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Current opinion in the regulation of small intestinal magnesium absorption

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Abstract

Ma^{2+} has an important role in numerous biological functions, and Mg^{2+} deficiency is associated with several diseases. Therefore, adequate intestinal absorption of Mg^{2+} is vital for health. The small intestine was previously thought to absorb digested Mg^{2+} exclusively through an unregulated paracellular mechanism, which is responsible for approximately 90% of total Mg^{2+} absorption. Recent studies, however, have revealed that the duodenum, jejunum, and ileum absorb Mg^{2+} through both transcellular and paracellular routes. Several regulatory factors of small-intestinal Mg^{2+} uptake also have been explored; *e.g.*, parathyroid hormone, fibroblast growth factor-23, apical acidity, proton pump inhibitor (PPI), and pH-sensing channel and receptors. The mechanistic factors underlying PPI suppression of small-intestinal Mg^{2+}, such as magnesiotropic protein dysfunction, higher mucosal bicarbonate secretion, Paneth cell dysfunction, and intestinal inflammation, are currently being explored. The potential role of small-intestinal microbiomes in Mg^{2+} absorption has also been proposed. In this article, we review the current knowledge on the mechanisms and regulatory factors of small-intestinal Mg^{2+} absorption.

Key Words: Hormone; Mg2+ absorption; Paneth cells; Proton pump inhibitor; Regulation; Small intestine

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Core Tip: Small-intestinal epithelium absorbs digested Mg2+ through both transcellular active and paracellular passive mechanisms. Several regulatory factors of small-intestinal Mg2+ uptake have been reported. Parathyroid hormone and fibroblast growth factor-23 directly inhibit transcellular Mg2+ absorption in the duodenum, jejunum, and ileum. The apical proton triggered acid-sensing ion-channel 1a and purinergic P2Y2 receptor activities, which stimulated mucosal bicarbonate secretion

(MBS). Secreted bicarbonate induced MgCO3 precipitation that suppressed small-intestinal Mg2+ absorption. Omeprazole suppressed Mg2+ absorption in the duodenum, jejunum, and ileum *via* higher MBS, lower mucosal TRPM6 and TRPM6/7 activity, Paneth cell dysfunction, and higher intestinal inflammation and villous atrophy.

INTRODUCTION

INTRODUCTION

Magnesium (Mg^{2+}) has an essential role in numerous cellular biochemical functions ranging from DNA structure stability and repairing, cell proliferation, neuronal excitability, bronchodilatation, vasodilatation, muscle contraction, myocardial excitability, bone hydroxyapatite formation, and anti-inflammatory function to exocrine and endocrine function of the pancreas [1]. Mg^{2+} deficiency has been implicated in several diseases, such as Alzheimer's disease [2], osteoporosis [3], hypertension [4], diabetes mellitus [5], and cancer [6]. Therefore, its plasma level is tightly regulated within a narrow range (0.7–1.1 mmol/L) by the collaborative actions of intestinally digested Mg^{2+} absorption, bone and muscle Mg^{2+} storage, and excess renal Mg^{2+} excretion [1]. The mechanism underlying regulation of transepithelial Mg^{2+} transport has been extensively explored in the renal tubular epithelium [1]. However, few research articles on the mechanism and regulatory factors of intestinal Mg^{2+} absorption have been published.

Since dietary intake is the sole source of Mg^{2+} in humans, adequate intestinal absorption of Mg^{2+} is vital for normal Mg^{2+} balance. It was previously hypothesized that bulk Mg^{2+} uptake occurs in the small intestine through an unregulated paracellular pathway, whereas fine-tuning of colonic Mg^{2+} absorption occurs through a regulated transcellular mechanism [1,7,8]. Colonic Mg^{2+} absorption can be modulated by dietary Mg^{2+} content and inulin fibers [7,9], but not by hormones [1,7]. In contrast, recent studies have provided new insights into the mechanisms and modulatory factors of small intestinal Mg^{2+} uptake. The aim of this article was to review the current knowledge of the mechanisms and regulatory factors of small-intestinal Mg^{2+} absorption.

