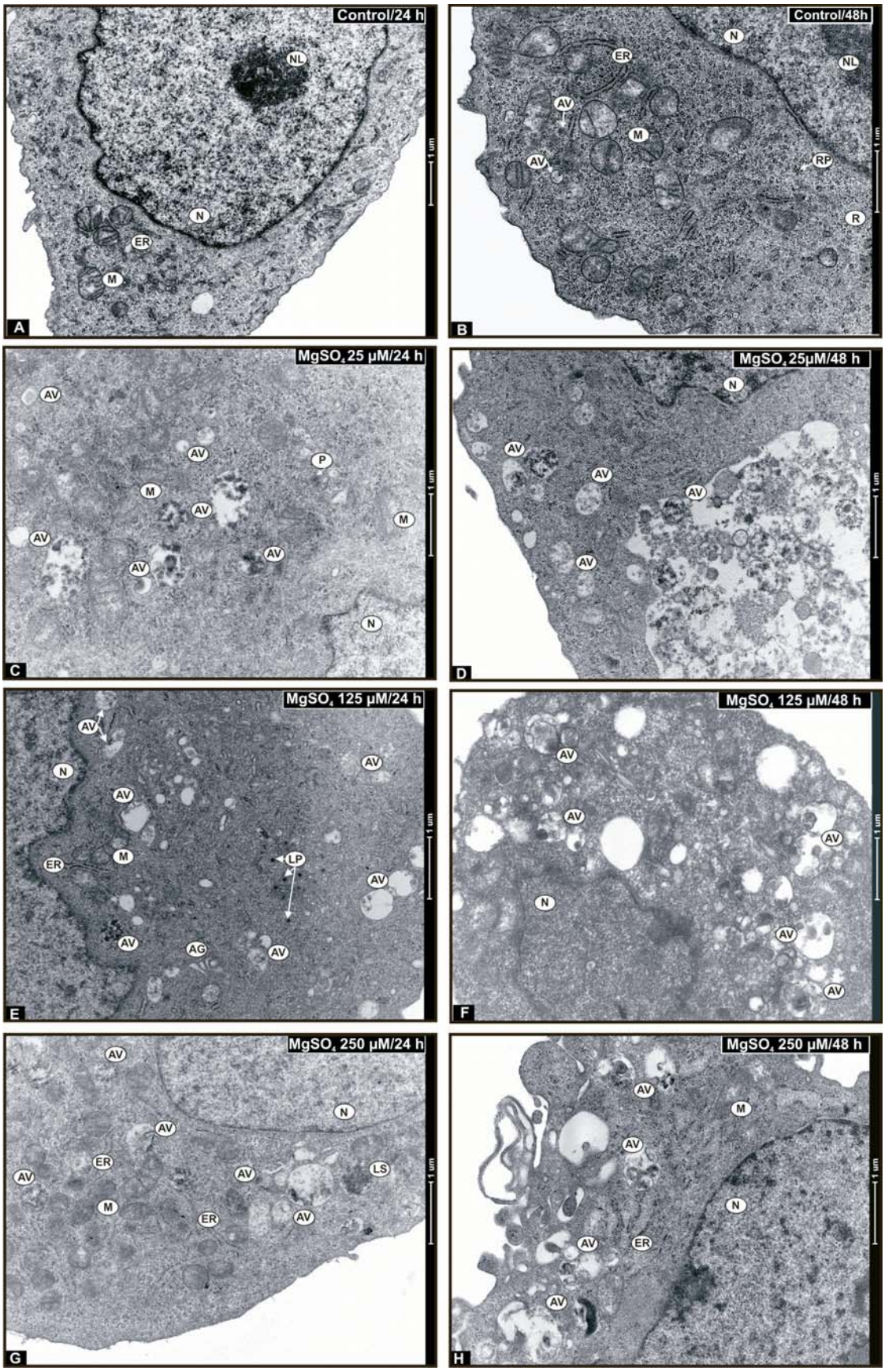

Fig. 1. Ultrastructure of CHO-K1 cells of the control groups (A,B) and cells exposed to the action of $MgSO_4$ at concentrations of 25 µM (C,D), 125 µM (E,F) and 250 µM (G,H) for 24 and 48 hours. The cytoplasm contains: nucleus (N), mitochondria (M), peroxisomes (P), endoplasmic reticulum (ER), numerous autophagic vacuoles (AV), primary (LP) and secondary lysosomes (LS). TEM – Magnification (A-H) × 16 500.

