ABNORMAL RENAL HANDLING OF MAGNESIUM

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ABSTRACT

Magnesium (Mg^{2+}) is the fourth most abundant cation in the human body and the second most common cation in the intracellular fluid. The strict control of plasma Mg^{2+} level is essential for many physiological processes such as cell permeability, neurotransmitter release, muscle contraction, hormone receptor binding, neuronal activity and cardiac excitability. It has a fundamental role as a co-factor in more than 300 enzymatic reactions involving energy metabolism and synthesis of nucleic acids. Plasma Mg^{2+} concentration is tightly regulated by the dynamic balance and interplay between intestinal absorption, exchange from bone and renal reabsorption. The kidney plays a central role in maintaining magnesium homeostasis. The majority of filtered Mg^{2+} is reabsorbed in the thick ascending limb of the loop of Henle by a passive paracellular transport, mediated by tight junction proteins claudin-16 and -19. Their mutations result in increased urinary Mg^{2+} excretion and hypomagnesemia. The "fine-tuning" of Mg^{2+} reabsorption takes place along the distal convoluted tubules where Mg^{2+} is reabsorbed by an active transcellular transport via transient receptor potential channel melastatin 6 (TRPM6). This channel regulates the apical entry of magnesium into epithelia and alters whole-body magnesium homeostasis by controlling urinary excretion. TRPM6 is controlled by numerous factors and hormones at the level of transcription, membrane expression, and function.

Key words: *magnesium reabsorption, claudin-16/19, TRPM6, hypomagnesemia*

INTRODUCTION

Magnesium (Mg^{2+}) is the fourth most abundant cation in the human body and the second most common cation in the intracellular fluid. The precise control of plasma Mg^{2+} level is essential for many physiological processes such as cell permeability, neurotransmitter release, muscle contraction, hormone receptor binding, neuronal activity and cardiac excitability [29]. Magnesium has a fundamental role as a co-factor in more than 300 enzymatic reactions involving energy metabolism, synthesis of proteins and nucleic acids [26]. In healthy people, plasma magnesium is carefully regulated within the narrow range of 0,7-1,1 mmol/l. This article reviews the role of the kidneys in magnesium homeostasis and also discusses genetic and drug-induced causes of renal Mg^{2+} wasting.

MAGNESIUM REABSORPTION ALONG RENAL TUBULES

Approximately 70 - 80 % of total plasma Mg^{2+} (20-30% is protein bound) is filtered in the glomerulus, which accounts about 2000-2400 mg per day. Under normal conditions 95-97% of filtered magnesium is reabsorbed in the renal tubules and only 3-5% is excreted in the urine i.e.~100 mg [8]. 10-25% of the filtered magnesium is reabsorbed in the proximal tubule (PT). The exact mechanisms are not known, magnesium is believed to be absorbed via passive paracellular transport, facilitated by the increased intraluminal magnesium concentration, created by water uptake via aquaporin 1 (AQP1) [27].

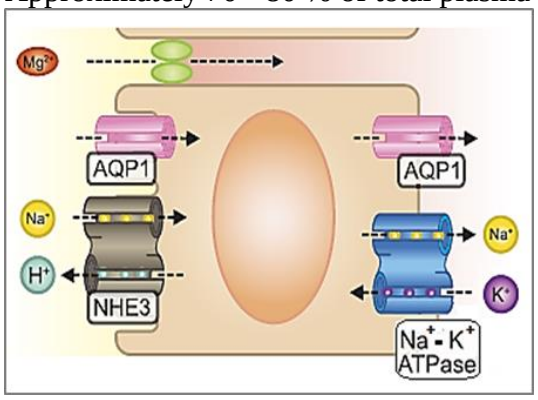

Figure 1. Mg^{2+} reabsorption in PT.

Mg^{2+} reabsorption mainly occurs in the late parts of the PT, where the concentration gradient is sufficient to favor the passive transport (fig. 1). Previous Na^{+} reabsorption is

required to drive water transport that is a prerequisite for Mg^{2+} reabsorption. Disturbances of proximal tubular Mg^{2+} reabsorption do not result in clinical symptoms, because distal tubules (DT) could compensate decreased Mg^{2+} uptake in PT [17].

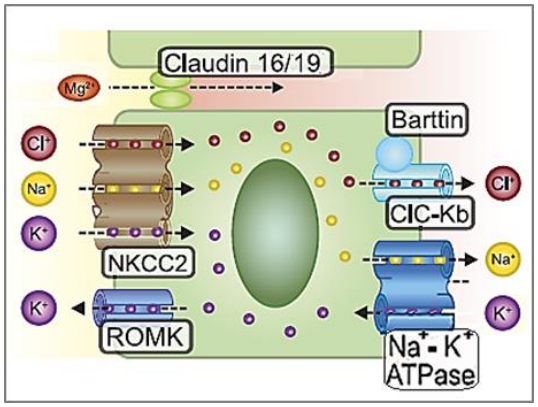

Figure 2. Mg^{2+} reabsorption in TAL.