MECHANISM OF SMALL INTESTINAL MG^{2+} ABSORPTION

The mechanism of small-intestinal Mg^{2+} absorption is currently under debate. One research group has proposed that transient receptor potential melastatin (TRPM)6 mRNA expression and transcellular Mg^{2+} absorption were not present in the small intestine [1,7,8]. However, a study from the same group showed positive immunofluorescence staining of TRPM6 protein in the absorptive cells along the brush-border membrane of the villi in the duodenum [10]. Another group has proposed that the small-intestinal epithelium absorbs Mg^{2+} through transcellular active and paracellular passive transport mechanisms [11-13]. In an Ussing chamber study, transport of transcellular and paracellular Mg^{2+} was detected in the duodenum, jejunum, and ileum [11-13]. The proposed mechanism of small-intestinal Mg^{2+} absorption is shown in Figure 1.

Transcellular Mg^{2+} absorption

Transcellular Mg^{2+} absorption occurs through mucosal Mg^{2+} uptake by TRPM6 and TRPM7, both of which were markedly detected in the small intestinal epithelium of human and murine [10-14]. In addition, recent mass spectrometric peptide sequence analysis confirmed the expressions of TRPM6 and TRPM7 in the duodenum and jejunum [15]. The channel activities of both homodimers of TRPM6 and of TRPM7 are negatively regulated by physiological Mg×ATP and Mg^{2+} levels [10,16-19]. A recent study reported the expression of a heterodimer TRPM6/7 channel in the plasma membrane of duodenal and jejunal epithelium [15]; therefore, Mg^{2+} enters the small-intestinal epithelial cells through TRPM6/7, TRMP6, and TRPM7. However, the *heterodimer* TRPM6/7 channels do not respond to physiological intracellular Mg^{2+} and Mg·ATP [17,19]; thus, continuous epithelial Mg^{2+} absorption can occur through the TRPM6/7 channel, regardless of intracellular Mg^{2+} and concentrations. Basolateral Mg^{2+} extrusion from the small-intestinal epithelium occurs through cystathionine β-synthase domain divalent metal cation transport mediator 4 (CNNM4)[11-13,20] by means of Na^{+} gradient-dependent

secondary active transport [20]. However, mutation of CNNM4 does not affect the plasma concentration in humans [21,22], suggesting that other Mg^{2+} extrusion mechanisms probably occur.

Paracellular Mg^{2+} absorption

It has been suggested that paracellular Mg^{2+} absorption is responsible for 90% of total intestinal Mg^{2+} uptake [23]. Paracellular permeability is regulated by the paracellular claudin (Cldn) channel of the tight junction [24]. In 1999, the first discovery of a paracellular channel at the tight junction was Cldn-19 or paracellin-1, which form a paracellular Mg^{2+} channel [25]. It is thought that paracellular Mg^{2+} channels in epithelial tissues are formed by Cldn-16 and -19 [25-27], mutations that lead to severe hypomagnesemia. The small-intestinal epithelium expresses Cldn-1–5, -7, -8, -12, and -15, but not -16 and -19 [28,29]. A previous study proposed that Cldn-7 and -12 modulated intestinal paracellular Mg^{2+} absorption [30]. However, the processes involving Cldn-regulated paracellular Mg^{2+} absorption in the small intestine still must be elucidated.

REGULATORY FACTORS OF SMALL INTESTINAL MG^{2+} ABSORPTION

Hormones

In general, hormones mainly modulate the transcellular electrolyte transport to regulate epithelial electrolyte absorption or secretion. Hormonal regulation of small-intestinal Mg^{2+} absorption also modulates transcellular Mg^{2+} absorption. A recent study reported that parathyroid hormone (PTH) and fibroblast growth factor-23 (FGF-23) systemically and directly inhibited transcellular, but not paracellular, Mg^{2+} absorption in the duodenum, jejunum, and ileum [13]. There was no additional effect of PTH and FGF-23, suggesting that they acted through the same intracellular signaling molecule. Both PTH and FGF-23 activate their corresponding receptors that further stimulate the same protein kinase c (PKC) pathway to suppress plasma membrane-associated TRPM6 expression (Figure 2). Since native TRPM6 primarily functions as a subunit of heteromeric TRPM6/7 channels [31], the suppression of plasma membrane TRPM6

probably suppresses plasma TRPM6/7 heterodimer expression. The suppression of plasma TRPM6 and TRPM6/7 activity leads to diminution of transcellular Mg^{2+} absorption [13]. The inhibitory effect of PTH and FGF-23 could be nullified by Gö 6850 [13], which inhibits the conventional (α, β1, β2, and γ) and novel PKC isoforms (δ and ε). However, the exact signaling pathway of ***PTH and FGF-23 inhibition of small-intestinal transcellular Mg^{2+} absorption requires further study***