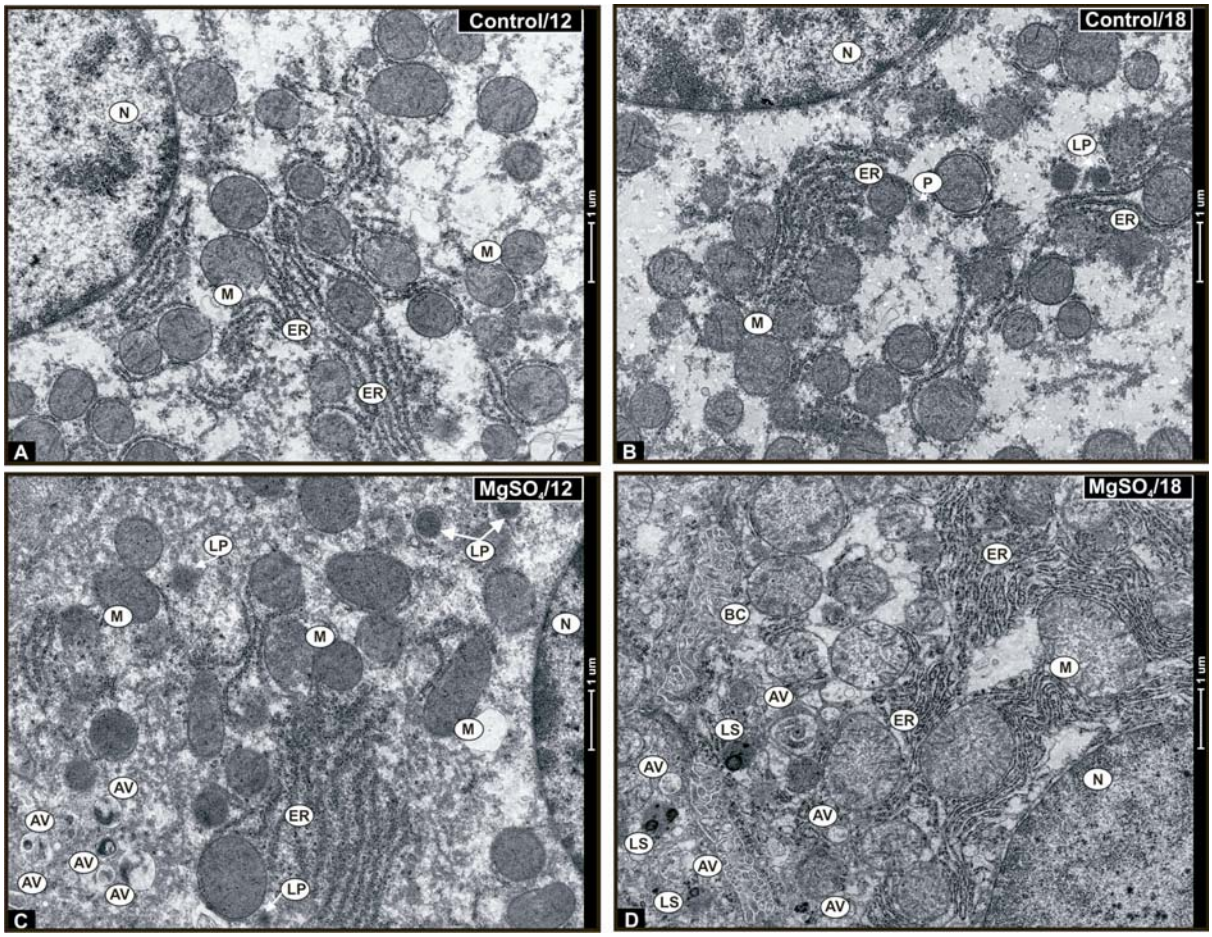

Fig. 2. Ultrastructure of rat liver cells of the control groups (A,B) and cells exposed to the action of $MgSO_4$ at the concentration of 0.06 mg Mg/ml (C,D) for 12 and 18 weeks. The cytoplasm contains: nucleus (N), endoplasmic reticulum (ER), mitochondria (M), bile canaliculi (BC), cellular membrane (CM), numerous autophagic vacuoles (AV), primary (LP) and secondary (LS) lysosomes. TEM – Magnification (A-D) × 11 500.

(Fig. 5). Increased cytoplasmic vacuolization was also observed in rat liver cells after the administration of $MgSO_4$ (Figs 4A-4D). It was shown that the range of changes in the studied cells, in relation to the control (Figs 4A-4B), was significantly increased as a consequence of extending the exposure time of animals to $MgSO_4$ to 18 weeks (Fig. 4D).

Activity of lysosomal hydrolases in response to the action of $MgSO_4$ *in vitro* and *in vivo*

Changes in the activity of the hydrolases of the lysosomal compartment were revealed *in vitro* (Figs 6A-6C). After 24 hours of exposure of CHO-K1 cells to $MgSO_4$ at concentrations of 25, 125 and 250 μM, increased activity of the model lysosomal enzymes was found (Figs 6A-6C). The most significant changes were observed in CHO-K1 cells loaded with $MgSO_4$ at a concentration of 250 μM (Fig. 6). An increase in the activity of the studied enzymes was also revealed in CHO-K1 cells as a result of 48 hour exposure to $MgSO_4$. Significant increases in the activity of Cath D/L, β-Gr and HeX were demonstrated after 48 hours of incubation of CHO-K1 cells with $MgSO_4$ at a concentration of 250 μM(Figs 6A-6C). For two studied lysosomal enzymes (Cath D/L and HeX), we found significant (in each case at $p<0.001$) main effects of time (24 hours vs 48 hours) and concentrations (control, 25 μM, 125 μM, 250 μM), as well as the interactions of these two factors (Table 1). The activity of the studied enzymes after 48 hours was significantly higher than after 24 hours (Figs 6A-6C). β-Gr did not show any dependence between the time and the concentrations (Table 1). The *in vivo* study demonstrated a significant effect of time was β-Gr and HeX (Table 2).

