Acta Alimentaria, Vol. 43 (3), pp. 419–425 (2014)
DOI: 10.1556/AAlim.43.2014.3.8

IN VITRO ANTIOXIDANT ACTIVITIES OF MAGNESIUM COMPOUNDS USED IN FOOD INDUSTRY

K. Szentmihályi[a]*, M. Szilágyi[b], J. Balla[c], L. Ujhelyi[c] and A. Blázovics[d]

[a] Institute of Materials and Environmental Chemistry, Research Center for Natural Sciences, Hungarian Academy of Sciences, H-1117 Budapest, Magyar tudósok körútja 2. Hungary
[b] Research Institute for Animal Breeding and Nutrition, H-2053 Herceghalom, Gesztenyés út 1. Hungary
[c] Medical and Health Science Centre, University of Debrecen, H-4032 Debrecen, Egyetem tér 1. Hungary
[d] Institute of Pharmacognosy, Semmelweis University, H-1085 Budapest, Üllői út 26. Hungary

(Received: 4 February 2013; accepted: 2 April 2013)

Magnesium participates in numerous enzymatic reactions in the human body and it has essential role in the maintenance of the antioxidant system. Since several magnesium compounds have been applied in the food and pharmaceutical industry, our purpose was to investigate the antioxidant/free radical scavenging activity of some magnesium compounds in vitro. The antioxidant/prooxidant effect of inorganic salts (e.g. $MgCl_2$) and organic complexes (e.g. Mg-gluconate) was determined with chemiluminometric method (H_2O_2/•OH-microperoxidase-luminol) and heme mediated LDL oxidation (LDL-heme-H_2O_2) in vitro. It has been stated that the chemiluminescence method and LDL (low density lipoprotein) oxidation measurement is applicable in the presence of magnesium salts and complexes. Most of the compounds do not generate free radicals and the antioxidant/prooxidant effect depends on the quality of the ligand and the concentration. In the concentration range used, some representatives of the magnesium compounds (MgO, Mg-gluconate, Mg-polygalacturonate) investigated showed radical generating activity measured with chemiluminescence method, whereas the LDL oxidation has not been affected. Magnesium citrate and malate proved to be antioxidants measured with the chemiluminescence method and they slightly accelerate the LDL oxidation in the system and in the concentration applied. In vitro some of the ligands of magnesium compounds showed antioxidant activities.

Keywords: magnesium, radical scavenger capacity, LDL oxidation, *in vitro*

Magnesium is an essential macroelement in the human body, it has fundamental role in the synthesis and metabolism of carbohydrates, lipids, proteins, and nucleic acids, for example, for synthesizing DNA and RNA in mitochondria (Siegel & Siegel, 1990; Fazekas et al., 1994). It is also required for the synthesis and maintenance of the antioxidant defence system, including enzymes and antioxidant molecules (Minnich et al., 1971; Kuzniar et al., 2004). Magnesium supposedly is an antioxidant in biological systems (Hans et al., 2003; Han et al., 2004) and antioxidant/prooxidant balance correlates with the magnesium concentration (Kurys et al., 2001).

Magnesium is often used in the food industry as a food additive or in the pharmaceutical industry as a supplement in e.g. citrate, fumarate, malate, glycinate, glutamate, succinate, tartarate, taurate, aspartate, chloride, citrate, oxide, ascorbate, lactate, sulphate form or as a nutrient. Nevertheless data on the antioxidant/prooxidant properties of them is available only sporadically. Free radical scavenging activity of magnesium lithospermate B, copper-induced oxidation of human low density lipoprotein of magnesium-pyridoxal-5'-phosphate-glutamate and free radical generating properties of some metal complexes were measured in vitro (Kögl

* To whom correspondence should be addressed.
Phone: +36-1438-4141/113; fax: +36-1438-1147; e-mail: szentmihalyi.klara@ttk.mta.hu

et al., 1994; JUN et al., 2000; SZENTMIHÁLYI et al., 2003). Therefore, our purpose was to investigate the antioxidant/prooxidant effect of some magnesium compounds/complexes in vitro to extend our knowledge in this field.

1. Materials and methods

1.1. Materials

The examined magnesium compounds were magnesium oxide, magnesium chloride, magnesium sulphate, magnesium citrate, magnesium gluconate, magnesium malate, magnesium polygalacturonate (magnesium complex of polygalacturonic acid made from pectin) (SZENTMIHÁLYI et al., 1998).