The proposed physiologically relevant magnesiotropic actions of PTH and FGF-23 are shown in Figure 3. During hypocalcemia, the parathyroid gland actively secretes PTH into the blood stream. PTH stimulates the bone resorption process, which increases plasma Ca^{2+}, Pi, and Mg^{2+} levels [32,33]. PTH stimulates renal 1,25-dihydroxy vitamin D_3 [1,25$(OH)_2D_3$] production, which subsequently induces small-intestinal Ca^{2+} absorption [34]. PTH also activates renal tubular Ca^{2+} and Mg^{2+} reabsorption[32]. Plasma Pi and PTH trigger bone-derived FGF-23 release, which acts as a negative feedback regulator to abolish 1,25$(OH)_2D_3$-induced intestinal Ca^{2+} absorption [33]. PTH and FGF-23 synergistically suppress the small-intestinal absorption of dietary Mg^{2+}[13] to prevent hypermagnesemia. PTH and FGF-23 downregulate the Na^{2+}-dependent P_i cotransporters, (NaPi)-IIa and NaPi-IIc, and increase urinary P_i excretion [32] to prevent hyperphosphatemia. Therefore, PTH and FGF-23 exert their calcemic effect by preventing hyperphosphatemia and hypermagnesemia.

Luminal acidity

The hypothesis that apical acidity and mucosal bicarbonate secretion (MBS) affect luminal Mg^{2+} solubility and intestinal Mg^{2+} absorption was previously proposed in 2014[11,35], which was confirmed in a recent review article [36]. The luminal acidity along the entire human and rodent small bowel varies from pH 5.0–7.3[12,37]. The luminal protons provide an appropriate environment for mineral absorption by stabilizing their ionized forms [38]. The elevation of luminal pH led to a lower soluble Mg^{2+}, which decreased from 79.61% of total luminal Mg content at pH 5.15 to 8.71% of total luminal Mg at pH 7.8 [39]. Therefore, luminal acidity enhances Mg^{2+} absorption in the human

small intestine[40] and epithelial-like Caco-2 monolayers[30,35]. The MBS and luminal pH elevation diminished duodenal, jejunal, and ileal Mg^{2+} absorption [11,12].

pH-sensing channel and receptor

Small-intestinal enterocytes are regularly exposed to strong gastric acid. When luminal protons are present in the duodenal lumen, the intestinal epithelium cells can directly detect and modulate their cellular response through the proton-sensing channels; *e.g.*, the acid-sensing ion-channel 1a (ASIC1a) or proton-sensing receptors, such as ovarian cancer G protein-coupled receptor 1 (OGR1) and P2Y purinoceptor [41-44].

OGR1, also known as GPR68, is expressed in the human small intestine, spleen, testes, brain, lungs, placenta, heart, and kidneys, but not in the colon[44]. OGR1 is a proton-sensitive receptor with pH values at half activation ($pH_{0.5}$) and full activation of 7.2 and 6.8, respectively [45-47]. When the luminal pH decreases to 6.5, OGR1 activity is inactivated [45]. Activation of OGR1 triggers the phospholipase C (PLC)–PKC signaling pathway to activate intestinal Mg^{2+} absorption [35] (Figure 4).

ASIC1a is a proton-sensitive Ca^{2+} channel with a $pH_{0.5}$ of 6.2[41,43]. Activation of ASIC1a activates intracellular Ca^{2+} signaling and subsequently induces MBS. In the intestinal epithelium, luminal proton stimulates ASIC1a activity that further activates MBS in Ca^{2+} signaling-cystic fibrosis transmembrane conductance regulator (CFTR)-dependent mechanism [35] (Figure 4). Secreted HCO_3^- has previously been found to reduce luminal protons [48] and induce precipitation of luminal free Mg^{2+}[49], thus reducing free soluble Mg^{2+} and suppressing intestinal Mg^{2+} absorption.