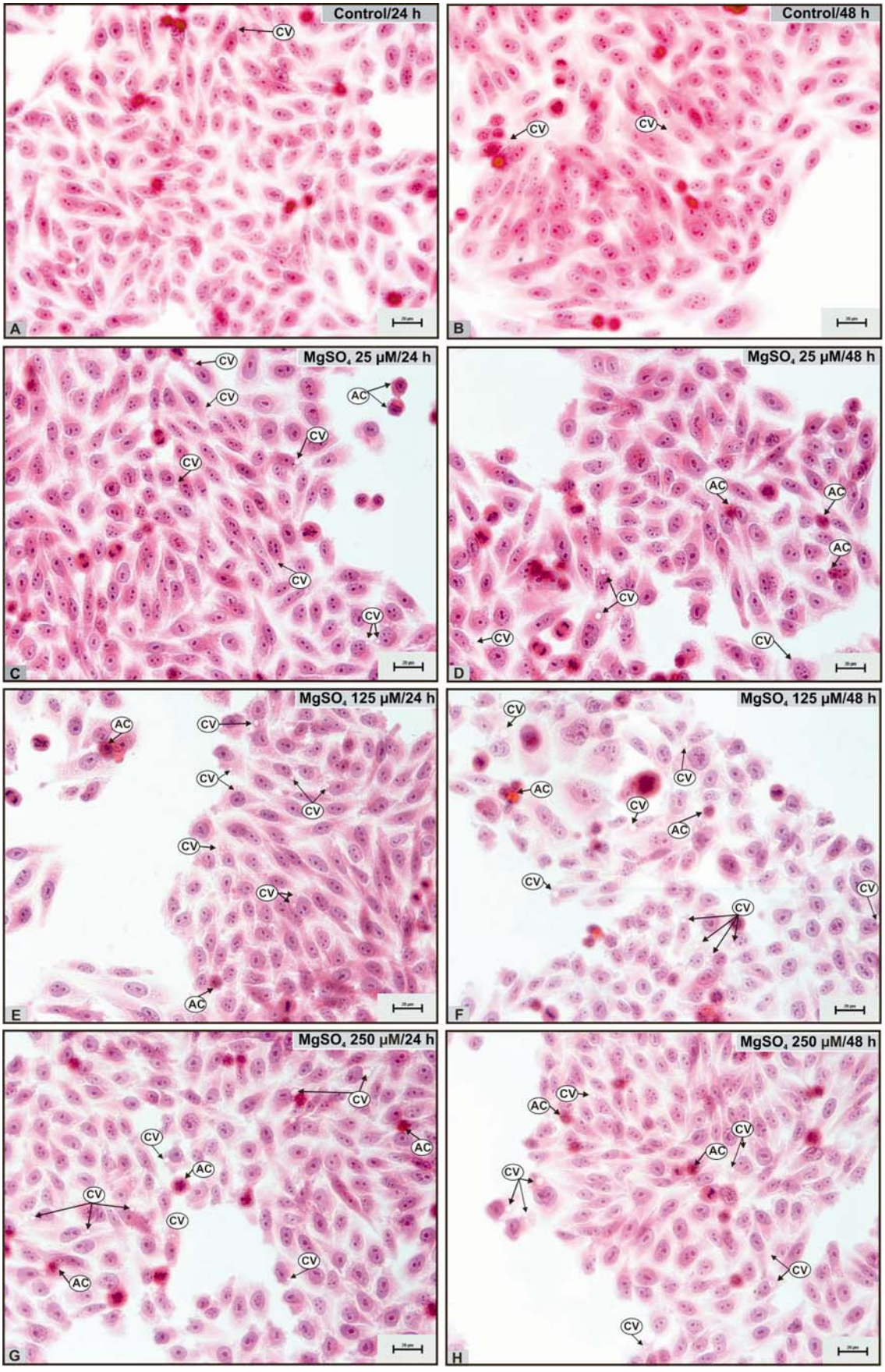

Fig. 3. Morphology of CHO-K1 cells of the control groups (A,B) and cells exposed to the action of $MgSO_4$, at concentrations of 25 µM (C,D), 125 µM (E,F) and 250 µM (G,H) for 24 and 48 hours. The image shows increased cytoplasmic vacuolization (CV) and single apoptotic cells (AC). Cells were stained with H+E – Magnification (A-H) × 400.

