

Research Article

Higher Absorption and Lower Urinary Elimination of a New Magnesium Rice Complex Compared to Two Other Organic Forms of Magnesium: A Pilot Study in Rats

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Abstract

Magnesium (Mg) supplementation may be required when certain health problems or conditions such as Crohn's disease or pregnancy cause Mg deficiency for which increasing dietary intake is insufficient.

Mg supplements are available as inorganic or organic forms, which are absorbed differently. Although inorganic salts are particularly rich in elemental Mg, organic forms are generally considered to be more bioavailable. This pilot study compared the pharmacokinetics and bioavailability following acute absorption in rats of two organic Mg forms to a new Mg rice complex made from an inorganic, marine Mg oxide and amino acids from a rice protein hydrolysate.

The Mg rice complex was well absorbed from the gastrointestinal tract providing a relatively stable serum Mg concentration for up to 12 hours. The 24-h AUC for Mg rice complex was similar to the other two preparations. Mg urinary elimination for Mg rice complex was significantly lower than Mg bisglycinate, although did not significantly differ from Mg glycerophosphate or vehicle. No difference in fecal elimination was observed. The results suggest that Mg rice complex is at least as bioavailable as the two other forms tested. Containing almost twice the amount of elemental Mg than the two other forms, exposure to elemental Mg from Mg rice complex would be approximately twice as high as either Mg bisglycinate or Mg glycerophosphate for the same dose of supplement ingested. Consuming a Mg formulation with a higher, bioavailable content of elemental Mg could be particularly useful for preventing and treating Mg deficiency associated with various health problems.

Keywords

Bioavailability; Bisglycinate; Glycerophosphate; Magnesium; Rat; Supplementation

Abbreviations

Mg : Magnesium

Introduction

Magnesium (Mg) is an essential mineral for the body. Numerous studies have shown that the intake is below the recommended dietary amount in a large proportion of the population in many countries including the US, Canada, China and several European countries [1-5]. Usually, Mg is acquired

from dietary sources such as vegetables and cereals and increasing the consumption of these products can improve Mg intake. However, certain health problems or conditions may cause either an excessive Mg loss or limit Mg absorption (e.g., stress, pregnancy and Crohn's disease [6]). In these cases, high Mg intake from ordinary food cannot be accomplished and Mg supplementation may be indicated.

Magnesium supplements are marketed in a large variety of chemical and pharmaceutical forms. In terms of chemical structure, Mg supplements are available as inorganic and organic salts. The terms inorganic and organic relate to the type of compound Mg is bound to, whether naturally or in a laboratory environment. Magnesium bound to a mineral salt are known as inorganic forms (e.g., Mg chloride, oxide or sulphate) whereas chelated Mg that is bound to molecules associated with living organisms, acids (e.g. Mg citrate, lactate, aspartate) or amino acids (e.g., Mg glycinate or taurate), are organic forms. The type of compound Mg is bound to affects the way Mg is absorbed. Organic salts are generally considered to have a better bioavailability than inorganic salts. This is partly because organic salts are more water soluble, so are often recommended for supplementation [6].

Currently, there is very little data available in the literature on the most appropriate form of Mg for supplementation. Also, many absorption studies comparing organic to inorganic salts provide conflicting results. A few published studies performed in animals [7,8] and humans [9-12] report a better bioavailability for organic salts than inorganic salts. Firoz and Graber compared the bioavailability of four commercially available Mg preparations in humans [9], two inorganic (Mg oxide, Mg chloride) and two organic salts (Mg lactate, Mg aspartate). They measured urinary excretion of Mg and concluded that Mg oxide had a relatively low bioavailability compared to the three other forms [9]. However, another study showed inorganic salts such as Mg oxide, Mg sulphate and Mg chloride were all effective in preserving the Mg status of rats compared to Mg gluconate and Mg citrate, two organic salts [13]. The discrepancy between the different study results might be partly explained by differences in the methodology and endpoints used for assessment.

Interestingly, despite the low bioavailability of inorganic salts, their content in elemental Mg is generally higher than that of organic salts. For example, Mg oxide contains 60% in mass of Mg whereas Mg gluconate contains only 5%.

Based on these observations, a new Mg amino acid chelate was developed with a marine Mg oxide (inorganic salt) combined with amino acids from a rice protein hydrolysate. This Mg rice complex has a high elemental Mg content, containing 33% in mass of Mg (according to supplier unpublished data). This study was designed to determine whether the pharmacokinetics of acute absorption and the bioavailability of the new Mg rice complex were comparable

to two forms commonly used in supplements, Mg bisglycinate, another amino acid chelate, and Mg glycerophosphate, an organic salt.

