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ORIGINAL ARTICLE

Omeprazole decreases magnesium transport across Caco-2 monolayers

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Abstract

AIM: To elucidate the effect and underlying mechanisms of omeprazole action on Mg^{2+} transport across the intestinal epithelium.

METHODS: Caco-2 monolayers were cultured in various dose omeprazole-containing media for 14 or 21 d before being inserted into a modified Ussing chamber apparatus to investigate the bi-directional Mg^{2+} transport and electrical parameters. Paracellular permeability of the monolayer was also observed by the dilution potential technique and a cation permeability study. An Arrhenius plot was performed to elucidate the activation energy of passive Mg^{2+} transport across the Caco-2 monolayers.

RESULTS: Both apical to basolateral and basolateral to apical passive Mg^{2+} fluxes of omeprazole-treated epithelium were decreased in a dose- and time-dependent manner. Omeprazole also decreased the paracellular cation selectivity and changed the paracellular selective permeability profile of Caco-2 epithelium to Li^+, Na^+, K^+, Rb^+, and Cs^+ from series Ⅶ to series Ⅵ of the Eisenman sequence. The Arrhenius plot revealed the higher activation energy for passive Mg^{2+} transport in omeprazole-treated epithelium than that of control epithelium, indicating that omeprazole affected the paracellular channel of Caco-2 epithelium in such a way that Mg^{2+} movement was impeded.

CONCLUSION: Omeprazole decreased paracellular cation permeability and increased the activation energy for passive Mg^{2+} transport of Caco-2 monolayers that led to the suppression of passive Mg^{2+} absorption.

Key words: Magnesium; Paracellular; Proton pump inhibitor; Transepithelial; Tight junction

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INTRODUCTION

Magnesium plays an important role in numerous biological functions. Mg^{2+} deficiency is associated with several diseases, e.g. Alzheimer's disease[1], osteoporosis[2], and hypertension[3]. Therefore, its plasma level is tightly regulated within a narrow range (0.7-1.1 mmol/L) by intestinal absorption and renal excretion[4]. In human intestine, fractional Mg^{2+} absorption varies from 11% to 65% depending on the amount of Mg^{2+} intake[5]. Intestinal epithelium absorbs Mg^{2+} *via* both saturable transcellular and non-saturable paracellular pathways. Transcellular Mg^{2+} transport is an

active process that requires the activity of transient receptor potential melastatin 6 (TRPM6) and the basolateral Na^+/Mg^{2+} exchanger[6,7]. On the other hand, paracellular Mg^{2+} transport is a passive mechanism and is implicated in about 90% of intestinal Mg^{2+} absorption[7]. The paracellular Mg^{2+} transport process is modulated by the tight junction proteins, i.e. Claudin-16 and Claudin-19[8].

Omeprazole is a common therapeutic tool for acid-peptic disorders. Its active sulphenamide form selectively and covalently interacts with the H^+/K^+-ATPase, particularly the extracellular cysteine 813, leading to potent inhibition of H^+/K^+-ATPase activity[9]. Previous reports demonstrated that prolonged omeprazole administration led to hypomagnesemia and hypomagnesuria in humans[10,11]. Withdrawal of omeprazole and intravenous Mg^{2+} replacement, but not high dose oral Mg^{2+} supplement, could normalize the plasma and urinary Mg^{2+} levels[10,12]. Renal Mg^{2+} handling was normal in patients with severe hypomagnesemia associated with long-term use of omeprazole[12-14]. This body of evidence suggested an inhibitory effect of omeprazole on intestinal Mg^{2+} absorption. However, the direct action of omeprazole on intestinal Mg^{2+} transport is still elusive. The present study, therefore, aimed to elucidate the effect of omeprazole as well as obtain information regarding possible mechanisms of omeprazole action on Mg^{2+} transport across the intestinal epithelium. This study employed a monolayer of Caco-2 cells which is a suitable *in vitro* model for studying intestinal transport of divalent cations, e.g. Ca^{2+}[15] and Mg^{2+}[16].

MATERIALS AND METHODS

Cell culture

Caco-2 cells (ATCC No. HTB-37) were grown in Dulbecco's modified Eagle medium (DMEM) (Sigma, St. Louis, MO, USA) supplemented with 15% fetal bovine serum (FBS-Gold) (PAA Laboratories GmbH, Pasching, Austria), 1% L-glutamine (Gibco, Grand Island, NY, USA), 1% non-essential amino acid (Sigma, St. Louis, MO, USA), and 1% antibiotic-antimycotic solution (Gibco, Grand Island, NY, USA) and maintained at a humidified atmosphere containing 5% CO_2 at 37℃. The Caco-2 monolayers were developed by seeding cells (5.0×10^5 cells/cm^2) onto permeable Snapwell^{tm} inserts (12-mm diameter and 0.4-μm pore size polyester filter) (Corning, Corning, NY, USA). In the omeprazole-treated group, Caco-2 monolayers were grown in 200, 400, 600, 800, or 1000 ng/mL omeprazole (Calbiochem, San Diego, CA, USA) containing culture media. The culture medium was changed three times a week. On day 14 or 21 after seeding, the Snapwell was inserted into a modified Ussing chamber (1.13 cm^2 exposed area).

Measurement of Mg^{2+} flux

In the Ussing chamber, the monolayer was equilibrated for 20 min in bathing solution at 37℃, pH 7.4, and osmolarity of 290-293 mmol/kg H_2O[17]. To avoid the unstirred water layer and to maintain pH at 7.4, the bathing solution in each hemi-chamber was continuously gassed with humidified 5% CO_2 in 95% O_2. After equilibration, the apical or basolateral bathing solution was replaced with 2.5, 5, 10, 20, 40, or 80 mmol/L $MgCl_2$-containing bathing solution, while the contralateral side was replaced with $MgCl_2$-free bathing solution. At 1 and 2 h, 500 μL solution was collected from the side that contained $MgCl_2$-free bathing solution and Mg^{2+} concentration was measured. Mg^{2+} flux (nmol/h per cm^2) was calculated using Equation (Eq. 1):

$$Mg^{2+} \text{ flux} = C_{Mg}/(t \times S) \quad (1)$$

Where C_{Mg} is Mg^{2+} concentration (nmol/L); t is time (h); and S is transport surface area (cm^2).