1.2. In vitro antioxidant activities

Chemiluminescence techniques were applied for studying of •OH radical generation and $^{\cdot}OH/H_2O_2$ radical scavenger activity of magnesium compounds. Light emission of luminol was measured by a method of BLÁZOVICS and co-workers (BLÁZOVICS et al., 1999) using a Berthold Lumat LB-9501 luminometer. The intensity of chemiluminescence light is given as the relative light unit (RLU).

The reaction mixture for measuring free radical generating ability was the following: hydrogen peroxide (10^4 dilution) 300 µl, luminol (7×10^{-7}M) 100 µl, the sample (1 mg metal compound in 1 ml solution) 100 µl and bidistilled water 500 µl (BLÁZOVICS et al., 1999).

The reaction mixture for measuring the hydroxyl scavenging activity was hydrogen peroxide (10^4 dilution) 300 µl, microperoxidase (3×10^{-7}M) 300 µl, luminol (7×10^{-7}M) 50 µl and the sample (1 mg ml^{-1}) 100 µl diluted with bidistilled water to 1 ml. The intensity of chemiluminescence light is given as the relative light unit (RLU) reduced by free radical scavenging substances.

For measurement of LDL oxidation**,** LDL fraction was prepared from blood. Reaction mixture: HEPES buffer (pH=7.4), LDL, heme, hydrogen peroxide and sample solution. The determination is based on the measurement of heme absorbance at 405 nm at 37 °C. The decrease of heme absorbance is inversely proportional to the increase of conjugated diene, lipid hydroperoxide and thiobarbituric acid reactive substance content. Results are expressed in the percentage of ΔTs (LDL+sample)/ΔT (LDL) (BALLA et al., 1991).

1.3. Statistical analysis

Means and standard deviations (SD) were calculated from the results. For comparison of the means one way analysis of variance (ANOVA) was used by GraphPAD software version 1.14 (1990).

2. Results and discussion

2.1. Radical generation ability

Earlier examination concerned the determination of possible radical generation ability of magnesium and other metal compounds in the system applied. It was found that the magnesium compounds did not or only in a small degree generate the decomposition of luminol in the luminol-H_2O_2 system (SZENTMIHÁLYI et al., 2003). Therefore, the radical

generation ability of the examined magnesium compounds is negligible in the given amount and under the given circumstances and the system is suitable for the examination of radical scavenging ability.

2.2. Radical scavenging activity

In the presence of microperoxidase as a catalyst, the free radical (H_2O_2/˙OH) scavenging ability of magnesium compounds in 0.1 μg magnesium ml^{-1} concentration was examined. The chemiluminescence intensity of magnesium compounds at the same concentration of magnesium differed significantly (ANOVA, $P<0.05$) from each other (Fig. 1). Some of the magnesium compounds, as Mg-malate and Mg-citrate, seemed to have antioxidant properties in the applied system, since they bound the hydroxyl radicals liberated from H_2O_2 by microperoxidase (the relative light units are under 100%). Magnesium oxide, -gluconate and –polygalacturonate had prooxidant properties in the applied concentration and system, because they generated hydroxyl radicals from H_2O_2. Magnesium chloride with the 100% RLU was not antioxidant nor prooxidant in the applied concentration, while magnesium sulphate was a very weak prooxidant with the 106% RLU.

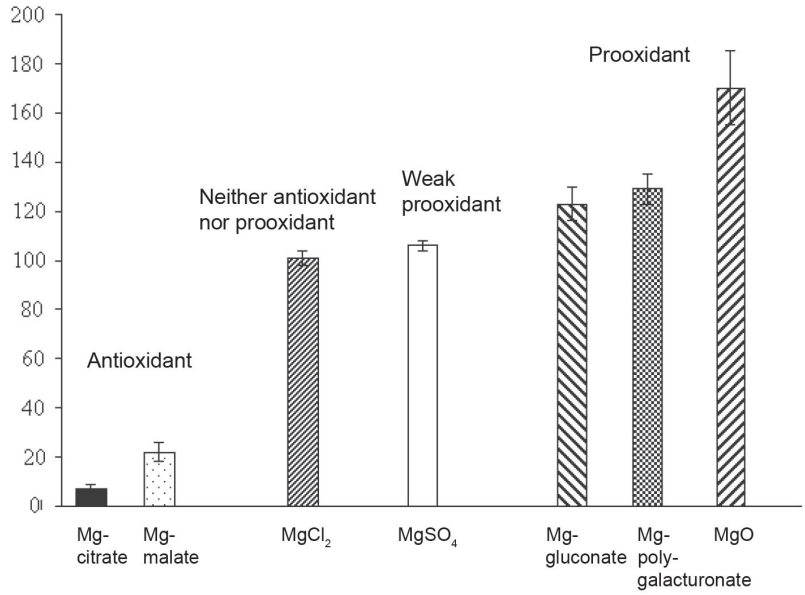

Fig. 1. Chemiluminescence intensity (RLU%) of magnesium compounds in the H_2O_2/˙OH-microperoxidase-luminol system (concentration: 0.1 μg magnesium ml^{-1}); RLU under 100% means antioxidant, while above 100% means prooxidant