Purinergic regulation of luminal pH and electrolyte transport in the small intestine have been described [50-52]. Duodenocyte regularly secretes ATP into its lumen. If luminal pH is low, luminal alkaline phosphatase (AP) activity is diminished and luminal ATP increase, which subsequently activates P2Y purinoceptor. Simultaneously, P2Y is a proton-sensitive receptor that is activated by luminal protons [42]. Active P2Y purinoceptors further activate MBS to increase luminal pH. A previous study showed that $P2Y_2$ activation induced MBS through a CFTR- and Na^+-HCO_3^- cotransporter-1

(NBCe1)-dependent mechanism, which subsequently suppressed intestinal Mg^{2+} absorption [53] (Figure 5).

Proton-pump inhibitor (PPI)

PPI-induced hypomagnesemia (PPIH) and hypomagnesuria in humans have been reported since 2006 [54-57]. Intravenous Mg^{2+} supplementation or withdrawal of PPI was able to rapidly normalize plasma and urinary Mg^{2+} levels in PPIH patients, oral Mg^{2+} supplementation could not. Clinical assessments have reported that PPIH patients had normal renal Mg^{2+} handling [54,56,57]. These findings suggest that PPI could suppress intestinal Mg^{2+} absorption. Our group has extensively studied the underlying mechanisms of PPI-suppressed intestinal Mg^{2+} absorption for a decade [11,12,15,30,35,53,58,59]. Our results suggest that PPI mainly suppressed small-intestinal Mg^{2+} absorption. Omeprazole, the first introduced PPI, significantly suppressed total, transcellular, and paracellular Mg^{2+} absorption in the duodenum, jejunum, ilium, and colon of PPIH rats [11,12]. Regarding the percentage suppression of total Mg^{2+} absorption in the duodenum (81.86%), jejunum (70.59%), ileum (69.45%), and colon (39.25%), the small intestine is the segment most adversely affected by prolonged PPI administration. However, previous articles have proposed that PPI mainly inhibits colonic Mg^{2+} absorption [36,60,61], but those study results remain controversial [60,61]. They also proposed that colonic fermentation of dietary fibers probably increased serum Mg^{2+} and cured patients with PPIH [36]. A previous study clearly showed that dietary inulin fibers significantly induced cecal and colonic fermentation, but not plasma Mg^{2+} levels, in control and PPIH mice [61]. In contrast, dietary inulin fibers significantly induced renal Mg^{2+} excretion in PPIH mice [61], which should aggravate hypomagnesemia in PPIH. Therefore, the large intestine may not be a suitable intestinal segment that should be modulated to counteract PPIH.

The proposed mechanism of PPI-suppression of small-intestinal Mg^{2+} absorption is shown in Figure 6. PPI markedly suppresses membranous TRPM7 and TRPM6/7[15]. Membranous TRPM6-channel activity is suppressed by hyper-phosphorylation at the

T1851 residue and hyper-oxidation at the M1755 residue [15]. Phosphorylation of the T1851 residue of the TRPM6 protein induces TRPM6-channel suppression by intracellular free Mg^{2+} and activated 5 C-kinase 1[62]. Oxidation of the M1755 residue in the TRPM6 protein also suppresses its channel permeability [63]. Suppression of membranous TRPM6, TRPM7, and TRPM6/7 disrupts mucosal Mg^{2+} entry into the small-intestinal epithelium and then inhibits transcellular Mg^{2+} absorption [11,12]. Plasma FGF-23 was markedly increased in PPIH rats [12]. The mechanism by which FGF-23 inhibits transcellular small-intestinal Mg^{2+} absorption is described in the above section [13]. Therefore, PPI-suppressed transcellular Mg^{2+} absorption is due, at least in part, to FGF-23.

PPI suppresses paracellular Mg^{2+} absorption (Figure 6). The small intestinal epithelium only expresses Cldn-1, -2, -3, -4, -5, -7, -8, -12, and -15 [28,29]. Over-expression of Cldn proteins and higher paracellular resistance have been demonstrated in the small intestines of PPIH rats [11,12]. Paracellular tight junction width was significantly decreased in the small intestine of PPIH rats [58]. PPI also suppresses epithelial paracellular Mg^{2+} permeability and cation selectivity [30,59]. These results shed light on the mechanism of PPI-suppressed paracellular Mg^{2+} absorption in the small intestine.