To elucidate the involvement of solvent drag-induced mechanism on Mg^{2+} transport, 100 μmol/L phlorizin (Fluka Chemie AG, Buchs, Switzerland) and 100 μmol/L phloretin (Calbiochem, San Diego, CA, USA) were added to the apical and basolateral solution, respectively. Mg^{2+} transport was also observed at different temperatures (15, 25, or 35℃) and the results were presented as an Arrhernius plot[18] (Eq. 2):

$$\ln(P_{Mg}) = (-E_a)/(RT) + \ln(E) \quad (2)$$

Where $\ln(P_{Mg})$ is the natural logarithm of Mg^{2+} permeability (cm/s); E_a is activation energy (kJ/mol); R is gas constant; T is absolute temperature (273+°C), E is pre-exponential factor. The temperature coefficient Q_{10} was determined as previously described[19].

Measurement of Mg^{2+} concentration

The concentration of Mg^{2+} was determined by Xylidyl Blue (Sigma, St. Louis, MO, USA) colorimetric assay, modified from the method of Tang and Goodenough[20]. In brief, the sample solutions were spun at 1000 × *g* for 10 min and a 200 μL sample of the upper solution was collected. An aliquot was added to 100 μL water, gently mixed, and then 200 μL of 1.25 mmol/L EGTA was added to the assay tube. After mixing well, 500 μL of Xylidyl Blue solution (pH 10.5) was added to the assay tube. After 5 min of incubation at room temperature, the assay solution was subjected to colorimetric analysis using a spectrophotometer at 520 nm (model UV-2550; Shimadzu, Kyoto, Japan).

Measurement of epithelial electrical parameters

Trans-epithelium resistance (TER), potential difference (PD), and short-circuit current (I_{SC}) were determined as previously described[21]. These electrical parameters were recorded after 20 min equilibration at 30 min intervals throughout the 2 h of Mg^{2+} flux study.

Ion permeability measurement

Absolute permeabilities of Na^+ (P_{Na}) and Cl^- (P_{Cl}), as well as the relative permeability of Na^+ to Cl^- (P_{Na}/P_{Cl}), of Caco-2 monolayers were obtained by the dilution potential technique as previously described[21]. The absolute permeability of group I alkaline metals (Li^+, K^+, Rb^+, and Cs^+), i.e. P_{Li}, P_K, P_{Rb}, and P_{Cs} was determined as previously described[21] using the same calculation as that used to obtain P_{Na}.

The Mg^{2+} permeability (P_{Mg}) of Caco-2 monolayers was calculated using Eq. 3:

$$P_{Mg} = Mg^{2+} \text{ flux}/\Delta C_{Mg} \quad (3)$$

Table 1 Kinetic data of $Mg_{B\to A}$ transport

	V_m (nmol/h per cm^2)	K_m (mmol/L)	m ($\times 10^{-3}$ cm/h)
14 d			
Control	57.22 ± 8.41	5.62 ± 1.83	2.18 ± 0.11
Omeprazole treated (ng/mL)			
200	58.12 ± 6.19	4.55 ± 1.23	1.80 ± 0.08[b]
400	62.82 ± 9.64	5.83 ± 2.22	1.54 ± 0.12[b]
600	57.01 ± 7.49	4.48 ± 1.44	1.28 ± 0.09[b]
800	59.35 ± 7.40	4.71 ± 1.40	0.83 ± 0.09[b]
1000	58.84 ± 7.52	5.84 ± 1.59	0.52 ± 0.10[b]
21 d			
Control	55.82 ± 8.02	5.09 ± 1.89	2.17 ± 0.10
Omeprazole treated (ng/mL)			
200	57.81 ± 10.41	7.48 ± 2.24	1.26 ± 0.12[b,d]
400	53.47 ± 7.59	5.10 ± 1.87	1.18 ± 0.10[b,d]
600	53.12 ± 7.53	4.42 ± 1.68	0.96 ± 0.09[b,d]
800	57.65 ± 6.76	5.97 ± 1.51	0.53 ± 0.08[b,d]
1000	57.99 ± 7.72	6.09 ± 1.48	0.49 ± 0.07[b]

[b]$P < 0.001$ *vs* the age-matched control group, [d]$P < 0.001$ *vs* the concentration-matched 14 d-omeprazole-treated groups.

Table 2 Electrical parameters of Caco-2 monolayers

	n	PD (mV)	I_{SC} (mA/cm^2)	TER ($\Omega.cm^2$)
14 d				
Control	9	0.99 ± 0.12	3.09 ± 0.36	322.19 ± 6.37
Omeprazole treated (ng/mL)				
200	9	1.03 ± 0.54	2.52 ± 0.39	413.64 ± 12.95
400	9	0.98 ± 0.14	2.24 ± 0.29	433.23 ± 17.66
600	9	1.08 ± 0.15	2.29 ± 0.31	470.35 ± 23.87[d]
800	9	1.09 ± 0.18	2.30 ± 0.41	483.22 ± 20.20[d]
1000	9	1.20 ± 0.11	2.41 ± 0.20	502.88 ± 30.99[d]
21 d				
Control	9	1.00 ± 0.15	3.18 ± 0.47	314.05 ± 4.64
Omeprazole treated (ng/mL)				
200	9	1.26 ± 0.20	2.48 ± 0.32	485.09 ± 24.36[b]
400	9	1.13 ± 0.19	2.20 ± 0.32	502.19 ± 27.47[d]
600	9	1.06 ± 0.13	2.21 ± 0.34	500.33 ± 32.97[d]
800	9	0.99 ± 0.19	1.97 ± 0.31	481.64 ± 25.48[d]
1000	9	1.07 ± 0.18	2.06 ± 0.30	500.84 ± 26.61[d]

[b]$P < 0.01$, [d]$P < 0.001$ *vs* the age-matched control group.

Where ΔC_{Mg} is the concentration difference of Mg^{2+} between the apical and basolateral solutions.

Mg^{2+} transport kinetic analysis

To estimate the kinetic values of the saturable active and non-saturable passive Mg^{2+} transport, the rate of apical to basolateral Mg^{2+} transport ($Mg_{A\to B}$ transport) was fitted to a modified Michaelis-Menten kinetic plus linear component as shown in Eq. 4:

$$Mg_{A\to B}\ \text{transport} = (V_m \times C_{mg})/(K_m + C_{mg}) + mC_{mg} \quad (4)$$

Where V_m is the maximal rate of saturable $Mg_{A\to B}$ transport; K_m is the rate constant of saturable $Mg_{A\to B}$ transport; m is the rate constant for non-saturable $Mg_{A\to B}$ transport; and C_{Mg} as mentioned above. This study was performed using a nonlinear regression program of GraphPad Prism version 5.0 for Window (GraphPad Software Inc., San Diego, CA, USA).