Methods and Materials

Rats

Twenty male Wistar rats weighing 265 ± 5 g were acquired from Janvier Labs (Le Genest-Saint-Isle, France) and delivered to the laboratory at least 5 days before the experiments to allow time to acclimatize to laboratory conditions. The animal house was maintained under artificial lighting between 7:00 and 19:00 (12 hours) in a controlled ambient temperature of $21 \pm 3^\circ\text{C}$ and relative humidity between 20-80%. Rats had free access to food (code 113 - SAFE, 89290 Augy, France) and water during the 5-day acclimatization period and only to water on the day of experiment. Rats were sacrificed by exposure to CO_2 after last blood withdrawal. The study was conducted in compliance with Animal Health regulations, in particular the Council Directive No. 2010/63/UE, September 22nd 2010 on the protection of animals used for scientific purposes and the French decree No. 2013-118, February 1st 2013 on animal protection; and in accordance with the recommendations of the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) of which the accreditation was granted in June 2012. Ethics committee approval was obtained before the study started.

Magnesium supplementation

Post-absorptive rats were randomized into four groups (5 rats per group) and received one of the following treatments: Mg rice complex (Hypro-ri[®] mag; 3i Nature, Saint Bonnet de Rochefort, France; batch no. 14F221T1; 33% of elemental Mg), Mg bisglycinate chelated buffered (Albion, Clearfield, USA; batch no. 13315201; 18% of elemental Mg), Mg glycerophosphate (Seppic, Puteaux, France; batch no. 3186988; 12.5% of elemental Mg) or the vehicle (Dimethyl Sulphoxide; DMSO). Mg powders were dissolved in pure DMSO (5% of the final volume). Each rat was administered a single oral dose of 5 mL/kg body weight of their allotted Mg treatment by gavage feeding, which contained 33 mg/kg body weight of elemental Mg (i.e., all rats received the same dose of elemental Mg). This amount corresponds to the Nutrient Reference Value (NRV) for humans (375 mg) adjusted for rats [14].

Blood, urine and feces collection

On the testing day, the rats were weighed and a pre-dose blood sample was collected via sublingual puncture. After the treatment was administered, each rat was immediately placed in a separate metabolic cage. Blood samples were collected at nine time points: 0.5, 1, 1.5, 2, 3, 4, 8, 12 and 24 hours post-dosing. At each time point, approximately 400 μL of blood were collected from the sublingual vein. The blood samples were immediately transferred into pre-serum gel tubes containing clot activator. After sealing each tube, the blood

samples were manually agitated and stored at room temperature for at least 30 minutes to allow the blood to clot. The samples were then centrifuged at room temperature, at 1500 g, for 10 minutes. The entire resultant serum obtained from each tube was immediately transferred into two, suitably labelled polypropylene tubes (100 µL in the first aliquot and the remaining volume in the second aliquot). The tubes were stored upright at $-20 \pm 5^\circ\text{C}$ and protected from light until analysis.

Urine samples were collected at 4, 8, 12 and 24 hours post-dosing and feces samples at 12 and 24 hours post-dosing. At each time point, the urine volume was measured (mL) and feces were weighed (g) and both were transferred into pre-labelled polypropylene tubes and stored upright at $-20 \pm 5^\circ\text{C}$ until analysis.

Magnesium determination

Magnesium concentrations were assayed by a colorimetric method using a Konelab analyzer in the serum and urine samples and by an ICP/MS method in the feces samples.

Statistical analysis

Serum Mg concentrations measured in mmol/L over the 24-hour period are expressed as means followed by the Standard Error of the Mean (SEM). The Area Under the Curve (AUC) was calculated according to the linear trapezoidal rule. The Mg concentration values in urine and feces (µmol) observed at each time point was added to obtain the cumulated Mg concentration in urine and feces at 24 hours. Statistical analyses were based on non-parametric tests, Mann-Whitney U-test and Kruskal-Wallis test. The results were subjected to repeated measures ANOVA. Sphericity assumption was verified with Mauchly's test. Depending on the outcome of this test, the main effects of Group (type of Mg formulation) and Time, as well as the effect of interaction Group*Time, were analyzed with univariate or multivariate F-tests. Whenever an effect was identified as significant, post-hoc analysis with the least significant difference test was conducted. Statistical characteristics of the results were presented as arithmetic means and their 95% confidence intervals (95% CIs). Calculations were carried out with Statistical 10 package (Stat Soft, Tulsa, OK, USA) with the threshold of statistical significance set at $p \leq 0.05$.