Approximately 50 - 70% of filtered Mg^{2+} is reabsorbed in the thick ascending limb of the loop of Henle (TAL) [19]. The passive paracellular transport in this segment is mediated by tight junction proteins claudin-16 and -19 and depends on lumen-positive transepithelial voltage (+10 mV). This potential gradient is created by the activity of the Na^+-K^+-$2Cl^-$ cotransporter (NKCC2) and the subsequent secretion of K^+ at the apical membrane [13]. Na^+ enters thick ascending loop cells via the apical KNKCC2. Basolateral Na^+-K^+-ATPase plays a key role in this transport, maintaining a low intracellular Na^+ concentration that provides a gradient for Na^+ entry. K^+ is recycled back into the luminal space via the renal outer medullary K^+ (ROMK) channel and is involved in generating and maintaining a lumen-positive potential required for paracellular magnesium transport. Efflux of chloride (Cl^-) occurs through basolateral channel CLCKb (fig. 2). Magnesium transport in TAL is influenced by the calcium-sensing receptor (CaSR) in the basolateral membrane.

Claudins are tight junction integral membrane proteins that are key regulators of the paracellular pathway. Claudin-16 is a highly negative charged protein. This negative charge contributes to the cationic selectivity of the reabsorptive paracellular pathway. The interaction between claudin-16 and claudin-19 is required for generating specific cation-permeable channels [16].

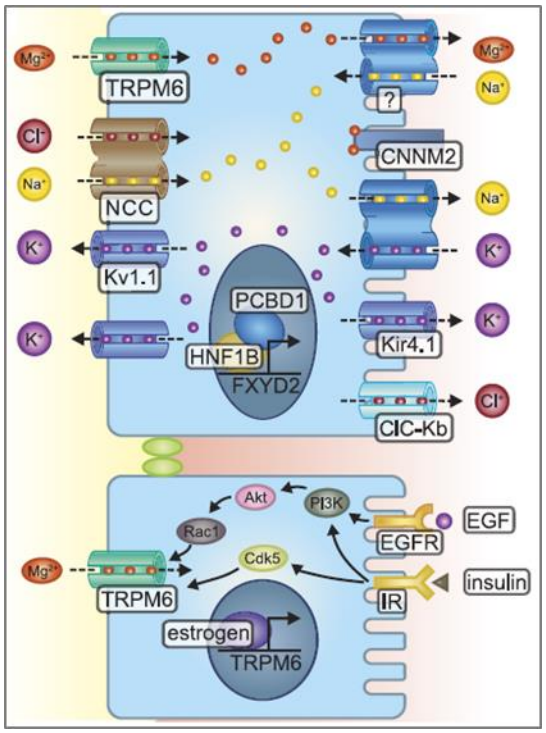

Figure 3. Mg^{2+} reabsorption in the DCT

The "fine-tuning" of Mg^{2+} reabsorption takes place along the distal convoluted tubules (DCT) as no reabsorption takes place beyond this segment. Approximately 10% of filtered Mg^{2+} is reabsorbed by an active transcellular transport via TRPM6 [11].

Luminal Mg^{2+} entry depends on the apical membrane potential in the DCT cells, which is negative (approximately -70 mV) and is maintained by the apical voltage-gated K^+ channel, Kv1.1 [12]. Because intracellular and extracellular Mg^{2+} concentrations are comparable, membrane potential provides the driving force for Mg^{2+} entry [36]. Na^+ enters the cell via Na^+-Cl^--cotransporter (NCC) expressed on the apical membrane of the DCT (23). Basolateral Na^+- K^+-ATP ase and K^+ recycling through Kir4.1 channel can alter Mg^{2+} reabsorption, regulating intracellular voltage needed for Mg^{2+} transport (fig. 3).

At the basolateral membrane, extrusion of Mg^{2+} occurs against a steep electrochemical gradient via a recently identified magnesium/sodium exchanger SLC41A1

family [10].

Mg^{2+} reabsorption in the DCT is tightly regulated by plasma Mg^{2+} levels [15]. The acid-base status of an individual affects the body's handling of Mg^{2+} through an alteration in levels of TRPM6 [24].

TRPM6 are regulated by numerous factors at the level of transcription, plasma membrane availability, and activity [5]. EGF and insulin act on TRPM6 by a PI3K-Akt-Rac1 dependent mechanism, increasing the insertion of TRPM6 in the membrane. Insulin may directly affect TRPM6 activity through cylin-dependent kinase 5 (cdk5)-dependent phosphorylation of the channel. Patients with reduced EGFR or insulin receptor (IR) activity are therefore more susceptible to hypomagnesemia [22]. Renal EGF acts in an autocrine or a paracrine manner to increase TRPM6 activity stimulating the Mg^{2+} reabsorption in DCT [21].

Estrogens also affect Mg^{2+} reabsorption in DCT by increasing the expression of TRPM6 (fig. 3). Experiments with ovariectomized rats showed a decrease in levels of TRPM6 (and magnesuria) that was normalized by administration of the hormone [14].

GENETIC AND DRUG-INDUCED CAUSES OF RENAL MAGNESIUM WASTING

Hypomagnesemia is defined as serum Mg^{2+} concentration below 0,70 mmol/l. Several inherited renal tubular disorders are associated with excessive urinary loss of magnesium. The genetic causes of hypomagnesemia are heterogeneous and comprise both recessive and dominant disorders.