Statistical analysis

Results were expressed as means ± SE. Two sets of data were compared using the unpaired Student's *t*-test. One-way analysis of variance (ANOVA) with Dunnett's posttest was employed for multiple sets of data. The level of significance was $P < 0.05$. Linear regression and slope analysis were performed to obtain the basolateral to apical Mg^{2+} transport ($Mg_{B\to A}$ transport)-Mg concentration relationship. The curve of P_{Mg}-Δmagnesium relationship was obtained using one phase exponential decay equation. All data were analyzed by GraphPad Prism (GraphPad Software Inc.).

RESULTS

$Mg_{A\to B}$ transport and P_{Mg} in both a dose- and time-dependent manner

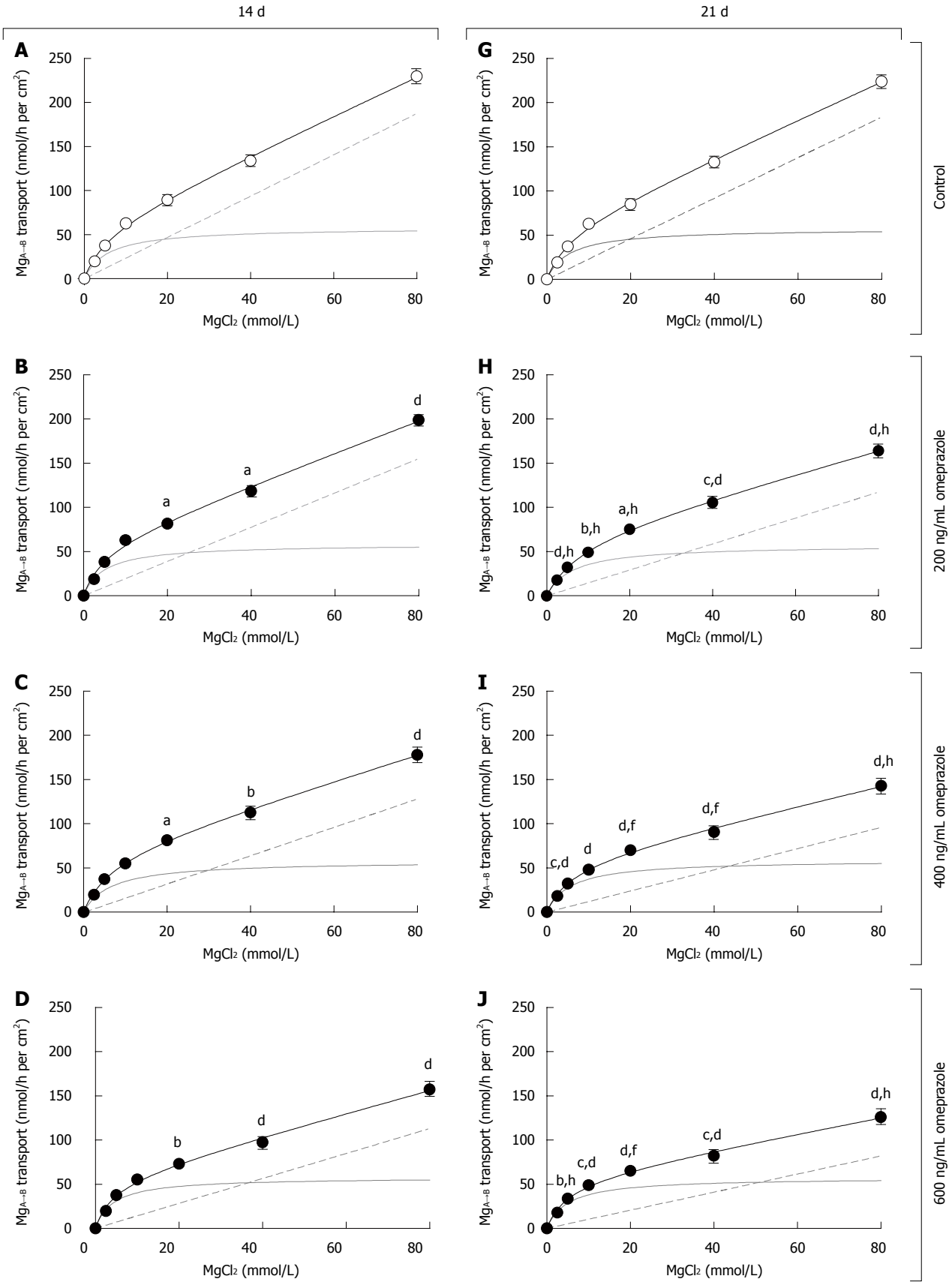
As demonstrated in Figure 1, the $Mg_{A\to B}$ transport *vs* Mg^{2+} concentration plots of Caco-2 monolayers were curvilinear similar to that reported in humans[5]. After 14 d in the omeprazole-treated groups, $Mg_{A\to B}$ transport was inhibited when compared with its corresponding untreated group (Figures 1A-F). The level of inhibition progressively increased with higher concentrations of omeprazole. Omeprazole selectively decreased non-saturable $Mg_{A\to B}$ transport, but not the saturated component, as clearly demonstrated by the lower rate constant for non-saturable $Mg_{A\to B}$ transport (Table 1). For 21 d omeprazole-treated groups, the results were similar to those of the 14 d omeprazole-treatment (Figure 1G-L, Table 1). When the same omeprazole concentration was considered, 21 d-treated groups showed a significantly lower $Mg_{A\to B}$ transport than the 14 d-treated groups (Figure 1, Table 1). Therefore, omeprazole decreased $Mg_{A\to B}$ transport in a dose- and time-dependent manner. According to the $Mg_{A\to B}$ transport, omeprazole also decreased the apical to basolateral P_{Mg} in a dose- and time-dependent mechanism (Figure 2). Moreover, omeprazole significantly increased TER, but not PD or I_{SC}, of Caco-2 monolayers (Table 2), indicating the lower paracellular permeability to ion transport.

Omeprazole decreased $Mg_{B\to A}$ transport

Since the $Mg_{B\to A}$ transport occurred solely through the paracellular pathway, the $Mg_{B\to A}$ transport *vs* Mg^{2+} concentration plot was linear (Figure 3A). Omeprazole significantly decreased the slope of the $Mg_{B\to A}$ transport-Mg^{2+} concentration plot. The slope progressively decreased with increased concentration of omeprazole (Figure 3A). In addition, omeprazole significantly suppressed the basolateral to apical P_{Mg} in a dose-dependent manner (Figure 3B). The collective results clearly showed that omeprazole suppressed paracellular passive Mg^{2+} transport across Caco-2 monolayers.