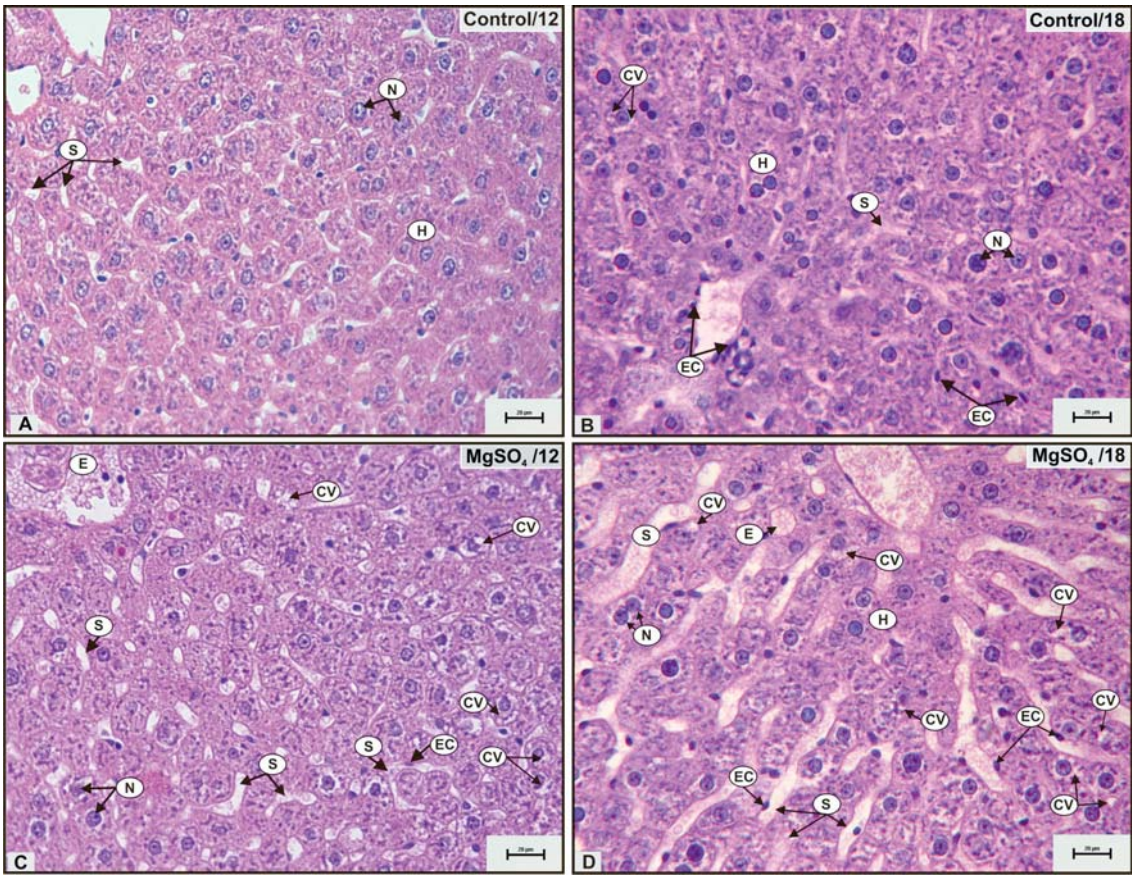

Fig. 4. Morphology of rat liver cells of the control groups (A,B) and cells exposed to the action of $MgSO_4$ at a concentration of 0.06 mg Mg/ml (C,D) for 12 and 18 weeks. The histological profile shows erythrocytes (E), hepatocytes (H), nucleus of hepatocytes (N), sinusoids (S), endothelium cells (EC) and increased cytoplasmic vacuolization (CV). Cells were stained with H+E – Magnification (A-D) × 400.

