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Bioactivity and cytotoxic effect of nanosized magnesium oxide in the *in vitro* experiments.

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ABSTRACT

Magnesium oxide nanoparticles were obtained from the magnesia concentrate during the enrichment of natural amorphous magnesite. These particles are bioaccessible and bioactive. Irrespective of the type, nucleated cells react to the change of magnesium oxide content, which obviously raise functional activity of the cells. As a result, it leads to the depositing of Mg^{2+}-free form. At the same time, nucleated red bone marrow cells demonstrate the change of viability depending on the concentration of the magnesium oxide nanoparticles in the cultural medium. Magnesium oxide at the concentration of 0.250 mM has a cytotoxic effect, but not general. Along with that, the concentration of 0.125 mM increases cells viability that indicates the activation of intracellular exchange processes as a result of this impact.

Keywords: magnesium oxide, red bone marrow cells, white blood cells, bioactivity, bioaccessibility, cytotoxicity

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INTRODUCTION

The maintenance of magnesium homeostasis in the organism is essential requirement for the human health [1, 2]. Magnesium takes part in control of functions of a numerous cells enzymes and carriers that provide energy production, cells growth and division [3, 4, 5, 6]. However, plasma membrane of the cells is impermeable for Mg^{2+} and energy-dependent transport systems provide the extrusion of Mg^{2+} against the concentration gradient [7]. Therefore, the topical issue is the search of natural chemical compounds containing the bioaccessible and bioactive magnesium, which allow developing of effective biologically active food additives for correction of the magnesium deficient state.

The purpose of the research is the estimation of the bioactivity of nanosized magnesium oxide (MgO) and its influence on the cells viability.

METHODS

A white powder of the magnesium oxide obtained from magnesia concentrate during the enrichment of natural amorphous magnesite (Khalilovsky deposit, Orenburg Region, Russia) was used in the work. The magnesium oxide was produced by using of plasma-chemical method with sputtering of the superdispersed magnesium nitrate powder in plasma arc [8]. Microstructure and elemental composition of the synthesized magnesium oxide powder were investigated by electron microscope JEOL JEM-2100 (Fig. 1) and X-ray diffractometer Rigaku Ultima IV (Fig. 2).

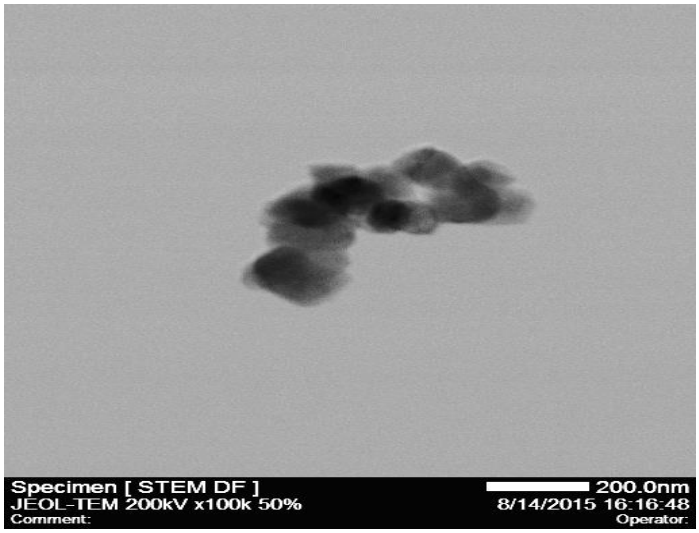

Figure 1. TEM image of MgO nanoparticles

Content of the base material in the synthesized nanosized magnesium oxide powder exceeded 99%.

In vitro test was carried out on red bone marrow cells and white blood cells which were obtained from the male Wistar rats weighting 250-300 grams (nursery “Stolbovaya”, Moscow region, Russia).