Omeprazole decreased paracellular cation selectivity

Similar to previous reports[21,22], Caco-2 monolayers


14 d
21 d
A
B
C
D
G
H
I
J
Control
200 ng/mL omeprazole
400 ng/mL omeprazole
600 ng/mL omeprazole
MgA→B transport (nmol/h per cm2)
MgCl2 (mmol/L)
0
20
40
60
80
50
100
150
200
250
a
b
d
a,h
b,h
c,d
d,h
d,f

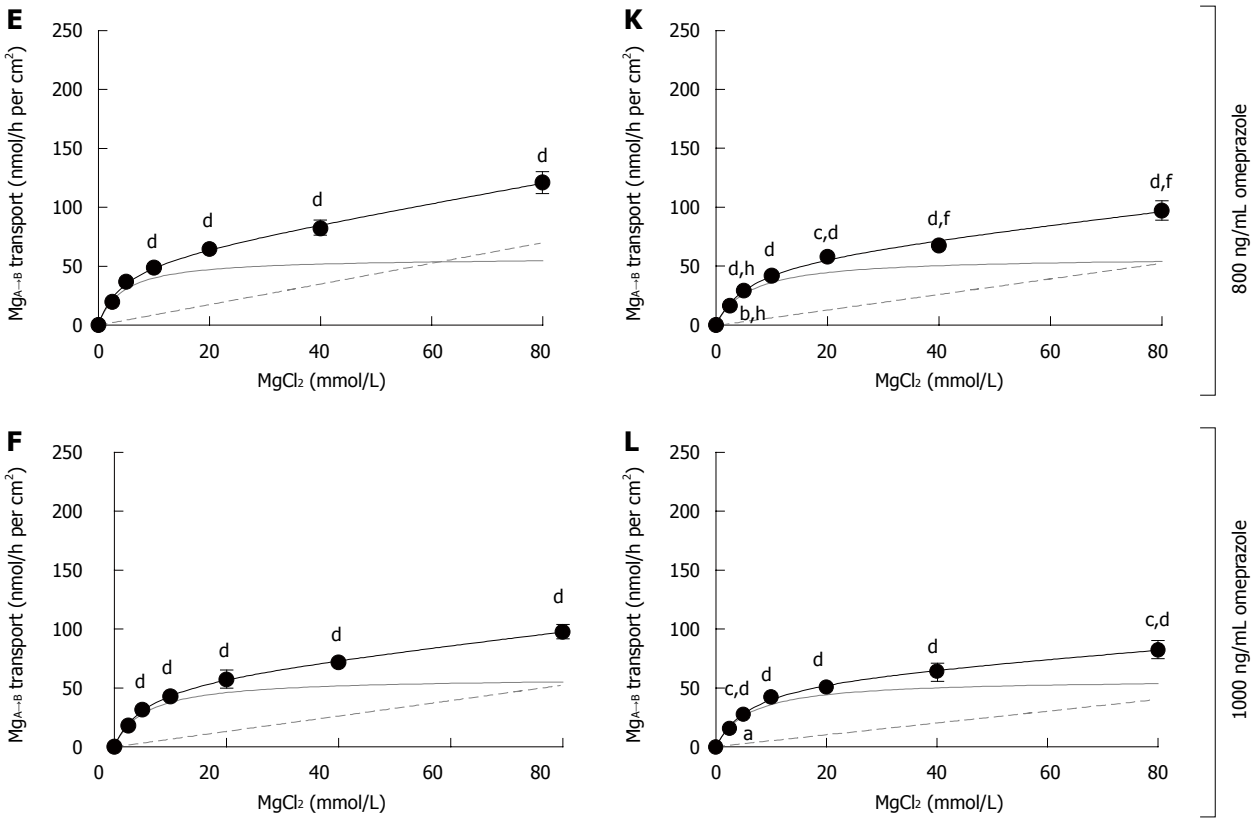


Figure 1 $Mg_{A\rightarrow B}$ transport across Caco-2 monolayers. For $Mg_{A\rightarrow B}$ transport of 14 d monolayers, A: Control; B: 200 ng/mL omeprazole-treated; C: 400 ng/mL omeprazole-treated; D: 600 ng/mL omeprazole-treated; E: 800 ng/mL omeprazole-treated; and F: 1000 ng/mL omeprazole-treated monolayers. For $Mg_{A\rightarrow B}$ transport of 21 d monolayers, G: control; H: 200 ng/mL omeprazole-treated; I: 400 ng/mL omeprazole-treated; J: 600 ng/mL omeprazole-treated; K: 800 ng/mL omeprazole-treated and L: 1000 ng/mL omeprazole-treated monolayers. Light solid lines represent the saturable component. Dashed lines represent the non-saturable component. $^aP < 0.05$, $^bP < 0.01$, $^dP < 0.001$ *vs* the age-matched control group, $^cP < 0.05$, $^fP < 0.01$, $^hP < 0.001$ *vs* the concentration-matched 14 d omeprazole-treated groups. For each data point, $n = 9$.

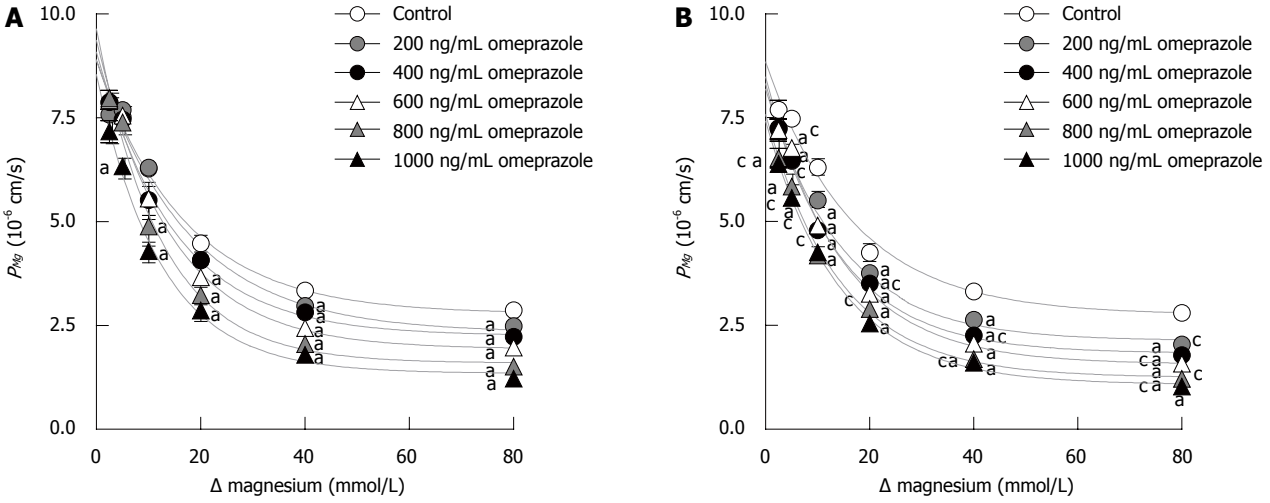


Figure 2 Apical to basolateral P_{Mg}. A: P_{Mg} of 14 d control and various dose omeprazole-treated monolayers; B: 21 d control and various dose omeprazole-treated monolayers. $^aP < 0.05$ *vs* the age-matched control group, $^cP < 0.05$ *vs* the concentration-matched 14 d omeprazole-treated group. For each data point, $n = 9$.

