Use of the Stable Isotopes Technique to Evaluate the Bioavailability of a Pharmaceutical Form of Magnesium in Man

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Received May 20, 1997; accepted October 31, 1997

KEY WORDS: magnesium; absolute bioavailability; stable isotopes; pharmacokinetics; ICP-MS.

INTRODUCTION

Magnesium deficiency may result in clinical disorders (neuromuscular, cardiovascular, osseous or renal). A therapeutic supply is then necessary which is provided by the administration of pharmaceutical forms whose bioavailability must be calculated. Conventional studies of bioavailability, with monitoring of plasma or urinary magnesium, are not possible because of the high endogenous plasma levels (20 mg/L) subject to rapid homeostasis after administration of exogenous magnesium. Absorption of magnesium salts has been studied by indirect methods of dietary balance (1,2), or by the increase in urinary excretion (3–5). Using isotopic methods, it is possible to differentiate exogenous and endogenous magnesium, thereby permitting estimation of absolute bioavailability of exogenous magnesium. The use of stable isotopes as labels is becoming an attractive tool for the study of magnesium behavior in humans (6–8). We have therefore used two stable isotopes of magnesium, ^{25}Mg and ^{26}Mg, to measure the absolute bioavailability of a pharmaceutical chewable form of magnesium.

MATERIALS AND METHODS

Materials

The pharmaceutical form was a chewable tablet of magnesium lactate and citrate (110/10, w/w), containing 120 mg of Mg^{2+}, named MgOR, enriched with ^{26}Mg (^{24}Mg: 13.2%, ^{25}Mg: 3.7% and ^{26}Mg: 83.2%). The reference form was a solution of magnesium lactate, named MgIV, enriched with ^{25}Mg (^{24}Mg: 8.7%, ^{25}Mg: 89.8% and ^{26}Mg: 1.5%). Chewable OROMAG® tablets and injectable preparations were prepared by Laboratoires THERAMEX with Mg lactate and citrate obtained from ^{25}MgO (94% purity) and ^{26}MgO (95% purity) provided by ISOTOPCHIM (Ganagobie-Peyrius, France). Analytical grade reagents were: concentrated nitric acid (Suprapur, Merck), 30% hydrogen peroxide (Medical extra pure, Merck) and de-ionized water (Milli-Q, Millipore). Magnesium standard solutions were prepared in 2% v/v HNO_3 using 1000 mg.l^{-1} solution (Specpure, Johnson Mattey). All other reagents and chemicals were of analytical grade.

Clinical Trial

The clinical trial protocol was approved by an Ethics Committee and the study was conducted in accordance with the Declaration of Helsinki and French regulations governing biomedical research. Six healthy male volunteers (age 27.8 ± 3.1 years, weight 69.5 ± 7.8 kg) gave their informed written consent. The health of volunteers was confirmed by a clinical examination on eating habits, electrocardiography and laboratory tests. Volunteers received simultaneously in a single dosing 1) by the oral route: 3 chewable tablets each containing 120 mg (i.e. total 360 mg) of Mg^{2+} enriched with ^{26}Mg, 2) by the intravenous route: 10 ml of the magnesium lactate solution containing 50 mg of Mg^{2+} enriched with ^{25}Mg. Blood was collected (20 ml) prior to administration, then at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 48, 72, 96 and 120 hours. Plasma was collected after centrifugation. Urine was collected prior to administration and then during the intervals 0–2, 2–4, 4–6, 6–8, 8–12, 12–24, 24–48, 48–72, 72–96 and 96–120 hours. Feces were collected prior to administration and for 5 days after administration, and were pooled by day of collection. Samples were stored at −20°C until analyzed.

Total Magnesium Determination

Total magnesium was assayed in plasma and urine samples using the Mg-Kit from BioMérieux, Lyon, France (limit of detection, less than 1 μg/mL). Total magnesium in feces was assayed by atomic absorption spectrophotometry after mineralization. The CV was less than 5% for assay reproducibility and the limit of detection less than 0.1 μg/mL.

Isotope Ratio Measurement

All determinations were made on a Fisons Instrument model VG PlasmaQuad PQ3 inductively coupled plasma mass spectrometer (Winsford, Cheshire, UK) equipped with a Gilson 220 autosampler.

ICP/MS operating conditions were as follows: Rf power, 1350 kW; Reflected power, <5 W; Aerosol carrier gas flow, 0.86 1 min^{-1}; Intermediate gas flow 1.0 1 min^{-1}; Outer gas flow, 14.0 1 min^{-1}; Sample uptake flow, 1.0 ml min^{-1}; Washing time, 120 s; Dwell time, 20 ms; Acquisition time, 20 s; Number of replicates, 12; Point across peak, 1; Acquisition mode, Peak hop.