Red bone marrow cells were washed out of the tibia by the physiological saline in the Petri dishes. After that the cells suspension was transferred to the tubes and adjusted to 10 ml final volume with 0.83% ammonium chloride solution for the anucleated cells lysis. Then the suspension was centrifuged at 1 000 g for 4 min. The supernatant of red bone marrow cells was obtained after the cells washing with the isotonic buffer solution and sedimentation by the centrifugation at 1 000 g for 2 min.

Peripheral blood was obtained after decapitation of the rat. The blood was centrifuged at 1 500 g for 10 min. The leukocyte ring between the plasma and erythrocyte mass was collected with syringe. The erythrocytes were removed from the suspension by adding of 10 ml of 0.83% ammonium chloride solution. After that the suspension was centrifuged at 1 000 g for 4 min. The cells were washed twice with isotonic buffer solution and after that centrifuged at 1 000 g for 2 min.

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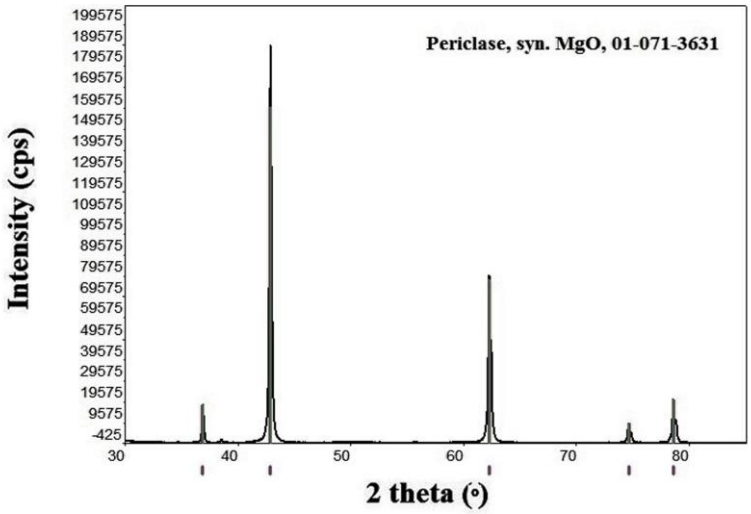

Figure 2. XRD pattern of MgO nanopowder

All further manipulations with the red bone marrow cells and white blood cells were carried out according to the scheme: 100 µl of cell suspension and 900 µl of medium 199 with Hanks' salts and glutamin (PanECO, Russia) were placed in the 35x10 mm Petri dishes (SPL Lifesciences, Korean). There were prepared 3 control probes with the red bone marrow cells and 3 control probes with the white blood cells; 12 experimental probes with the red bone marrow cells and 6 experimental probes with the white blood cells.

Estimation of the bioaccessibility was performed by adding the magnesium oxide at the concentration of 0.250 mM (3 probes with the red bone marrow cells, 3 probes with white blood cells) and the magnesium oxide at the concentration of 0.125 mM in the medium for cell cultivation (3 probes with the red bone marrow cells). Identification of the magnesium in the cells was implemented by fluorescent dye – Magnesium Green, AM (M3735, Molecular probes, USA). Stock solution of Magnesium Green was prepared according to the manufacturer's instruction. After that 30 µl of fluorescent dye on Mg^{2+} were added to the 1 000 µl of medium 199 containing the cells. The cells were incubated at 37°C for 60 min in the presence of 5% CO_2.