showed high P_{Na}/P_{Cl} (3.79 ± 0.15 in 14 d monolayers; 3.96 ± 0.22 in 21 d monolayers) from the higher P_{Na} (8.28 ± 0.20 in 14 d monolayers; 8.11 ± 0.25 in 21 d monolayers) than P_{Cl} (2.08 ± 0.09 in 14 d monolayers; 2.07 ± 0.11 in 21 d monolayers) (Figure 4A-C). Therefore, the Caco-2 monolayer was a cation selective epithelium. In 14 d- as well as 21 d-omeprazole-treated groups, omeprazole significantly suppressed P_{Na}/P_{Cl} and P_{Na} but enhanced P_{Cl} in a dose-dependent manner (Figure 4A-C), indicating that omeprazole decreased cation selectivity of Caco-2 monolayers.

Moreover, the present study also examined the paracellular permeability to monovalent cations, i.e. Li^+, Na^+,

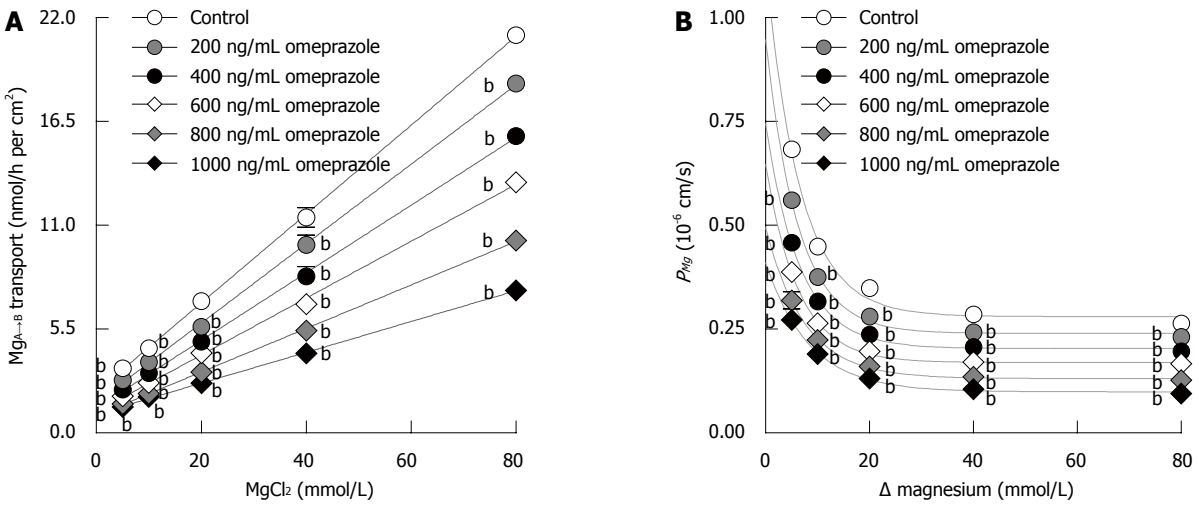


Figure 3 $Mg_{B\rightarrow A}$ transport and basolateral to apical P_{Mg}. A: $Mg_{B\rightarrow A}$ transport; B: Basolateral to apical P_{Mg} of 14 d control and various dose omeprazole-treated monolayers. $^{b}P < 0.01$ *vs* the control group. For each data point, $n = 6$.

K^+, Rb^+, and Cs^+. In control conditions, Caco-2 monolayers showed the following selective sequence: P_{Na} (8.62 ± 0.18) > P_K (7.99 ± 0.19) > P_{Rb} (5.84 ± 0.08) > P_{Cs} (4.82 ± 0.07) > P_{Li} (3.98 ± 0.12) (Figure 4D). Interestingly, 14 d-omeprazole (600 ng/mL)-exposed monolayers showed a different permeability sequence as follows: $P_K > P_{Na} > P_{Rb} > P_{Cs} > P_{Li}$ (Figure 4D). In addition, omeprazole also inhibited Caco-2 permeability to all of these monovalent cations in a dose-dependent manner.

In a parallel study, TER was simultaneously recorded when the monolayers were exposed to group I alkaline metals containing solution. In control conditions, Caco-2 monolayers showed the highest conductance (lowest TER) to Na^+ (Figure 4E). The TER-Pauling radii relationship showed a V-shaped profile. Omeprazole-treated Caco-2 monolayers showed the lowest TER when the primary ion was K^+ (Figure 4E). Omeprazole also changed the TER-Pauling radii graph to a U-shape relationship and increased TER in all groups.

Omeprazole inhibited paracellular Mg^{2+} transport

Theoretically, ions can move transversely across Caco-2 monolayer *via* four transport mechanisms, i.e. solvent drag-induced active, voltage dependent active, transcellular active, and paracellular passive transport. Therefore, the present experiment aimed to identify the relative involvement of each mechanism in Mg^{2+} transport across Caco-2 monolayers. Inhibitors of solvent drag-induced ion transport (phlorizin and phloretin) had no effect on $Mg_{A\rightarrow B}$ transport (40 mmol/L Mg^{2+} concentration gradient) in both control and omeprazole-treated monolayers (Figure 5D). In another set of experiments, Caco-2 monolayers received continuous application of *Isc*, simultaneously with the Mg^{2+} flux study, to nullify trans-epithelial PD and to abolish voltage dependent Mg^{2+} transport. The $Mg_{A\rightarrow B}$ transport in both control and omeprazole-treated monolayers were unaffected by *Isc*, (Figure 5A). The results indicated that solvent drag-induced and voltage dependent $Mg_{A\rightarrow B}$ transport were negligible.