PPI-induced small-intestinal MBS (Figure 6) has been reported in humans [64], PPIH rats [11], and PPI-treated Caco-2 monolayers [35,53]. PPI has also been shown to significantly increase ASIC1a and $P2Y_2$ expression in PPI-treated epithelium [35,53]. Active ASIC1a and $P2Y_2$ trigger MBS. Higher secreted HCO_3^- in PPIH small intestines reduces free soluble Mg^{2+}, which disrupts Mg^{2+} absorption (Figure 6). Inhibition of MBS significantly increases duodenal Mg^{2+} absorption in PPIH rats [11].

In addition to the change in magnesiotropic protein expression and function and MBS, PPI has been shown to induce structural change in the absorptive epithelium of the small intestine [58]. Prolonged PPI administration markedly decreased the villous length and absorptive area in the duodenal, jejunal, and ilial epithelium of PPIH rats. The underlying mechanism involves Paneth cell dysfunction in the small intestine [58]. Paneth cells have an important role in host-microorganism homeostasis in the small

intestine by providing antimicrobial α-defensin peptides [65,66]. Disruption of the secretory function of Paneth cells leads to infection and chronic inflammation of the small intestine [65,66]. In PPIH rats, a reduction in secretory granules and metaplasia of Paneth cells occurs in the duodenum, jejunum, and ileum, suggesting Paneth cell secretory dysfunction [58]. Chronic inflammation in the small-intestinal epithelium leads to villous atrophy and reduction of the absorptive area in the small intestine of PPIH rats [58].

Gut microbiota

The potential role of gut microbiota in colonic Mg^{2+} absorption has previously been proposed [36]. However, it is currently unknown how the small-intestinal microbiome affects small-intestinal Mg^{2+} absorption. Previous studies have shown that the small intestine is colonized by the complex gut microbiota community, and is less numerous and diverse (approximately 10^3–10^7 microbial cells/gram) than in the colon (approximately 10^{12} microbial cells/gram)[67]. The dominant bacterial phyla in the small intestine are *Streptococcus sp.*, *Lactobacillaceae* and *Enterobacteriaceae*, whereas in the colon, the dominant phyla are *Bacteroidaceae, Prevotellaceae, Rikenellaceae, Lachnospiraceae*, and *Ruminococcaceae* [68,69]. Prolonged PPI treatment can lead to gut microbiota dysbiosis, such as the reduction of *Actinobacteria* and *Bifidobacteria spp.*, which are responsible for maintaining the mucosal barrier function [68]. Furthermore, long-term treatment with PPIs causes small-intestinal bacterial overgrowth (SIBO) because of the loss of the gastric acid defensive barrier [70]. The jejunal samples of SIBO patients regularly showed increased production of toxic agents, such as serum endotoxin and bacterial compounds that stimulate the secretion of proinflammatory cytokines [71]. Apart from these findings, our previous study showed Paneth dysfunction and chronic inflammation in the small intestine of PPIH rats [58]. From the perspective of gut microbiota relevant, Paneth cell defects have been found to be associated with increased *Bacteroidetes* and *Enterococcus* and decreased *Bifidobacterium* [72], whereas *Bifidobacterium longum* has been found to promote cell proliferation and expression of Lgr5 and Wnt3a

in intestinal organoids and alleviate microbiota dysbiosis by regulating the functions of Paneth cells [73]. It is also possible that the synthesis of gut microbiota metabolites could lead to changes in the absorptive surface in the gut and/or stimulate gene expression [74].

In the colon, bifidobacterial fermentation leads to acidification of the colon, which shows beneficial absorption of Mg^{2+}[9,61,75]. In humans, small-intestinal microbiota can also ferment the available carbohydrates and induce intestinal acidification[76]. In the human small intestine, a dominant bacterial phylum is *Streptococcus sp.*[77,78], which is an anaerobe that can ferment relatively simple carbohydrates at a high rate [79]. According to the above, luminal acidity markedly induces small-intestinal Mg^{2+} absorption. Therefore, small-intestinal fermentation should induce small-intestinal Mg^{2+} absorption.

CONCLUSION

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