Estimation of the cytotoxicity was carried out according to the same scheme by adding magnesium oxide at the concentration of 0.250 mM (3 probes with the red bone marrow cells, 3 probes with white blood cells) and the magnesium oxide at the concentration of 0.125 mM into the medium for cultivation (3 probes with the red bone marrow cells). The cytotoxicity effect was determined by calculation of the number of the viable cells in the suspension with help of double staining using ethidium bromide (Sigma-Aldrich, USA) and calcein acetoxymethyl ester (Sigma-Aldrich, USA) [7]. Stock solutions of dyes were prepared according to the manufacturer's instructions. The work solution was obtained from the stock solutions: 4 µM of ethidium bromide and 2 µM of calcein acetoxymethyl ester were dissolved in the 1.5 ml of Dulbecco's saline (PanECO, Russia). After that 30 µl of double-component solution of the fluorescent dyes were added to the 1 000 µl of the control and experimental groups cells suspensions in the medium 199. The cells were incubated at 37°C for 60 min in the presence of 5% CO_2. After the incubation period the cells were washed twice from the dyes and the fresh medium 199 was added. The number of viable cells (green fluorescence) and dead cells (red fluorescence) were calculated. The percent of viability was calculated with formula: % viability = (live cell count / total cell count) × 100 [9].

All probes were investigated by using of fluorescent microscope Eclipse Ti-U (Nikon, Japan). Identification and documentation were carried out by the program NIS-Elements D (Nikon, Japan). Fluorescence intensity was determined by software EZ-C1 FreeViewer Ver 3.90 (Nikon, Japan).
The results were obtained by statistical computer programs using Student's t-test for unpaired data.

MAIN PART

It was determined that the red bone marrow cells and white blood cells reacted equally on the adding of the nanosized magnesium oxide in cultural medium (Fig. 3).

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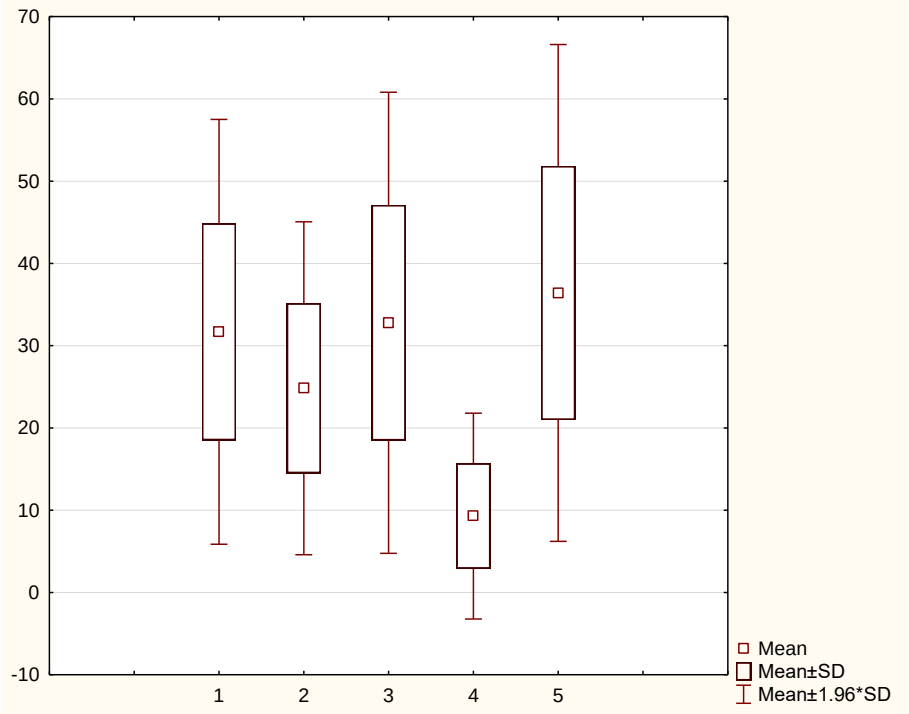

Figure 3. Graph of the fluorescence intensity of the magnesium ions (in procedure defined units (p.d.u.))