Since the antioxidant/prooxidant property depends on the concentration applied, this property of some magnesium compounds was also examined in the function of concentration in H_2O_2/˙OH-microperoxidase-luminol-system. The hydroxyl radical scavenging ability of magnesium compounds changed as the function of concentration (Fig. 2). Magnesium gluconate and magnesium polygalacturonate had no hydroxyl radical scavenging ability and both compounds became prooxidant in higher concentrations, while magnesium malate

showed significant hydroxyl radical scavenging ability in the higher concentration range (Fig. 2). Further examination of magnesium polygalacturonate in larger concentrations was inhibited by the solubility of the complex. The standard deviations of parallel measurements were below 2% in each case.

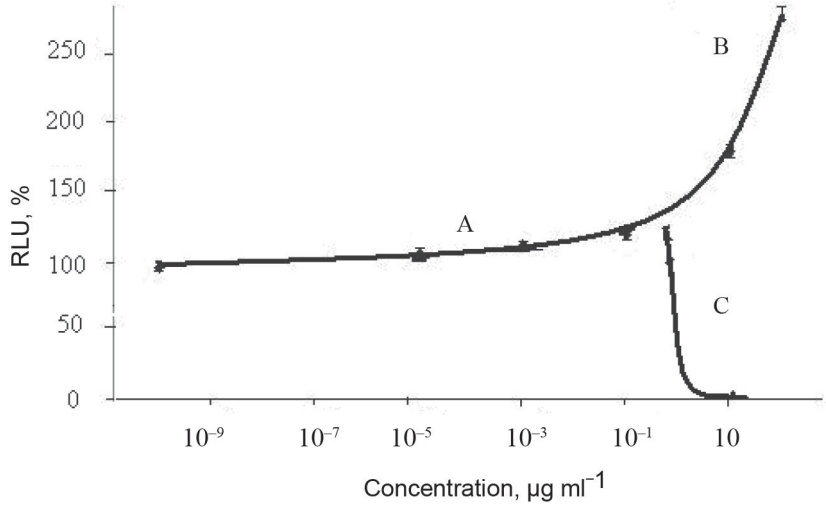

Fig. 2. Concentration dependent hydroxyl radical scavenging ability of magnesium gluconate (A) between 10^{-5} and 10^{-1} µg ml^{-1}, magnesium polygalacturonate (B) and magnesium malate (C) between 10^{-10} and 100 µg ml^{-1} in H_2O_2/·OH-microperoxidase-luminol-system

Since the ligand of magnesium may also have antioxidant/prooxidant properties, for the determination of it and to differentiate the free radical scavenging ability of magnesium and magnesium compounds, some ligands were also measured. Malic acid showed characteristic and significant hydroxyl radical scavenging ability in microperoxidase-luminol-H_2O_2 system as a function of concentration, while polygalacturonic acid may be characterized as a weak antioxidant in the higher concentration range (Fig. 3).