Results

Magnesium concentration in serum

The serum concentration of Mg rose to a level significantly higher than baseline 1.5 h after administration of the Mg rice complex (Figure 1). It remained elevated until 12 h except for a decrease at 4 h. This resulted in two apparent peaks in the serum concentration of Mg: a first peak at 2 h at a concentration of 0.99 ± 0.02 mmol/L and a second peak observed at 12 h with a higher concentration ($C_{\text{max}} = 1.03 \pm 0.02$ mmol/L; Figure 1). Magnesium concentration then decreased progressively from 12 h until returning to basal value at 24 h.

This bimodal profile was also observed with Mg bisglycinate with the first peak at 3 hours (0.97 ± 0.02 mmol/L) and a second higher one at 12 h ($C_{\text{max}} = 0.99 \pm 0.03$ mmol/L). Contrary to the results observed with Mg rice complex and Mg bisglycinate, the pharmacokinetics of serum Mg concentration following Mg glycerophosphate administration was monophasic, increasing progressively until reaching C_{max} at 12 h (1.02 ± 0.01 mmol/L) and returning to basal value at 24 h. At 2 h, the Mg rice complex concentration was significantly higher than that observed for Mg glycerophosphate and vehicle ($p < 0.05$). At 12 h, C_{max} obtained for Mg rice complex and Mg glycerophosphate were significantly higher than the serum Mg concentrations measured in the vehicle group ($p < 0.05$).

Mg rice complex did not differ significantly from the remaining forms and vehicle in terms of Mg serum dynamics (Group*Time: $F = 1.316$, $p = 0.250$).

The 24-h AUCs for total serum Mg following Mg rice complex (23.6 ± 0.46 mmol/L/h) and Mg bisglycinate (22.9 ± 0.45 mmol/L/h) administration were significantly greater than after the administration of vehicle (21.6 ± 0.46 mmol/L/h, $p = 0.005$ and $p = 0.044$, respectively); the difference in AUCs for Mg glycerophosphate (23.26 ± 0.27 mmol/L/h) and vehicle (21.6 ± 0.46 mmol/L/h) was at a threshold of statistical significance ($p = 0.059$). The AUC for Mg rice complex was not significantly greater than the AUC for Mg bisglycinate or Mg glycerophosphate ($p = 0.298$ and $p = 0.234$, respectively; Figure 1).

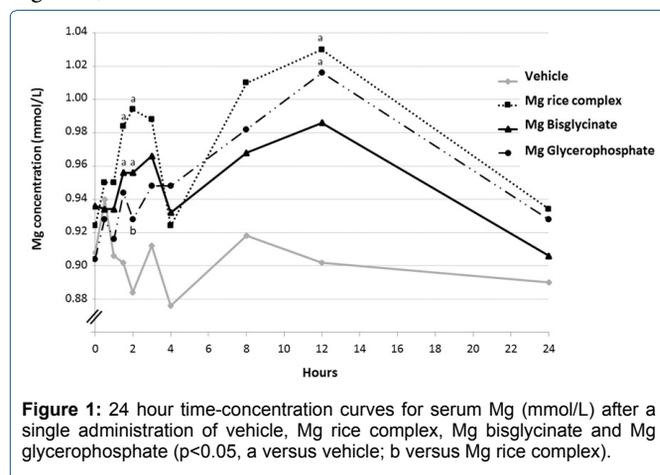


Figure 1: 24 hour time-concentration curves for serum Mg (mmol/L) after a single administration of vehicle, Mg rice complex, Mg bisglycinate and Mg glycerophosphate ($p < 0.05$, a versus vehicle; b versus Mg rice complex).