Since transcellular Mg^{2+} transport required apical Mg^{2+} influx, inhibition of Mg^{2+} influx should abolish Mg^{2+} transport. When 20 μmol/L ruthenium red (RR), a TRPM6 inhibitor[23], was added to the apical solution, a linear relationship between $Mg_{A\rightarrow B}$ transport and Mg^{2+} concentration was observed (Figure 5B). The rate constant for non-saturable $Mg_{A\rightarrow B}$ transport of control monolayers (2.18 ± 0.13) was not different from the slope of $Mg_{A\rightarrow B}$ transport (2.09 ± 0.06) of RR-treated control monolayers. In parallel experiments, 14 d-600 ng/mL omeprazole-treated monolayers were bathed in bathing solution with or without 20 μmol/L RR (Figure 5C). Similar to control conditions, RR inhibited the saturable component, but not the non-saturable component, of $Mg_{A\rightarrow B}$ transport in omeprazole-treated monolayers. In RR-treated monolayers, the 14 d-omeprazole-treated group showed a less steep slope when compared with that of the control group (1.29 ± 0.04 *vs* 2.09 ± 0.06, $P < 0.001$, Figure 5B and C). Therefore, omeprazole suppressed the non-saturable passive Mg^{2+} transport across Caco-2 monolayers.

Temperature dependent Mg^{2+} permeability

To elucidate the temperature dependent Mg^{2+} transport, Caco-2 monolayers were bathed in 40 mmol/L $MgCl_2$ containing apical solution, while the basolateral solution had no $MgCl_2$. As shown by the Arrhenius plot (Figure 5E), the ln(P_{Mg}) decreased in lower temperatures. The control monolayers showed E_a of 14.28 ± 1.19 kJ/mol and Q_{10} of 1.22 ± 0.04. Fourteen days of 600 ng/mL omeprazole exposure significantly suppressed Mg^{2+} transport and increased E_a (19.24 ± 1.98 kj/mol, $P < 0.05$), but not Q_{10} (1.31 ± 0.05), of Caco-2 monolayers.

DISCUSSION

The present study demonstrated the effect of omeprazole on Mg^{2+} transport across Caco-2 intestinal epithelium. Omeprazole-treated monolayers showed a dose- and time-dependent decrease in Mg^{2+} transport and P_{Mg} (Figures 1-3).

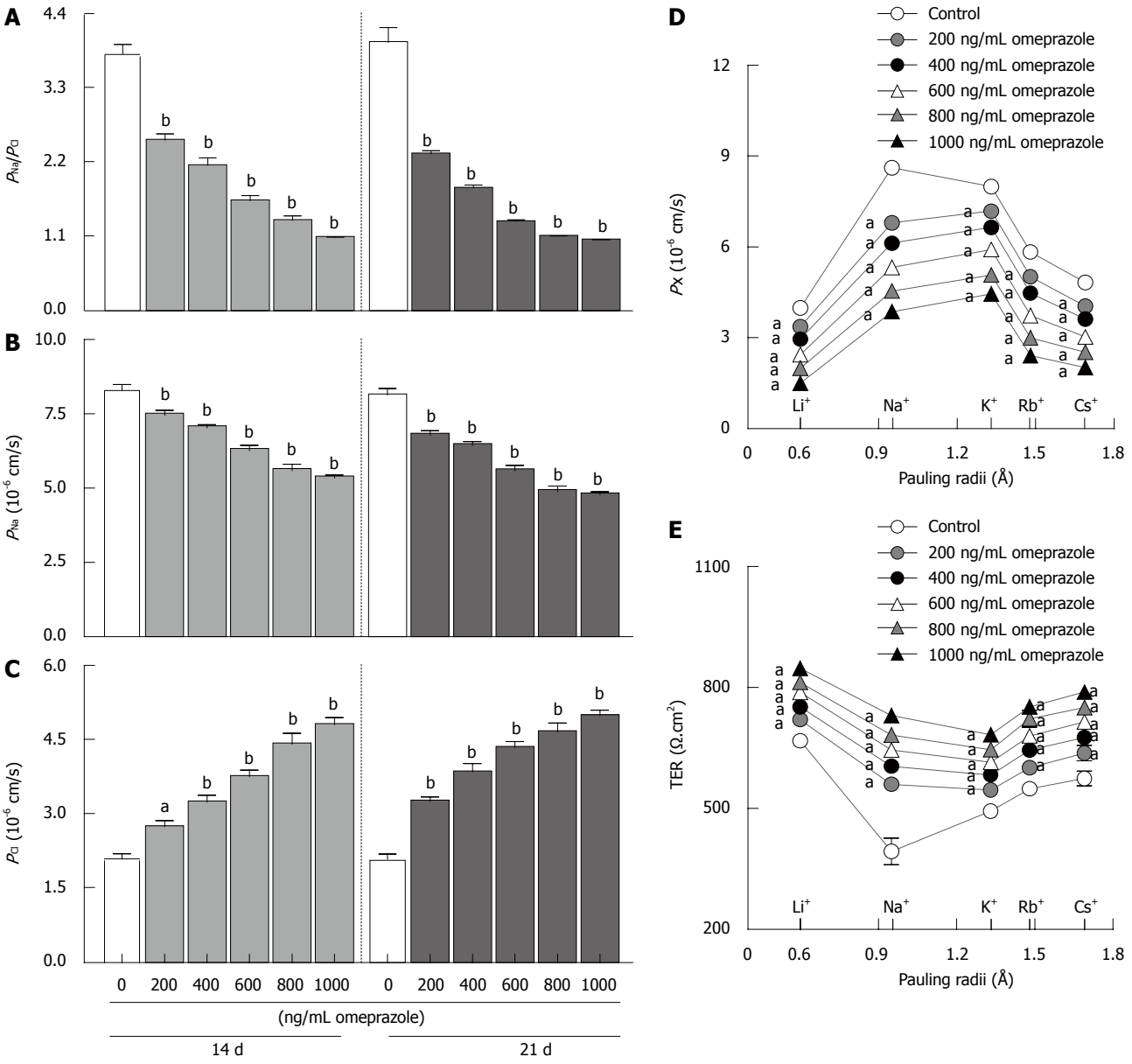


Figure 4 Paracellular charge selectivity and selective permeability profile. A: P_{Na}/P_{Cl}; B: P_{Na}; C: P_{Cl} of 14 and 21 d control and various dose omeprazole-treated monolayers; D: Absolute alkaline metal ions (Li^+, Na^+, K^+, Rb^+, and Cs^+) permeability; E: Trans-epithelium resistance (TER) of 14 d control and 600 ng/mL omeprazole-treated monolayers. $^aP < 0.05$, $^bP < 0.01$ *vs* the age matched control group. For each data point, $n = 6$.