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ABBREVIATIONS: ICP-MS, inductively coupled plasma—mass spectrometry; MgOR, exogenous magnesium given orally (mainly ^{26}Mg); MgIV, exogenous magnesium given by the i.v. route (mainly ^{25}Mg).

0724-8741/98/0200-0347$15.00/0

Plasma (2ml), fecal (500mg) and urinary (5ml) samples were mineralized to dryness in a Teflon beaker with 2 ml of 2% v/v HNO_3 and 0.5 ml of H_2O_2 on a hot plate. This operation was repeated several times to yield a clear solution of the residue in 10% v/v HNO_3 in deionized water. The final volume was 10 ml. Blank solutions were prepared under the same conditions. These solutions were diluted ×10 (plasma and urine) or ×2000 (feces) with 2% v/v HNO_3 for analysis by ICP/MS.

Sample isotope ratios, for net intensities, $^{25}Mg/^{24}Mg$ and $^{26}Mg/^{24}Mg$ were corrected with the bias factor calculated for the standard solution according to the theoretical values of natural magnesium isotope ratios. Relative percentages of $^{24}Mg/^{25}Mg/^{26}Mg$ were then calculated with these ratios.

Calculation of Magnesium Originating from Oral and I.V. Dosing

Since the 3 isotopes of magnesium were present in the 3 forms of magnesium (i.e.: MgNAT, MgOR and MgIV), the percentages of magnesium originating from MgNAT, MgOR and MgIV were calculated from the relative percentage of $^{24}Mg/^{25}Mg/^{26}Mg$ in each sample according to the three following equations:

$$\begin{array}{ll} & \text{MgNAT.:}\quad \text{MgIIV:}\quad \text{MgOR:} \\ ^{24}\text{Mg:} & a.X_1 + 0.087.X_2 + 0.132X_3 = d.\text{MgTotal} \\ ^{25}\text{Mg:} & b.X_1 + 0.898.X_2 + 0.37X_3 = e.\text{MgTotal} \\ ^{26}\text{Mg:} & a.X_1 + 0.015.X_2 + 0.832X_3 = f.\text{MgTotal} \end{array}$$

where: 1) 0.087, 0.898 and 0.015 are the respective relative proportions of ^{24}Mg, ^{25}Mg and ^{26}Mg in the intravenous MgIV form, 2) 0.132, 0.037 and 0.832 are the respective relative proportions in the oral MgOR form, 3) a, b and c are the respective relative proportions in the samples collected prior to administration (for example, 0.792, 0.102 and 0.106) and corresponding to natural relative isotopic proportions measured in this experiment and in this biological medium, 4) d, e and f are the respective relative proportions measured in the sample (for example, 0.750, 0.122, 0.128), 5) MgTotal is the total amount of magnesium (for example, 20 mg/L in plasma), 6) X_1, X_2 and X_3 are the relative percentages of MgNAT, MgIV and MgOR to be determined. These last values were calculated by solving the system of 3 equations with 3 unknowns.

The mean accuracy of the isotopic percentages calculated in the samples taken before dosing was 1) 100.5% for ^{25}Mg and 99.9% for ^{26}Mg in plasma, 2) 100.7% for ^{25}Mg and 100.6% for ^{26}Mg in urine, and 3) 99.8% for ^{25}Mg and 102.7% for ^{26}Mg in feces. The minimum relative percentage of ^{25}Mg and ^{26}Mg distinguishable from the natural percentage was 0.1%, which corresponded to a relative percentage of 1% for both MgIV and MgOR. In terms of concentration, the limit of quantification (LOQ) also depended on the total magnesium content of the sample. In plasma (magnesium levels are always around 20 mg/L), the LOQ was 0.02 mg/L, for both ^{25}Mg and ^{26}Mg. The mean CV % for reproducibility was 5% for ^{25}Mg and 6% for ^{26}Mg in plasma, 2% for both ^{25}Mg and ^{26}Mg in urine, and 0.3% for ^{26}Mg in feces. Quality controls were included in each analytical series.

Bioavailability Calculation

Plasma and urinary concentrations (in mg/L) of MgIV and MgOR were calculated by multiplying the total magnesium concentration by the relative percentages of MgOR/MgIV/MgTotal previously determined. Urinary and fecal amounts eliminated were obtained by multiplying concentrations by sample volume or weight.