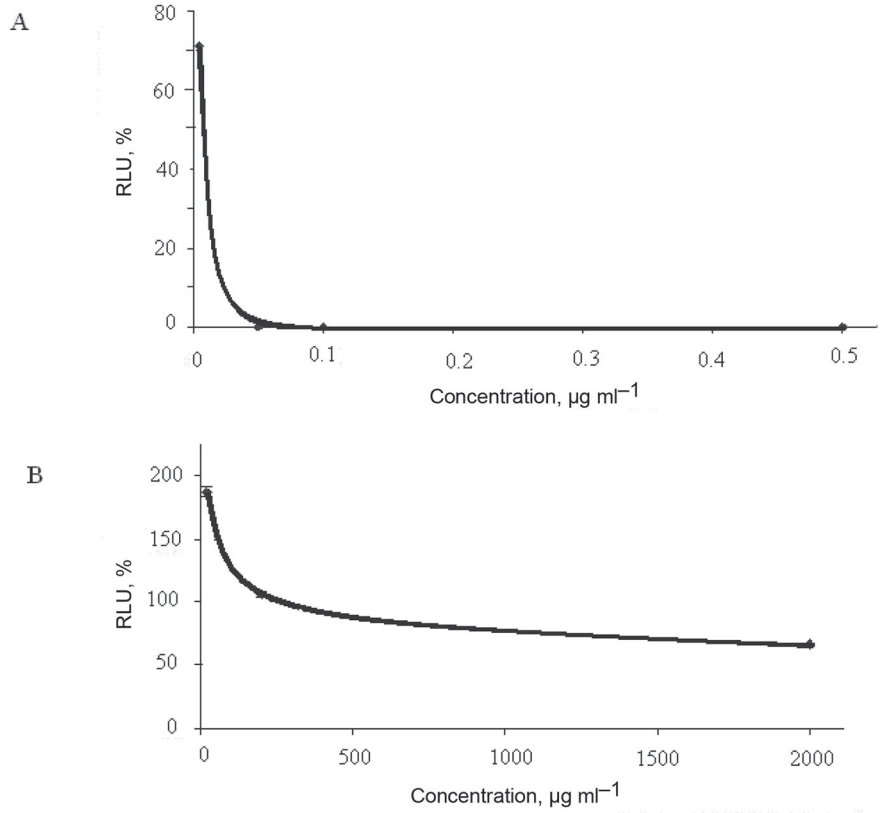

Fig. 3. Concentration dependent hydroxyl radical scavenging ability of malic acid (A) and polygalacturonic acid (B) in H_2O_2/·OH-microperoxidase-luminol-system

2.3. LDL oxidation

The magnesium compounds hardly affect the LDL oxidation time compared to the LDL oxidation time without any magnesium compounds (100%), although the values of magnesium compounds are significantly different (ANOVA, $P<0.05$) (Fig. 4). The LDL oxidation occurred in a shorter time in the presence of magnesium malate (4940±320 sec, ΔT_{Vmax}=6100 s), which points to some prooxidant effects, while the other magnesium compounds slightly extended the LDL oxidation. These insignificant effects hardly changed with the concentration in the cases of magnesium chloride and gluconate (Fig. 5), since the LDL oxidation time showed some alteration as a function of concentration only in the case of magnesium malate or malic acid.

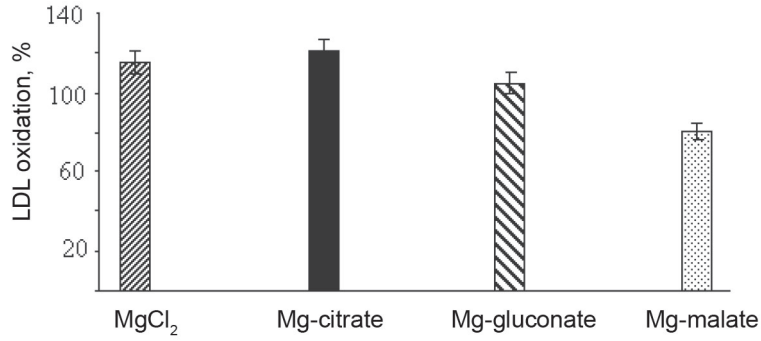

Fig. 4. Inhibition of LDL oxidation (%) of magnesium compounds in 0.6 μg magnesium ml^{-1} concentration

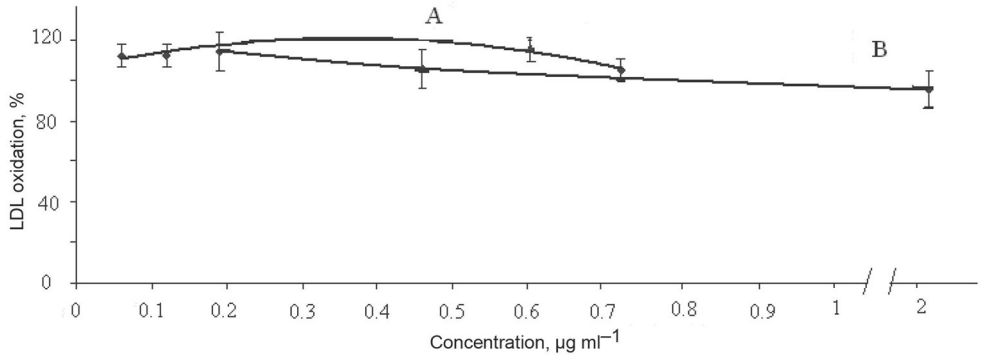

Fig. 5. Inhibition of LDL oxidation (%) of magnesium chloride (A) and magnesium gluconate (B) as the function of magnesium concentration