Magnesium concentration in urine and feces

The different forms had different Mg urinary elimination rates (Group*Time: $F = 4.248$, $p = 0.002$; Table 1). The Mg urinary elimination rate for the Mg rice complex was significantly lower than Mg bisglycinate, especially between 4 h and 8 h after administration. Post-hoc analysis showed that cumulated amount of Mg in urine after a single administration of Mg rice complex was lower than after administrating Mg bisglycinate ($p = 0.005$), but did not differ significantly compared to Mg glycerophosphate and vehicle ($p = 0.511$ and $p = 0.794$,

Time point	4 hour	8 hour	12 hour	24 hour	Cumulated
Urine (µmol)					
Mg rice complex	11.22 ± 4.76	42.84 ± 22.03	22.75 ± 6.22	44.11 ± 6.88	120.93 ± 35.36
Mg bisglycinate	26.74 ± 8.33	53.79 ± 26.96	34.61 ± 3.80	70.71 ± 4.59	185.86 ± 38.64
Mg glycerophosphate	10.22 ± 2.99	19.87 ± 6.28	31.48 ± 3.29	56.10 ± 4.66	117.67 ± 13.81
Vehicle	16.50 ± 1.88	20.77 ± 8.28	21.68 ± 2.28	46.24 ± 3.60	105.19 ± 9.61
Feces (µmol)					
Mg rice complex	-	-	303.02 ± 33.67	115.48 ± 23.79	418.50 ± 49.00
Mg bisglycinate	-	-	413.49 ± 67.97	66.62 ± 4.89	480.11 ± 71.72
Mg glycerophosphate	-	-	340.95 ± 72.90	72.18 ± 21.38	413.13 ± 79.41
Vehicle	-	-	262.18 ± 27.56	40.67 ± 8.34	302.85 ± 21.42

Table 1: Amounts of Mg (µmol) in urine and feces samples after a single administration of Mg rice complex, Mg bisglycinate, Mg glycerophosphate or vehicle.

respectively). The cumulated amount of Mg in urine after Mg bisglycinate administration was also significantly higher than for Mg glycerophosphate and vehicle (p=0.001 and p=0.003, respectively; Table 1).

The type of form affected the Mg fecal elimination rate (Group*Time: F=4.265, p=0.022). However, post-hoc analysis showed that the only statistically significant difference pertained to cumulated amount of Mg in feces after administration of the vehicle, being significantly lower than after the administration of Mg rice complex (p=0.001), Mg bisglycinate (p=0.001) and Mg glycerophosphate (p=0.005). Mg rice complex did not differ significantly from Mg bisglycinate (p=0.895) and Mg glycerophosphate (p=0.378) in terms of the cumulative fecal concentration of Mg (Table 1).

Discussion

The findings of this pilot study suggest that the pharmacokinetic profile of the Mg rice complex may differ from that of Mg bisglycinate and Mg glycerophosphate. Firstly, although there was no significant difference in 24-hour serum Mg levels between the three Mg forms, 24-hour serum concentrations of Mg after a single dose of Mg rice complex was significantly higher than after the administration of vehicle whereas the 24-hour serum Mg concentration after a single dose of Mg glycerophosphate did not differ from the vehicle. This suggests that Mg rice complex may have a higher gastrointestinal uptake. Secondly, Mg urinary elimination rate for the Mg rice complex was significantly lower than Mg bisglycinate. This observation suggests that a higher proportion of Mg remains in the tissues with Mg rice complex. Altogether, the Mg rice complex is well absorbed from the gastrointestinal tract following oral administration with a lower urinary excretion. This provides a relatively stable serum Mg concentration for up to 12 hours. These results point to Mg rice complex as a promising alternative for Mg supplementation.

One potential limitation of this study is small sample size. The study was underpowered, so perhaps many relationships at a threshold of statistical significance would become significant in a larger data set. Therefore, this study should be considered

as a preliminary research. Furthermore, the reason behind the evident drop off in serum Mg at 4 h is unclear. This was unlikely related to the pharmacokinetics of the forms analyzed since the same phenomenon, albeit less obvious, was also observed in the vehicle group.

These preliminary results suggest that Mg rice complex is at least as bioavailable as the two other forms tested. Also, the Mg rice complex contains almost twice the amount of elemental Mg than the other forms. Therefore, we can expect that for the same dose of supplement ingested, the exposure to elemental Mg from Mg rice complex would be approximately twice as high as either Mg bisglycinate or Mg glycerophosphate. Consuming a Mg form with a higher, bioavailable content of elemental Mg could be particularly useful for preventing and treating Mg deficiency associated with various health problems. Magnesium is involved in many biochemical reactions that are crucial to a wide range of fundamental biological processes such as energy metabolism, nerve transmission, protein synthesis and skeletal structure [15]. Low dietary intake of Mg and low serum Mg levels have been associated with major public health problems such as type 2 diabetes [16], pre-eclampsia and eclampsia [17], cardiac arrhythmia [18] or migraine [19]. Magnesium supplementation is indicated for the prophylactic or curative treatment of these pathologies. Supplementation studies in Mg deficient subjects would confirm the interest of Mg rice complex.

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