The pharmacokinetic parameters C_{max} (highest concentration) and t_{max} (corresponding time) were obtained from experimental plasma data. The area under the curve of the plasma concentrations as a function of time from 0 to 120 h (AUC_{0-120h}) was determined by the trapezoidal calculation (linlinear interpolation). Individual plasma data were fitted using a two-compartment model, with Pharm NCA software (Simed, Créteil, France). This allows determination of the half-life of distribution ($t_{1/2a}$) and elimination ($t_{1/2b}$), the total clearance (Cl_t) and the AUC from 0 to infinity ($AUC_{0-\infty}$). The absolute bioavailability (%) of the oral form was given by the following equation:

$$\frac{(AUC_{0-120h}MgOR) + 50mg}{(AUC_{0-120h}MgIV) + 360mg} \times 100$$

For urinary data, the absolute bioavailability (%) of the oral form was given by the following equation:

$$\frac{(Ae_{0-120h}MgOR) + 50}{(Ae_{0-120h}MgIV) + 360} \times 100$$

where $Ae_{0-120h}MgOR$ and Ae_{0-120h} MgIV were, respectively, the cumulated amounts of MgOR and MgIV eliminated in urine over 120 h.

For fecal elimination, absorption of the oral form was given by the following equation:

$$(1\text{-}[QF_{0-120h}MgOR/360]) \times 100$$

where $QF_{0-120h}MgOR$ is the cumulated amount of MgOR eliminated in the feces over 120 h.

RESULTS

Plasma Analysis

Mean values of plasma levels of MgIV and MgOR are presented in Fig. 1. Individual and mean pharmacokinetic parameters are summarized in Table I after i.v. and oral dosing. The plasma levels of MgIV decreased with time in a bi-exponential manner ($t_{1/2\alpha}$: 1.9 ± 0.3 h and $t_{1/2b}$: 59 ± 11 h).

After dosing of the oral form, C_{max} (1.10 ± 0.74 mg/L) was reached in 0.5 to 6 h (mean: 2.8 ± 2.2 h). In 4 of the 6 subjects, two plasma peaks were seen (see the mean in Fig. 1). The absolute bioavailability was calculated from AUC_{0-120h} and extrapolation from 120 hours to infinity was discarded, as previously suggested for long half-live compounds (9), due to the

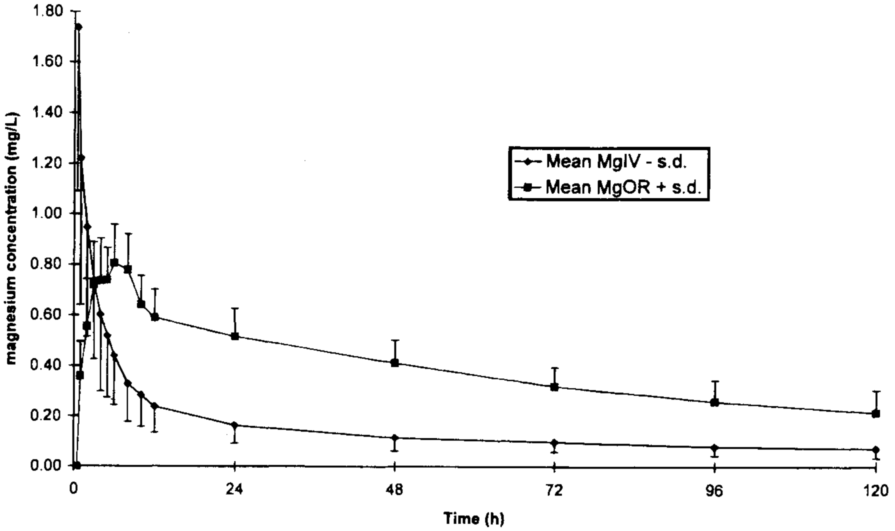

Fig. 1. Plasma magnesium concentration time-course of MgIV (89.8% ^{25}Mg) and MgOR (83.2% ^{26}Mg) after simultaneous administrations of ^{25}Mg-enriched Mg lactate (50 mg, i.v.) and ^{26}Mg-enriched Mg lactate and citrate(360 mg, orally).

imprecision of the extrapolation. Indeed, the last plasma concentrations were near the limit of quantification and, despite the five-day duration of the sampling schedule, AUC extrapolated values in all subjects were higher than 20%. We report as complementary data the $AUC_{0\text{-inf}}$ values computed using the fitted model (Table I). Bioavailability calculated from AUC_{0-120h}, ranged between 13.7 and 62.8%, with a mean of 34.5 ± 18.8% (n = 6) (mean ± SD) and, calculated using the model ranged between 18.5 and 69.0% with a mean of 43.3 ± 22.8% (n = 5). Absorption and absolute bioavailability are summarized in Table II.

