

MURINE STUDIES REGARDING THE TISSUE INTAKE OF DIFFERENT MAGNESIUM COMPOUNDS

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Abstract

The content of magnesium in different mice tissues (heart, liver, spleen, kidney and lung) after administration of different magnesium compounds was studied. Doses of 25, 50, 100, 200 and 300 mg of magnesium -orotate, -sulphate, -oxide, -carbonate, -citrate and -chloride were orally administered to groups of 2 mice for each dose and compound. Tests results showed that the magnesium levels decreased in the following order: orotate > sulphate > chloride > carbonate > citrate > oxide. No side effects were observed in the case of orotate, carbonate and chloride; in the case of oxide and citrate a moderate laxative effect was observed, while in the case of sulphate an intense laxation was noticed. The highest concentrations of magnesium were found in the liver, while the lowest concentrations were found in the lung. It is possible that the chemical structure of magnesium orotate facilitates the bioavailability and higher tisular concentrations of magnesium.

Rezumat

În această lucrare a fost studiată concentrația tisulară a magneziului, prin determinarea conținutului de magneziu în diferite organe (inimă, ficat, splină, rinichi și plămân) la șoareci, în urma administrării diferiților compuși ai magneziului. Au fost administrate doze de 25, 50, 100, 200 și 300 mg orotat, sulfat, oxid, carbonat, citrat și clorură de magneziu, prin gavaj, la grupuri a câte 2 șoareci, pentru fiecare doză și compus. Rezultatele au arătat o scădere a concentrației de magneziu în ordine: orotat > sulfat > clorura > carbonat > citrat > oxid. În cazul administrării de orotat, carbonat și clorură de magneziu nu au fost observate efecte secundare, în timp ce la administrarea de oxid și citrat de magneziu s-a observat o ușoară laxație, iar în urma administrării de sulfat de magneziu efectul laxativ a fost mai pronunțat. Concentrația cea mai mare de magneziu a fost determinată în ficat, iar concentrația cea mai scăzută în plămâni. Este foarte probabil că structura chimică a orotatului de magneziu să permită o mai bună biodisponibilitate a ionilor de magneziu și apariția implicită a unei concentrații mai mari de magneziu la nivel tisular, comparativ cu ceilalți compuși de magneziu studiați.

Keywords: magnesium compounds, intracellular absorption

Introduction

Magnesium (Mg) is the second most abundant intracellular cation after potassium and the fourth most abundant mineral in the body after calcium, potassium and sodium [10]. The total Mg content in the adult human body is reported to be ~ 20 g compared with 1000 g of total calcium [8, 9]. Of the total Mg found in the organism, 65% is located in bones, 33% in the intracellular space and only a small amount in the extracellular space (2%) and intravascular compartment (< 1%) [7].

Mg is a regulator of electrolytes and other substances passage through cellular membranes and the "biological competitor" of calcium. Furthermore, Mg is a structural component of various tissues especially bones. Adequate intracellular Mg levels are essential for life, since Mg is a fundamental co-factor for vital

biochemical reactions, mainly those which depend upon adenosine triphosphate and it is an important controller of the Krebs cycle and glycolysis [1, 5, 12, 16].

The Mg deficiency is caused mainly by depletion of the mineral content in soils and, consequently, in fruits and vegetables. For this reason, a large part of the population turns to Mg supplements to increase the intake of this important mineral for the human body. Mg is absorbed in the intestine, is mainly stored in bones and excreted by the kidney and faeces. Generally, the dietary supplements are ingested to supply the nutrients that are lacking in the diet, strengthen body immunity, support individuals with genetic biochemical and physiological variations and fight disease conditions [2, 11, 17]. Mg supplements contain various Mg compounds with

different bioavailability. The most commonly Mg compounds found in supplements are chloride and sulphate; however, sulphate has the least advantageous pharmacological properties (poor absorption, poor cellular penetration, membrane effects and anti-hypoxic effects). Moreover, sulphate infusion leads to a considerable increase in the urinary excretion of magnesium, calcium and potassium [6, 14]. Furthermore, Mg chloride and Mg sulphate have different behaviour regarding the ionic exchanges through the membrane: Mg chloride interacts with all the exchangers, while Mg sulphate is limited to paracellular components, except the Na^+/H^+ antiporter [3, 13].