1 – control probe with the white blood cells; 2 – experimental probe with the white blood cells with addition of magnesium oxide at a concentration of 0.250 mM; 3 – control probe with the red bone marrow cells; 4 – experimental probe with the red bone marrow cells with addition of magnesium oxide at a concentration of 0.250 mM; 5 – experimental probe with the red bone marrow cells with addition of magnesium oxide at a concentration of 0.125 mM

It was registered that the fluorescence intensity of the cells decrease (9.29 ± 6.38 p.d.u.) after addition of magnesium oxide at the concentration of 0.250 mM to the probe with the red bone marrow cells in comparison with the control probes (32.79 ± 14.30 p.d.u.) and experimental probes (36.41 ± 15.41 p.d.u.) with the magnesium oxide concentration of 0.125 mM ($p<0.01$) (Fig. 4).

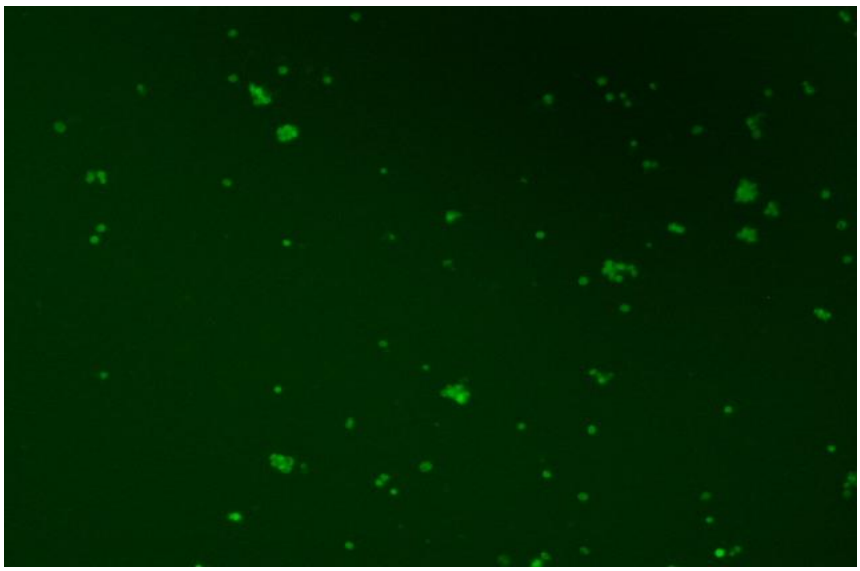

Figure 4. Fluorescence of magnesium ions in the red bone marrow cells: experimental probe with addition of magnesium oxide at a concentration of 0.250 mM (fluorescent microscope Eclipse Ti-U, Nikon, Japan)

Decrease of the fluorescence intensity was registered in the experimental probes with the white blood cells after addition of the magnesium oxide at the concentration of 0.250 mM into the cultural medium (24.83 ± 10.33 p.d.u.) in comparison with the control probes (31.69 ± 13.18 p.d.u.) ($p<0.01$) (Fig. 2).

Reduction of the Mg^{2+} fluorescence in red bone marrow cells and white blood cells after addition of the magnesium oxide at a concentration of 0.250 mM in the cultural medium could be associated with the stimulation of exchange processes in the cells as a consequence of increase of the extracellular concentration of the element [10] and that led to the change of magnesium from the free state to the bound state. But, the decrease of fluorescence intensity in the experimental probes with the red bone marrow cells with addition of magnesium oxide at a concentration of 0.125 mM was not determined in comparison with the control probes.
Analysis of the number of viable and dead cells in the presence of nanosized magnesium oxide in the cultural medium have shown that the number of viable red bone marrow cells were greater than the number of viable white blood cells. The number of viable red bone marrow cells was greater in the probes with magnesium oxide concentration 0.125 mM (100.20 ± 15.53 cells) in comparison with the probes with magnesium oxide concentration 0.250 mM (58.10 ± 30.49 cells) and with the control probes (85.20 ± 18.32 cells) ($p<0,01$). The number of viable white blood cells after addition of the magnesium oxide at the concentration of 0.250 mM was authentically decreased and (46.30 ± 11.18 cells), in comparison with control probes (80.31 ± 11.02 cells) ($p<0,01$).