Omeprazole selectively inhibited the non-saturable passive component, but not the saturable active component, of transepithelial Mg^{2+} transport (Table 1 and Figure 5). The paracellular cation selectivity of the monolayers was also reduced after prolonged exposure to omeprazole (Figure 4). Results of the Arrhenius plot (Figure 5) showed the higher E_a in the omeprazole-treated group, indicating impediment of the paracellular channel to Mg^{2+} movement.

In humans, intestinal Mg^{2+} absorption *vs* Mg^{2+} intake exhibited a curvilinear relationship[5] from the combination of saturable active and non-saturable passive absorption. Moreover, lower intestinal passive Mg^{2+} absorption as compared with passive Ca^{2+} absorption was also demonstrated[5,24]. Similarly, in Caco-2 monolayers, a plot of $Mg_{A\rightarrow B}$ transport (representing Mg^{2+} absorption) against Mg^{2+} concentration (in apical solution) was also curvilinear (Figure 1) and $Mg_{A\rightarrow B}$ transport was lower than the apical to basolateral Ca^{2+} transport[21]. Therefore, the Caco-2 monolayer was a suitable *in vitro* model of intestinal Mg^{2+} absorption[16].

Several case reports demonstrated severe hypomagnesemia associated with prolonged omeprazole usage[10-14], suggesting that intestinal Mg^{2+} absorption, but not renal Mg^{2+} handling, was defective. On the other hand, short-term omeprazole administration had no effect on intestinal Mg^{2+} absorption[25] because its bioavailability was low and its half-life was short[9,26]. Therefore, the later development of hypomagnesemia was probably associated with the depletion of Mg^{2+} store in the human body[13]. The present study demonstrated an inhibitory effect of omeprazole on Mg^{2+} fluxes across 14 and 21 d-omeprazole-treated Caco-2 monolayers, suggesting that the intes-

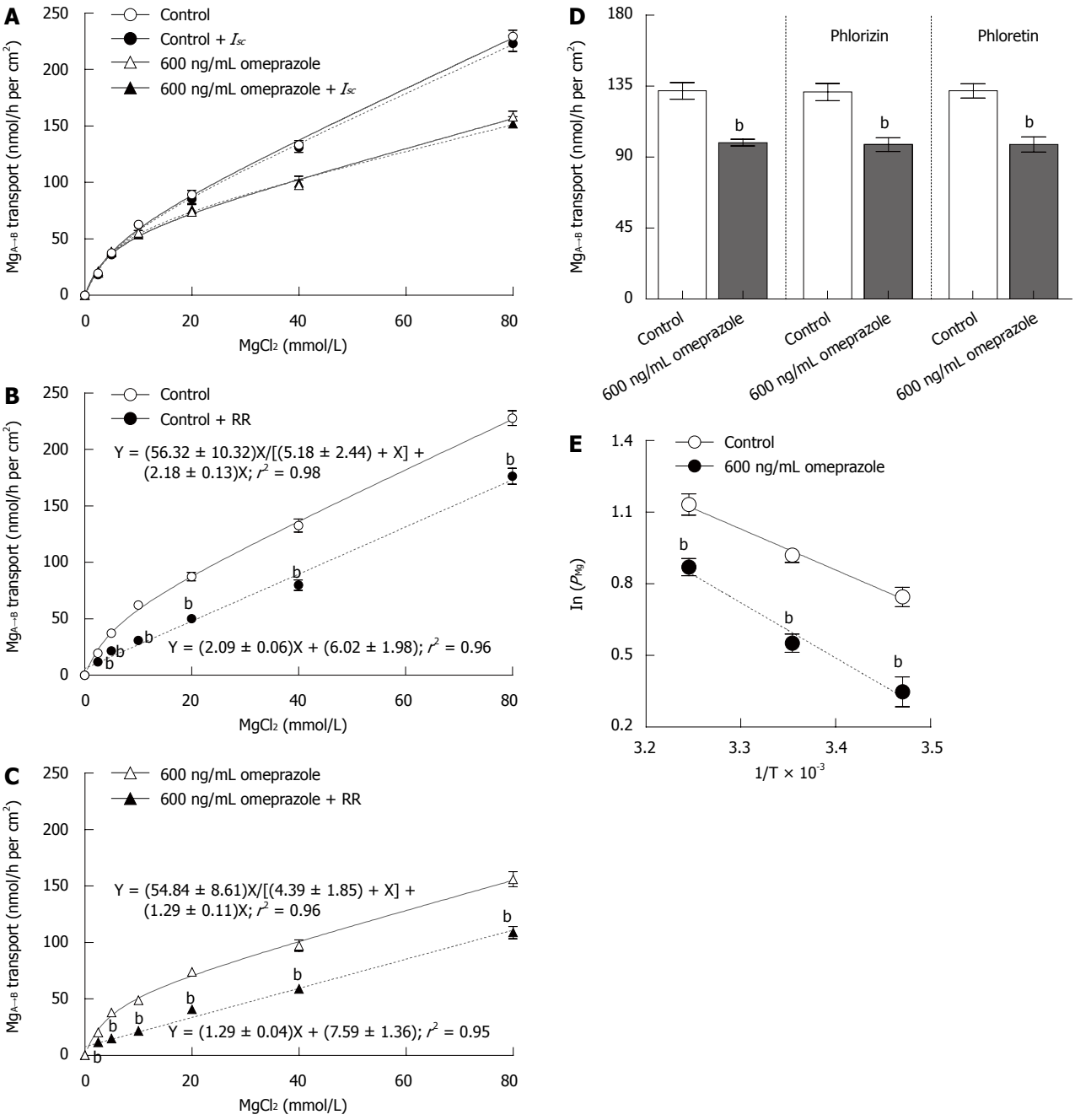


Figure 5 Mechanism of $Mg_{A\rightarrow B}$ transport. A: $Mg_{A\rightarrow B}$ transport 14 d control and 600 ng/mL omeprazole-treated monolayers with or without *Isc*; B and C: 20 μmol/L ruthenium red (RR); D: 100 μmol/L phlorizin or 100 μmol/L phloretin. For each data point, $n = 9$ in A; $n = 6$ in B and C. E, Arrhenius plot of 14 d control and 600 ng/mL omeprazole-treated monolayers. $^{b}P < 0.01$ *vs* the control group. For each data point, $n = 6$.

tinal Mg^{2+} flux defect could not be responsible for later development of hypomagnesemia in omeprazole use.