Urinary excretion

The mean cumulated amounts of MgIV and MgOR eliminated in urine are presented in Fig. 2. The percentage of the i.v. dose excreted in urine, over 5 days, ranged between 2.94 and 14.0%, with a mean of 7.40 ± 4.10% (mean ± SD), and that of the oral dose ranged from 1.76 and 2.84%, with a mean of 2.22 ± 0.43%. The absolute bioavailability was calculated as between 13.4% and 72.9% with a mean of 39.8 ± 24.3% (mean ± SD).

Fecal Data

MgIV was not found in the feces. The elimination of MgOR was greatest between the 12th and 48th hours. Fecal excretion of MgOR was complete after 72 h, except in one subject (subject 2) who was constipated and excluded from the calculation of the mean. In subject 1, absorption was negligible, although some MgOR was found in plasma and urine. Intestinal absorption was likely lower in this subject. The absorption for

Table I. Pharmacokinetic Parameters of Magnesium Given Intravenously and Orally After Simultaneous Administrations of ^{25}Mg-enriched Mg Lactate (50mg, i.v.) and ^{26}Mg-enriched Mg Lactate and Citrate (360 mg, orally)

	Parameters obtained after i.v. dosing of 50 mg of Mg^{2+}								Parameters obtained after oral dosing of 360 mg of Mg^{2+}					
Subject No.	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	Cl_t (l/h)	Vd (l)	MRT (h)	AUC_{0-120h} (h.mg/l)	$AUC_{0-inf.}$[a] (h.mg/l)	Ae_{120h} (% dose)	C_{max} (mg/l)	t_{max} (h)	ka (h^{-1})	AUC_{0-120h} (h.mg/l)	AUC_{0-inf}[a] (h.mg/l)	Ae_{0-120h} (% dose)
1	2.3	80	1.11	128	103	29.9	43.0	14.00	2.53	0.5	0.43	29.6	57.4	1.88
2	2.0	55	1.37	110	71	28.4	36.2	9.54	0.68	6.0	0.43	48.8	1509[b]	2.61
3	1.6	49	4.06	288	62	10.2	12.6	2.94	0.89	1.0	0.04	38.0	62.8	1.98
4	1.8	51	4.01	297	63	10.2	12.6	3.90	1.04	2.0	1.40	46.3	59.9	2.84
5	1.7	60	1.82	158	81	20.5	27.6	5.72	1.01	5.0	2.50	44.1	67.9	1.76
6	1.7	57	2.17	177	78	17.3	22.8	8.32	0.43	2.0	0.43	30.6	47.5	2.24
Mean	1.9	59	2.42	193	76	19.4	25.8	7.40	1.10	2.8	0.87	39.6	59.1	2.22
S.D.	0.3	11	1.30	81	15	8.6	12.4	4.10	0.74	2.2	0.92	8.2	7.6	0.43
S.D.(%)	14	19	54	42	20	44	48	55	67	81	105	21	13	19

[a] Parameters computerd using a 2 compartments model
[b] Excluded from the calculation of the mean

Table II. Bioavailability of the Chewable Form of Magnesium (%) Calculated From Plasma and Urine Data and Absorption Calculated From Fecal Data, After Simultaneous Administrations of ^{25}Mg-enriched Mg Lactate (50 mg, i.v.) and ^{26}Mg-enriched Mg Lactate and Citrate(360 mg, orally)

Subjects N°	AUC_{0-120h}	Ae_{0-120h}	AUC_{2cpt}	Faeces
1	13.7	13.4	18.5	0
2	23.9	27.4	ND	ND
3	51.8	67.3	69.0	85.6
4	62.8	72.9	66.1	20.6
5	29.9	30.7	34.2	14.7
6	24.6	26.9	28.9	6.9
Mean	34.5	39.8	43.3[a]	25.6
S.D.	18.8	24.3	22.8	34.5
S.D(%)	55	61	53	135

ND: not determined
AUC_{0-120h}: non compartmental determination of AUC from 0 to 120 hr.
Ae_{0-120h}: non compartmental urinary excretion from 0 to 120 hr.
AUC_{2cpt}: compartment analysis of plasma concentrations
Faeces: fecal excretion from 0 to 120 hr.
[a] mean calculated with n = 5.

the 3 other subjects (4, 5, and 6) was between 6.9 and 20.6% of the oral dose.