It was determined that the cell viability index after cultivation the cells with the magnesium oxide nanoparticles was higher in the probes with the red bone marrow cells than in probes with the white blood cells (Fig. 5).

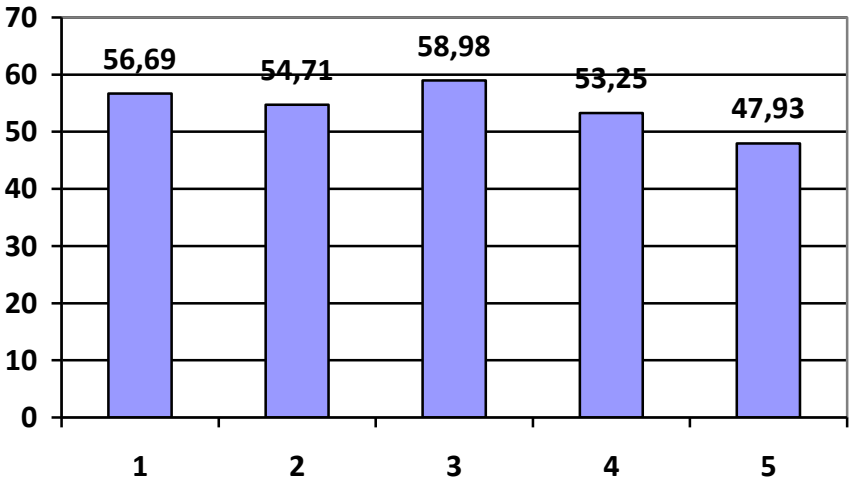

Figure 5. Bar graph showing the cells viability in the presence of nanosized magnesium oxide in cultural medium (%)

1 – control probe with the red bone marrow cells; 2 – experimental probe with the red bone marrow cells with addition of magnesium oxide at the concentration of 0.250 mM; 3 – experimental probe with the red bone marrow cells with addition of magnesium oxide at the concentration of 0.125 mM; 4 – control probe with the white blood cells; 5 – experimental probe with the white blood cells with addition of magnesium oxide at the concentration of 0.250 mM

Analysis of the data obtained during investigation of the cells viability showed that the viability of red bone marrow cells and white blood cells decreased after addition of magnesium oxide at the concentration of 0.250 mM. But the increase of viability of red bone marrow cells after addition of magnesium oxide at the concentration of 0.125 mM in the cultural medium in comparison with the control probes and experimental probes where magnesium oxide concentration is 0.250 mM.

CONCLUSION

As a result of this work the data indicating the high biological activity of the magnesium oxide in the nanoparticle form dissolved in cultural medium was obtained. Various types of nucleated cells react to the changes of magnesium oxide content in the simple manner, which evidently increases functional activity of the cells and leads to the Mg^{2+} free form depositing. The nucleated red bone marrow cells demonstrate the changes of viability depending on the concentration of magnesium oxide nanoparticles in the cultural medium. It was showed that addition magnesium oxide at the concentration of 0.250 mM to the cultural medium has the cytotoxic effect, but not general. Along with that, the concentration of 0.125 mM increases cells viability that indicated on the activation of the intracellular exchange processes under that influence.

Magnesium oxide nanoparticles are bioaccessible and bioactive, but it is necessary to consider the concentration of magnesium oxide nanoparticles during the investigation of its effects on other objects.

The main results can be summarized as follows:

- Magnesium oxide nanoparticles are bioaccessible and bioactive.
- It is determined that the nucleated blood cells and red bone marrow cells react equally to the change of magnesium oxide concentration in the cultural medium.
- Red bone marrow cells demonstrate increasing of viability after addition of magnesium oxide at the concentration of 0.125 mM in the cultural medium and decreasing of viability after addition of magnesium oxide at a concentration of 0.250 mM.

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