3. Conclusion

Mg-malate and Mg-citrate showed •OH/H_2O_2 radical scavenging activity in the microperoxidase-luminol-H_2O_2 system with the presence of microperoxidase as a catalyst. In this in vitro system the effect may not be due to magnesium, it may rather be attributed to the antioxidant properties of the ligands: malic and citric acid, since the •OH/H_2O_2 radical scavenging activity of magnesium compounds of the same magnesium concentration differed significantly and the radical scavenger activities of organic acids were found to be higher than those of magnesium compounds. At the same time $MgSO_4$ did not inhibit the basic chemiluminescent reaction. Chemiluminescence measurement, therefore intensity obtained, greatly depends on the pH value since this is a chemical system. Acidic compounds may show better antioxidant activities because these compounds are able to bind the hydroxyl radical liberated from the hydrogen peroxide.

Magnesium compounds hardly affect LDL oxidation, which supports the mechanism described above.

In summary, in vitro antioxidant activities of some magnesium compounds examined may have a direct effect, presumably caused by the chemical reactions of hydroxyl radical, hydrogen peroxide and the ligands.

References

Balla, G., Jacob, H.S., Eaton, J.W., Belcher, J.D. & Vercellotti, G.M. (1991): Hemin: A possible physiologic mediator of low density lipoprotein oxidation and endothelial injury. *Arterioscler. Thromb*., *11*, 1700–1711.

Blázovics, A., Kovács, Á., Lugasi, A., Hagymási, K., Bíró, L. & Fehér, J. (1999): Antioxidant defense in erythrocytes and plasma of patients with active and quiescent Crohn disease and ulcerative colitis: A chemiluminescent study. *Clin. Chem*., *6*, 45.

Fazekas, T., Selmeczi, B. & Stefanovits, P. (1994): *Magnesium in biological systems*. Akadémiai Kiadó, Budapest, pp. 1–300.

Han, B**.**, Yoon, S., Su, J., Han, H.R., Wang, M., Qu, W. & Zhong, D. (2004): Effects of selenium, copper and magnesium on antioxidant enzymes and lipid peroxidation in bovine fluorosis. *Asian-Aust. J. Anim. Sci., 17,* 1695–1699.

Hans, C.P., Chaudhary, D.P. & Bansal, D.D. (2003): Effect of magnesium supplementation on oxidative stress in alloxanic diabetic rats. *Magnes. Res*., *16,* 13–19.

Jun, W.X., Ping, W.Y., Wei, W., Kang, S.W, Ming, X.Y. & Jiang, X.L. (2000): Free radical scavenging and inhibition of lipid peroxidation by magnesium lithospermate B. *Acta Pharmacol. Sinica, 21,* 855–858.

Kögl, C., Schneider, W. & Elstner, E.F. (1994): The influence of magnesium-pyridoxal-5'-phosphate-glutamate in comparison with probucol, α-tocopherol and trolox on copper-induced oxidation of human low density lipoprotein *in vitro*. *Biochem. Pharmacol*., *47,* 2201–2206.

Kurys, E**.,** Kurys, P., Kuźniar, A. & Kieszko, R. **(**2001): Analysis of antioxidant enzyme activity and magnesium level in chronic obstructive pulmonary disease (COPD). *Ann. Univ. Mariae Curie Sklodowska Med.*, *56*, 261–266.

Kuzniar, A., Mitura, P., Kurys, P., Szymonik-Lesiuk, S., Florianczyk, B. & Stryjecka-Zimmer, M. (2004): The influence of hypomagnesemia on erythrocyte antioxidant enzyme defence system in mice. *BioMetals, 16,* 349–357.

Minnich, V., Smith, M.B., Brauner, M.J. & Majerus, P.W. (1971): Glutathione biosynthesis in human erythrocytes I. Identification of the enzymes of glutathione synthesis in hemolysates. *J. Clin. Invest., 50,* 507–513.

Siegel, A. & Siegel, H. (1990): Compendium on magnesium and its role in biology, nutrition and physiology. *Metal Ions Biol. Syst.*, *26*, 1–771.

Szentmihályi, K., Lakatos, B., Sándor, Z., Hajdú, M. & Vinkler, P. (1998): Preparation of magnesium polygalacturonate complexes for magnesium supplementation and their physicochemical characterization. -in: Kiss A.S. (Ed.): *Magnesium. Magnesium and interaction of magnesium with trace elements*. Hungarian Chemical Society, Budapest, pp. 241–244.

Szentmihályi, K., Blázovics, A. & Vinkler, P. (2003): Free radical properties of metal complexes. *Acta Biol. Szegediensis, 47,* 107–109.