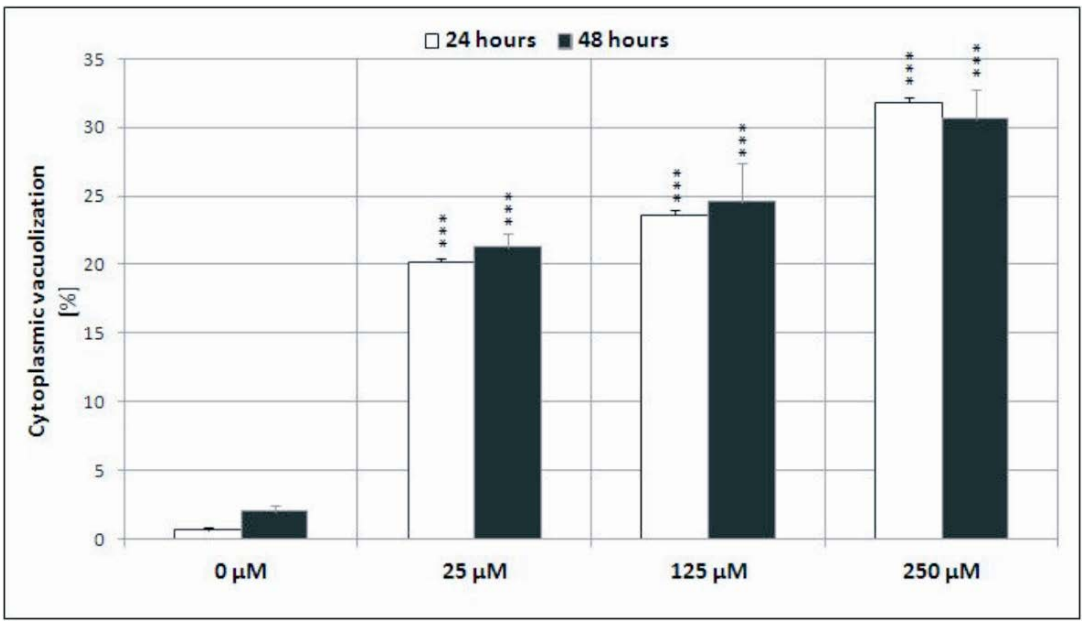

Fig. 5. Cytoplasmic vacuolization of CHO-K1 cells after 24 and 48 hour exposure to the action of $MgSO_4$ at concentrations of 25, 125 and 250 µM (N = 5000 cells/concentration/time of incubation). Statistically significant differences confirmed by the $\chi 2$ test in relation to the control with: ***$p<0.001$. The data are presented as a percentage ± SD.

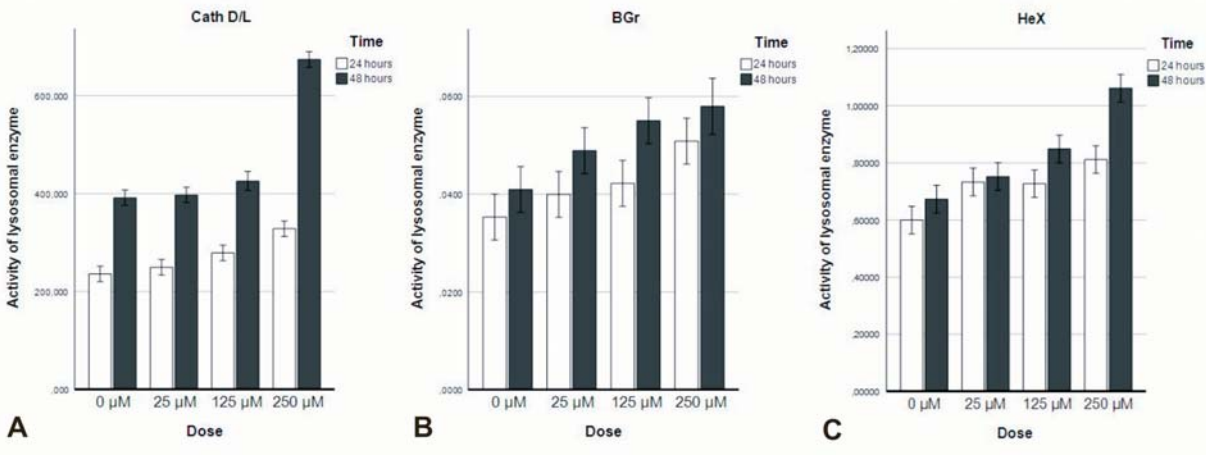

Fig. 6. Activity of cathepsin D and L (Cath D/L) (A), β-D-glucuronidase (β-Gr) (B) and β-N-acetylhexosaminidase (HeX) (C) in CHO-K1 cells after 24 and 48 hour exposure to the action of $MgSO_4$ at concentrations of 25, 125 and 250 µM. Error bars represent 95% confidence intervals.