As the assay of plasma Mg gives information about less than 1% of Mg in the body, we evaluated Mg concentrations in various mice organs (spleen, lung, kidney, heart and liver) in order to identify the compounds with the best tissue availability.

Materials and Methods

Reagents

Magnesium orotate ($\text{C}_{10}\text{H}_6\text{MgN}_4\text{O}_8$), magnesium sulphate ($\text{MgSO}_4 \times 7\text{H}_2\text{O}$), magnesium oxide (MgO), magnesium chloride ($\text{MgCl}_2 \times 6\text{H}_2\text{O}$), magnesium carbonate (MgCO_3), magnesium citrate ($\text{Mg}_3(\text{C}_6\text{H}_5\text{O}_7)_2$), HNO_3 65% and H_2O_2 30% were purchased from Merck, Germany. A multi-elemental standard solution of 1000 mg/L supplied from Merck, Germany was used for the calibration of inductively coupled plasma optical emission spectrometer (ICP-OES). All dilutions were prepared using ultrapure water (18.2 M Ω /cm) obtained from a Millipore Direct-Q3 UV Ultrapure water system (Millipore, France).

Animals

Adult male CD1 mice ($n = 62$) with an average weight of 26.92 ± 0.21 g were used after quarantine and acclimatization. The animals were maintained in environmentally controlled rooms (standard temperature of $22 \pm 2^\circ\text{C}$, relative humidity of $55 \pm 10\%$) in a 12/12-hour light/dark cycle with a light intensity of 300 lx in open-top cages [4]. The groups were randomized in different cages. All experimental protocols were approved by the Ethics Committee of "Iuliu Hațieganu" University of Medicine and Pharmacy and were conducted in accordance with the EU Directive 63/2010. The study was performed in accordance with the ARRIVE Guidelines for reporting animal research and the animals were cared according to the European guidelines.

Procedure and data collection

For each individual, amounts of 25, 50, 100, 200 or 300 mg of different Mg compounds were dissolved (orotate, sulphate, chloride and citrate) or suspended (carbonate and oxide) in distilled water and administered by gavage using flexible plastic feeding tubes. For each treatment, groups of 2 individuals were used. In case of the control group distilled water was administered. 2 hours after administration the animals were euthanized using the cervical dislocation method, followed by the harvesting of internal organs (heart, lungs, liver, spleen, kidney). The weight of each organ was recorded. The samples digestion was performed using a mixture of HNO_3 65% and H_2O_2 30% (10:1, v:v) in closed polytetrafluoroethylene vessel microwave digestion system (Berghof MWS-3+, Eningen, Germany) according to the method described by Zhao *et al* [18]. The digested samples were quantitatively transferred into 10 mL volumetric flasks and diluted to the mark with ultrapure water. The Mg concentration (mg/L) in each organ was determined by ICP-OES Optima 5300 DV (Perkin Elmer, USA), after digestion, followed by conversion to mg/kg. The calibration standards (0.1, 0.2, 0.5, 1, 2, 4 and 6 mg/L) were prepared by diluting the stock standard solution (1000 mg/L) in 0.5% (v/v) HNO_3 .

Results and Discussion

The average weights of the studied organs were: 0.169 ± 0.031 g heart, 0.206 ± 0.027 g, liver 1.606 ± 0.144 g, spleen 0.155 ± 0.038 g and kidney 0.338 ± 0.032 g. The Mg content was calculated as the difference between Mg content determined in the organs of test and control individuals. Table I shows the average Mg content determined in each organ, for the administered doses and compounds. The Mg content varied widely in all tissues. The highest Mg content was found in spleen, in the case of administering 200 mg of Mg-citrate, while the lowest in lungs, in case of administering 25 mg of Mg-oxide. Mg values decreased in the following order: spleen > kidney > liver > heart > lung. The high content of Mg in kidney could be explained by the fact that Mg homeostasis is maintained mainly by the kidneys by regulating the excretion rate [12]. The content of Mg in administered compounds was calculated based on the chemical formula, considering the molecular weight. Thus, the lowest Mg content (1.8 mg) was found in 25 mg of Mg-orotate, while the highest (181 mg) in 300 mg of Mg-oxide.