There are two transport mechanisms for Mg^{2+} absorption, i.e. transcellular active and paracellular passive transport, across the intestinal epithelium[7]. Previous reports suggested that omeprazole inhibited active intestinal Mg^{2+} absorption and TRPM6 activity because high dose oral Mg^{2+} supplement partially[13] and totally[14] resolved hypomagnesemia in prolonged omeprazole use. On the other hand, other reports showed different results i.e. high dose oral Mg^{2+} supplement, but not intravenous Mg^{2+} replacement and withdrawal of omeprazole, failed to normalize plasma and urinary Mg^{2+} levels[10,12]. The later evidence indicated that omeprazole inhibited passive Mg^{2+} absorption, which agreed with the present findings. In the present study, omeprazole inhibited the non-saturable passive, but not saturable active, Mg^{2+} transport across Caco-2 monolayers (Table 1). In addition, the role of transcellular active Mg^{2+} transport was examined using the TRPM6 inhibitor RR. Inhibition of TRPM6 in Caco-2 cells[27] abolished the saturable active Mg^{2+} transport and revealed the inhibition of non-saturable passive Mg^{2+} transport in omeprazole-treated monolayers (Figure 5B and C). Therefore, the paracellular passive Mg^{2+} absorption defect should be recognized in omeprazole usage.

Consistent with previous findings that the paracellular

passive transport of cations, such as Na^+, Cs^+, H^+, Ca^{2+}, and Mg^{2+}, was a temperature variance mechanism[18,20], the present Arrhenius plot (Figure 5E) showed the temperature-dependent Mg^{2+} transport. Since the temperature coefficient Q_{10} of passive ion diffusion through the open ion channel ranged from 1.2 to 1.4[28] and the paracellular pore of the tight junction behaved as the channel[20], therefore, the Q_{10} of control (1.22) and omeprazole treated (1.31) monolayers indicated that Mg^{2+} mainly moved through the paracellular channels of Caco-2 epithelium. The paracellular passive H^+ transport occurred *via* the claudin-8 channel of MDCK II epithelium[18]. The paracellular claudin-8 channel was found to impede H^+ transport by increasing the E_a[18]. Therefore, the higher E_a of omeprazole-treated Caco-2 epithelium suggested that the paracellular channel of Caco-2 epithelium impeded Mg^{2+} transport. In addition, the higher TER (Table 2) also indicated lower paracellular permeability. The present study supported a previous report by Hou *et al*[29], who demonstrated that the epithelium with higher TER showed lower passive Mg^{2+} transport.

The paracellular transport of Mg^{2+} was regulated by the paracellular charge selectivity, i.e. cation selectivity, of the tight junction[29,30]. Caco-2 epithelium was a cation selective epithelium (Figure 4A-C)[21,22] that favored the transport of cations through the paracellular pathway. Similar to a previous report[21], the paracellular selective permeability profile of Caco-2 monolayers to monovalent cations was $Na^+ > K^+ > Rb^+ > Cs^+ > Li^+$ (Figure 4D) which was classified as series Ⅶ of the Eisenman sequence[31]. Series Ⅶ indicated the presence of moderate negative electrical field strength in the paracellular channel of Caco-2 epithelium. However, omeprazole changed the selective permeability profile to series Ⅵ of the Eisenman sequence ($K^+ > Na^+ > Rb^+ > Cs^+ > Li^+$)[31]. Since series Ⅵ was characterized by lower negative electrical field strength than that of series Ⅶ[31], the paracellular cation selectivity was decreased when the monolayers were exposed to omeprazole (Figure 4A-C). Hou *et al*[29] also demonstrated lower paracellular Mg^{2+} transport due to lower paracellular cation selectivity of the epithelium. Thereby, omeprazole-induced suppression of paracellular cation selectivity led to the inhibition of paracellular Mg^{2+} transport across Caco-2 epithelium.

The present study demonstrated the inhibitory effect of omeprazole on passive Mg^{2+} transport which was consistent with previous reports[29,30,32-34]. The paracellular passive Mg^{2+} transport was mainly mediated by claudins at the tight junction[29,30], the distribution of which could be affected by the change in extracellular fluid pH. Inhibition of H^+/K^+-ATPase activity in Caco-2 cells[35] by omeprazole might decrease the extracellular H^+ concentration, which in turn increased the sensitivity of the extracellular calcium sensing receptor (CaSR)[32, 33], which was expressed in Caco-2 cells[36,37]. Ikari *et al*[34] clearly demonstrated that the activation of CaSR led to the translocation of claudin-16 from the tight junction into the cell, thus inhibiting paracellular Mg^{2+} transport. Therefore, omeprazole-inhibited passive Mg^{2+} transport appeared to involve the CaSR-tight junction-dependent mechanism.

In conclusion, omeprazole inhibited paracellular passive Mg^{2+} transport across Caco-2 epithelium in a dose- and time-dependent fashion. The inhibition of passive Mg^{2+} transport was due to the decrease in paracellular cation selectivity. The results from the present study provided evidence for the regulation of intestinal Mg^{2+} absorption. However, the underlying mechanism of omeprazole inhibiting passive Mg^{2+} transport requires further study.

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COMMENTS

Background

Previously, it was widely believed that intestinal Mg^{2+} transport in humans depended on the amount of Mg^{2+} intake and was not tightly regulated by any hormones. Omeprazole, a common therapeutic drug for acid-peptic disorders, has been found to have effects on Mg^{2+} metabolism.

Research frontiers

Several previous reports have demonstrated an association between severe hypomagnesemia and prolonged omeprazole usage in humans. Those patients had normal renal Mg^{2+} handling, suggesting that a defect in intestinal Mg^{2+} absorption may be responsible for hypomagnesemia. However, the direct action of omeprazole on intestinal Mg^{2+} absorption is unknown. In this manuscript, an inhibitory effect of omeprazole on intestinal passive Mg^{2+} absorption is demonstrated.

Innovations and breakthroughs

In this manuscript, the authors reported a direct inhibitory action of prolonged omeprazole treatment on paracellular passive Mg^{2+} absorption across the intestinal epithelium. This finding provides an explanation on how prolonged usage of omeprazole could lead to hypomagnesemia.

Applications

Acid-peptic disorders, e.g. gastro-oesophageal reflux disease, erosive oesophagitis, heartburn, and Barrett's disease, are chronic diseases that require prolonged omeprazole administration. Therefore, plasma Mg^{2+} assessment should help prevent hypomagnesemia in these patients.

Terminology

The paracellular charge selectivity is a property of epithelium that is selectively permeable to specific charged molecules, e.g. ions. This property is regulated by proteins of the tight junction, i.e. claudins. Alterations in claudin expression in the tight junction directly affect the charge selectivity and the paracellular ion transport across the epithelium.

Peer review

This is an interesting paper investigating the inhibitory action of omeprazole on magnesium transepithelial transport at intestinal level. The study is well-done, the rationale is clear, the experimental design correct, and the results shown convincingly support the conclusions drawn.

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