Table 1

Effect of time of action and concentrations of MgSO4 on the activity of lysosomal enzymes in CHO-K1 cells

Enzymes	Two-way ANOVA analysis		
	Effect of time	Effect of concetrations	Effect of interaction (time vs concentrations)
Cath D/L	F = 1231.510; p = 0.000	F = 250.293; p = 0.000	F = 77.542; p = 0.000
β-Gr	F = 26.051; p = 0.000	F = 15.788; p = 0.000	F = 0.878; p = 0.461
HeX	F = 46.974; p = 0.000	F = 54.332; p = 0.000	F = 8.470; p = 0.000

Table 2

Effect of time of action and factor on the activity of lysosomal enzymes in rat liver cells

Enzymes	Two-way ANOVA analysis		
	Effect of time	Effect of factor	Effect of interaction (time vs factor)
Cath D/L	F = 0.178; p = 0.676	F = 0.036; p = 0.851	F = 0.095; p = 0.760
β-Gr	F = 22.320; p = 0.000	F = 0.344; p = 0.562	F = 0.199; p = 0.658
HeX	F = 28.423; p = 0.000	F = 0.220; p = 0.642	F = 0.135; p = 0.716

Discussion

In the last decade interest in the influence of magnesium on the human body has increased significantly. This is probably due to the fact that published reports on the biotherapeutic effects of this element, especially it's antitumor activity, are often rather ambiguous. In addition, most of the

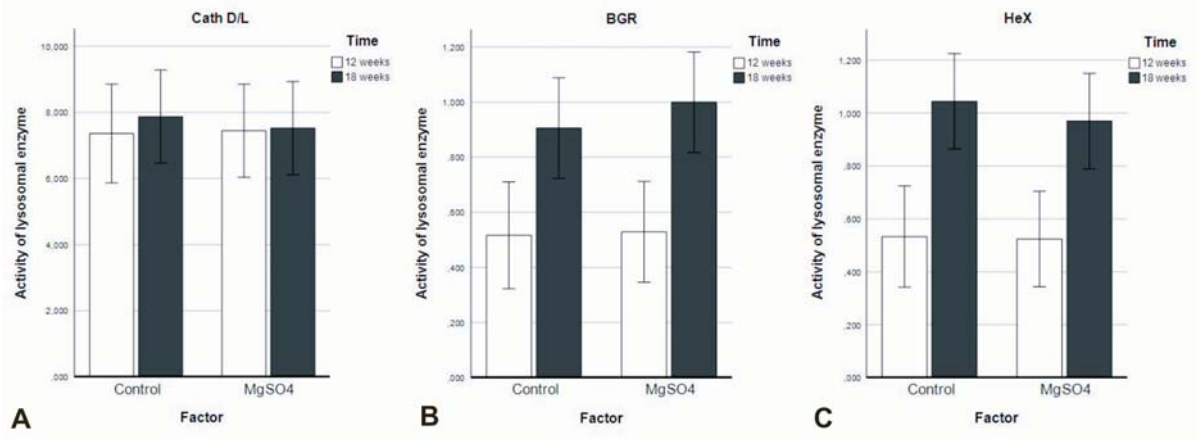

Fig. 7. Activity of cathepsin D and L (Cath D/L) (A), β-D-glucuronidase (β-Gr) (B) and β-N-acetylhexosaminidase (HeX) (C) in rat liver cells after 12 and 18 weeks of exposure to the action of $MgSO_4$ at a concentration of 0.06 mg Mg/ml. Error bars represent 95% confidence intervals.

work, both demonstrative and experimental, refer to the biological effects resulting mainly from the lack of Mg^{2+} ions (NASULEWICZ *et al.* 2004; VAN ORDEN *et al.* 2006; KILLILEA & MAIER 2008).