DISCUSSION

The aim of this study was to show the interest of using a simultaneous administration of two isotopes of magnesium to determine the absolute bioavailability of an oral chewable pharmaceutical form of magnesium (360 mg of Mg^{2+}). The results obtained using ICP-MS complemented and refined a previous study describing another less precise mass spectrometry method using electronic impact (10).

The use of stable ^{25}Mg and ^{26}Mg isotopes offered considerable advantages over the radioactive ^{28}Mg isotope and was also preferable in regulatory, ethical and observational (radioactive half-life around 20 h) terms: 1) ^{26}Mg is incorporated in the oral pharmaceutical form and consequently allows accurate monitoring of magnesium under the same conditions as the pharmacokinetics of an exogenous active principle; 2) The simultaneous administration of oral and intravenous forms enriched with two different isotopes circumvented intrasubject variability and reduced variations in pharmacokinetic parameters due to analytical measurements or the sampling schedule.

The pharmacokinetic results of this study indicate negligible intestinal secretion, low urinary elimination (less than 14% of the dose, 120 h after i.v. dosing) and multi-compartment plasma kinetics. These findings are consistent with previously published results on magnesium behavior (11–13).

Estimations of the F value showed it to be heterogeneous between subjects but equivalent between plasma (AUC_{0-120h}) and urinary (Ae_{0-120h}) data for each subject. Bioavailability was low (13.7% AUC_{0-120h} and 13.4% Ae_{0-120h}) for one subject (S1), around 25% (AUC_{0-120h}) and 28% (Ae_{0-120h}) for three subjects (S2, S5 and S6), and higher than 50% (AUC_{0-120h}) and 60% (Ae_{0-120h}) for two subjects (S3 and S4). Plausible explanations for this between-subject variability, which had been previously reviewed (14), are as follows: i) magnesium absorption or excretion mechanisms are complex functions of the magnesium pools, which are difficult to evaluate, ii) intestinal transit varied from one subject to another (one subject had slight diarrhea (S1) and another was constipated (S2) during the trial).

CONCLUSIONS

This double isotopes procedure has been successfully applied to the evaluation of the absolute bioavailability of a chewable pharmaceutical form of magnesium using either plasma or urinary data. Fecal data seemed less reliable for such a purpose. As it is known that magnesium absorption is related

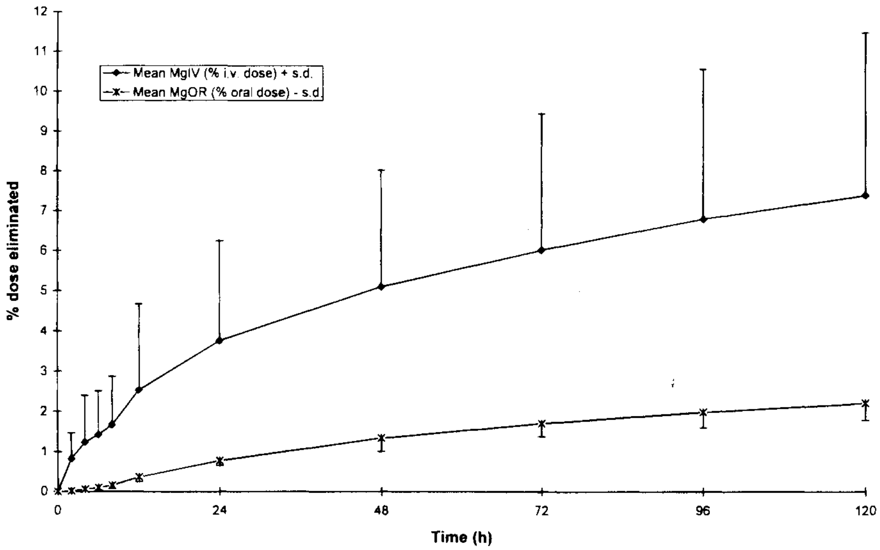

Fig. 2. Cumulated urinary magnesium MgIV (mainly ^{25}Mg) and MgOR (mainly ^{26}Mg) after simultaneous administrations of ^{25}Mg-enriched Mg lactate (50 mg, i.v.) and ^{26}Mg-enriched Mg lactate and citrate (360 mg, orally).

to the dose, or to the magnesium pools, the supply of therapeutic magnesium during long-term treatment could also be evaluated at the steady-state or at other magnesium oral doses.

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