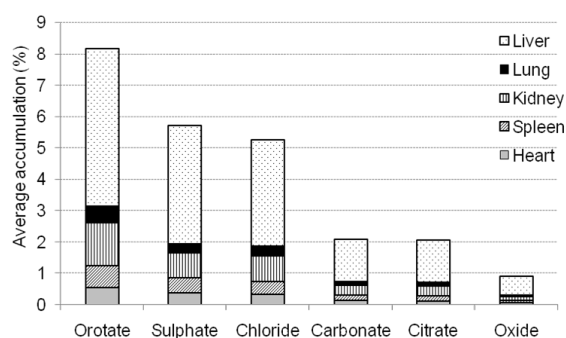
Table I

Mg content in studied organs in function of the administered Mg-compound and dose

Mg-compound	Dose (mg)	Mg content (mg/g)				
		Heart	Spleen	Kidney	Lung	Liver
Orotate	25	0.145	0.200	0.180	0.116	0.161
	50	0.150	0.210	0.186	0.121	0.166
	100	0.160	0.220	0.195	0.135	0.179
	200	0.171	0.230	0.210	0.141	0.191
	300	0.168	0.220	0.198	0.151	0.201
Sulphate	25	0.130	0.193	0.145	0.079	0.145
	50	0.140	0.210	0.150	0.088	0.149
	100	0.155	0.153	0.160	0.090	0.151
	200	0.160	0.229	0.180	0.102	0.160
	300	0.135	0.220	0.130	0.089	0.145
Oxide	25	0.122	0.191	0.130	0.076	0.142
	50	0.125	0.200	0.131	0.084	0.152
	100	0.144	0.152	0.130	0.088	0.157
	200	0.154	0.228	0.150	0.098	0.158
	300	0.130	0.216	0.139	0.090	0.113
Chloride	25	0.140	0.196	0.175	0.110	0.155
	50	0.145	0.210	0.185	0.115	0.161
	100	0.160	0.218	0.203	0.122	0.172
	200	0.170	0.225	0.215	0.132	0.185
	300	0.165	0.220	0.201	0.121	0.165
Carbonate	25	0.137	0.194	0.170	0.105	0.150
	50	0.140	0.200	0.173	0.111	0.155
	100	0.150	0.215	0.181	0.120	0.160
	200	0.161	0.220	0.190	0.125	0.175
	300	0.155	0.215	0.185	0.115	0.161
Citrate	25	0.152	0.210	0.185	0.120	0.170
	50	0.160	0.220	0.201	0.132	0.179
	100	0.170	0.232	0.218	0.162	0.192
	200	0.185	0.243	0.235	0.171	0.220
	300	0.180	0.239	0.221	0.160	0.216

The accumulation of Mg in different organs was calculated as the percentage of Mg content in each organ and Mg content in administered compound and dose. The lowest Mg% was found for 300 mg Mg-oxide (0.01%), while the highest Mg% for 25 mg Mg-orotate (10.2%). These data suggest that the chemical compound is more important than the administered dose, in the case of Mg supplements selection. The average accumulation in the targeted organs of each Mg compound, regardless the administered dose, is presented in Figure 1. The accumulation efficiency decreases in the following order: orotate > sulphate > chloride > carbonate ≥ citrate > oxide. The highest average accumulation was found in the hepatic tissue. The low solubility of orotic acid in aqueous medium and the chemical structure of Mg-orotate favour its intracellular dissociation. Thus, the orotic acid acts as a transporter that carries Mg into the cells [2, 15]. This is a possible explanation for the higher tisular

concentration of Mg originating from Mg-orotate compared with the other used Mg salts.

**Figure 1.**

Average accumulation in target organs of Mg compounds

The Mg accumulation in the studied organs, for each Mg compound in function of the administered dose of active substance is presented in Figure 2.