The published literature indicates an important role of the lysosomal system in the evaluation of cellular changes induced by the action of compounds with potential anticancer properties (KRÓL 1998a,b; KRÓL *et al.* 2008). The key importance of the lysosomal system involved in the process of intracellular degradation and various cellular pathways was also reported by TRYBUS *et al.* (2014, 2017a,b). Lysosomes are considered to be signaling centers, which are the first to react to the action of various factors of the extra- and intracellular environment (AITS *et al.* 2015). Therefore, their important role in numerous pathological processes, including neoplastics, is often emphasized (FENNELLY & AMARAVADI 2017). It seems interesting to understand the role of $MgSO_4$ in relation to the activity of the lysosomal compartment, in both *in vitro* and *in vivo* studies.

Magnesium is involved in the activation of over 300 different types of enzymes (PASTERNAK *et al.* 2010; DE BAAIJ *et al.* 2015). According to our research, magnesium also induces the activity of the model enzymes of the lysosomal compartment (Figs 6,7). Both *in vitro* and *in vivo* studies revealed changes in the activity of the examined lysosomal hydrolases (Figs 6, 7). The intensification of enzyme synthesis was dependent on the dose and duration of the action of $MgSO_4$ (Figs 6,7). In the *in vitro* conditions, the most significant increase of the activity of enzymes was demonstrated after 48-hour exposure of cells to the action of $MgSO_4$ at a concentration of 250 μM (Figs 6). The obtained results indicate activation of the lysosomal system. The revealed biochemical changes are confirmed by the ultrastructural changes of the examined cells, including an increase in the number of primary and secondary lysosomes (Figs 1E, 1G, 2C, 2D), as well as an increased number of very diverse autophagic vacuoles, including ones containing partially degraded cytoplasmic components (Figs 1C-1H, 2C-2D). In this study increased cytoplasmic vacuolization was also found (Figs 3C-3D, 4C-4H) and it was reflected in the results of the quantitative analyses performed (Fig. 5). The increase in the number of *in vitro* cells with visible vacuolization in the cytoplasm was dependent on the applied concentrations and the time of exposure to $MgSO_4$ (Fig. 5).

According to the literature, lysosomes may play a significant role in the mechanism of cell death caused by a multifactorial permeabilization of the lysosomal membranes (GUICCIARDI *et al.* 2004; BOYA & KROEMER 2008). In our ultrastructural (Figs 1C-1H; 2C-2D) and morphological (Figs 2C-2H; 3C-3D) studies of *in vitro* and *in vivo* cells, intensification of apoptotic features was not observed, which may indicate the stabilizing effect of $MgSO_4$ on the lysosomal membranes, probably resulting from the antioxidant properties of the studied compound.

The currently available literature contains little information about the effect of magnesium and its compounds on the lysosomal system. Our research suggests that $MgSO_4$ induces changes of lysosomal compartments in both *in vitro* and *in vivo* conditions. Certain compounds with anticancer properties stimulating the lysosomal system in cells are currently known (KRÓL *et al.* 1994; KRÓL *et al.* 1998a; HSU *et al*. 2009; PARK *et al.* 2016).

Also promising are results indicating the applicability of the compounds which activate lysosomes in a combined therapy with other cytostatics (KOSKELA *et al.* 2016) or other cancer treatment methods (LI *et al.* 2016), increasing the effectiveness of anticancer pharmacotherapy. It is possible that $MgSO_4$, may also be used in the future in anticancer therapy, as a compound stimulating the lysosomal system, in combination with other preparations. For this purpose, further research is required.

Conclusion

Our results indicate that $MgSO_4$ activates the lysosomal system in both *in vitro* and *in vivo* cells. This is demonstrated by changes in the lysosomal compartment of cells, including an increased number of autophagic vacuoles and autophagolysosomes, an intensification of cytoplasmic vacuolization, and increased activity of the lysosomal enzymes.

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Author Contributions

Research concept and design: H.Z.; Collection and/or assembly of data: E.T., W.T., K.K, E.K.; Data analysis and interpretation: A.K.-B.; Writing the article: A.K.-B.; Critical revision of the article: T.K.; Final approval of article: T.K.

Conflict of Interest

The authors declare no conflict of interest.

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