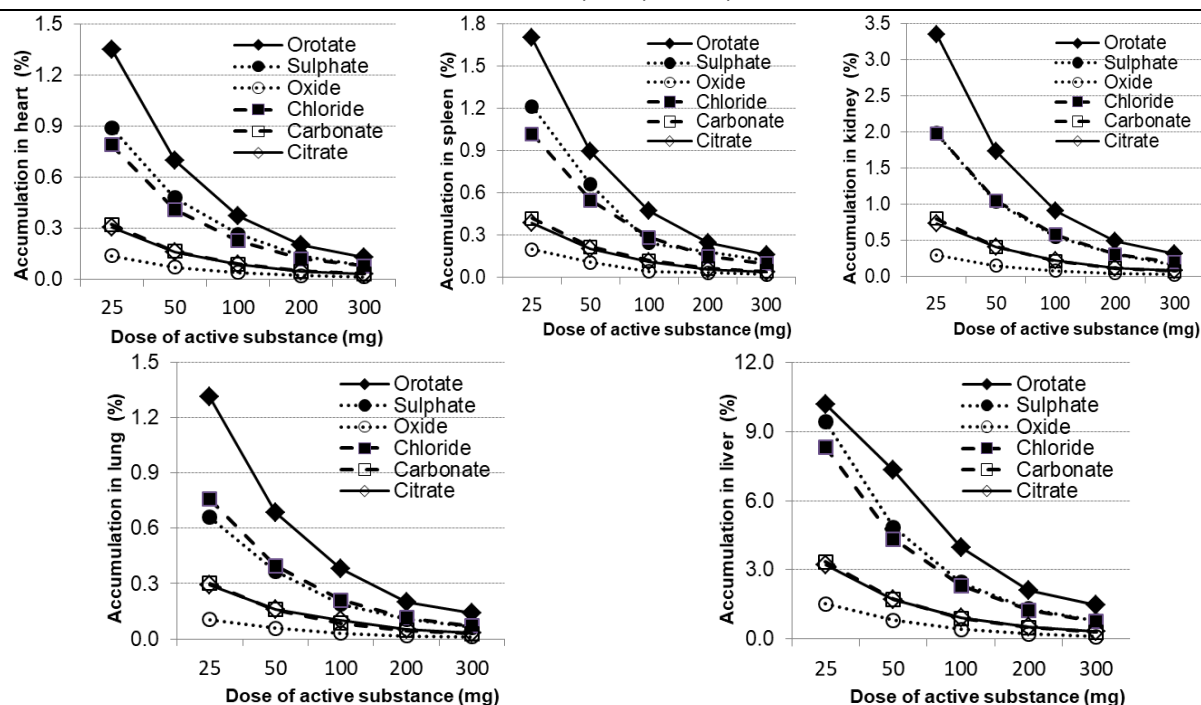


Figure 2.

Mg accumulation in tissues considering of the dose of active substance

The accumulated Mg (%) decreased with the increase of the administered doses for all tissues, suggesting that supplements are more effective in low rather than in high doses. A possible explanation of this fact could be that the body can store limited amounts of Mg and the absorption is higher when the Mg level is lower.

In the selection of the Mg therapy, an important role is also played by the side effects that may occur. In the present study, changes in behaviour, motility and reflectivity did not occur. However, in case of Mg-sulphate, between administration and organ harvest, the laxative effect was observed.

Conclusions

Mg concentrations in the mice tissues are low regardless of the administered dose and vary in the following order: liver > kidney > spleen > heart > lung. Mg concentration in all investigated organs decreases with the increase of Mg quantity orally administered as follows: orotate > sulphate > chloride > carbonate ≥ citrate > oxide. Following the oral administration of studied Mg compounds, the gastrointestinal side effects occurred for Mg sulphate, citrate and oxide and intensify with the increase of the administered dose, while for Mg orotate, chloride and carbonate, these effects were absent. The lack of side effects, as well as the high Mg concentrations measured after Mg-orotate administration, indicating Mg orotate as a source of Mg that can be used to supplement the oligoelement deficiency in the diet.

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