Recessive mutations in CLDN16 (encoding claudin-16) and CLDN19 (encoding claudin-19) are the most frequent cause of hypercalciuric hypomagnesemia, because disrupt the pore selectivity of the tight junction in the TAL, impairing paracellular reabsorption of Ca^{2+} and Mg^{2+}. Patients suffer from hypomagnesemia and its associated symptoms, childhood nephrocalcinosis possibly due to the hypercalciuria and polyuria with polydipsia due to additional sodium and volume loss [18, 40].

Gitelman's syndrome is caused by recessive mutations in SLC12A3, the gene encoding the Na^+-Cl^--cotransporter (NCC) expressed on the apical membrane of the DCT. It is characterized by

hypomagnesemia, hypokalemic metabolic alkalosis, hypocalciuria and commonly observed periods of muscle weakness and tetany [33].

Mutations in the gene encoding the calcium sensing receptor CaSR (CASR) are associated with hypercalciuric hypocalcemia and occasionally with hypomagnesemia [39].

Hypomagnesemia is also caused by mutation on the FXYD2 gene that encodes the γ-subunit of the Na^+-K^+-ATPase. The γ-subunit has an important role in modulating the activity of the Na^+-K^+-ATPase, which maintains the membrane potential and the Na^+ gradient. Suboptimal Na^+-K^+-ATPase activity leads to depolarization of the DCT due to the $3Na^+$-to-$2K^+$ exchange ratio, which reduces Mg^{2+} reabsorption via TRPM6. Patients suffered from convulsions, probably due to the low serum Mg^{2+} levels (<0,4mmol/l) and other hypomagnesemia-related symptoms [2, 7, 9].

Mutation in the EGF gene is characterized by hypomagnesemia (0,53-0,66mmol/l), epileptic seizures, moderate mental retardation, and normal Ca^{2+} handling. The EGF gene encodes for pro-EGF, a small peptide hormone expressed in gastrointestinal tract, respiratory tract, and kidney (primarily DCT). After insertion at the basolateral membrane, pro-EGF is processed into EGF that is able to stimulate locally the EGF receptor. EGF was defined as the first magnesiotropic hormone that significantly stimulate the activity of TRPM6 and Mg^{2+} transport. Some studies reported that EGF increases TRPM6 cell surface abundance by redistributing the channel from intracellular vesicles to the plasma membrane[34].

Mutations in the gene encoding apical TRPM6 Mg^{2+} channel in DCT cause the most profound

genetic hypomagnesemia [38]. A defect in the TRPM6 channel impairs epithelial Mg^{2+} resorption in the DCT, causing severe hypomagnesemia (0,1-0,4 mmol/l), secondary hypocalcemia, disturbed neuromuscular excitability, muscle spasms, tetany and convulsions [37].

Mutations in the KCNJ10 gene, encoding an inwardly rectifying K^+ channel (Kir4.1), also causes hypomagnesemia. Kir4.1channels are located at the basolateral membrane of the DCT and allows K^+ recycling, required for normal activity of the Na^+-K^+- ATPase. Impairment of the basolateral Na^+-K^+-ATPase due to the loss of Kir4.1 function may result in depolarization of the apical membrane and therefore reduction of the Mg^{2+} influx via TRPM6. Affected patients suffered from seizures, sensorineural deafness, ataxia, mental retardation, and electrolyte imbalance (SeSAME) [6,31].

Mutation in the KCNA1 gene, encoding the voltage-gated potassium (K^+) channels Kv1.1

is characterized by low serum Mg^{2+} levels (0,28- 0,37 mmol/l), recurrent muscle cramps, tetany, tremor, muscle weakness, cerebellar atrophy, and myokymia. Kv1.1 channels are localized in DCT and establish a favorable luminal membrane potential in tubular cells required to control TRPM6-mediated Mg^{2+} reabsorption [12].

Hypomagnesemia is present in some patients with Bartter's syndrome, that is caused by mutations in NKCC2, or ROMK, or ClC-Kb, or Barttin (an essential b-subunit for ClC-Ka and ClC-Kb chloride channels) [33].

Acquired hypomagnesaemia is a well-known side effect of a number of different medications, such as thiazide diuretics, cisplatin, aminoglycoside antibiotics, calcineurin inhibitors or antibodies against epidermal growth factor receptor. Inhibition of NKCC2 by furosemide diuretics decreases TAL Mg^{2+} reabsorption. Furosemide inhibits the activity of NKCC2, reducing the positive transepithelial membrane potential that drives paracellular Mg^{2+} transport in TAL. This results in loss of lumen-positive potential, decreasing the driving force for paracellular magnesium reabsorption via claudin-16 and claudin-19 [28].

The use of thiazide diuretics, which inhibit NCC in DCT, frequently induces renal Mg^{2+} wasting [23]. In mice, thiazide treatment reduces the renal expression of TRPM6, which can explain the high urinary Mg^{2+} excretion [25].

The use of the EGFR inhibitor cetuximab can result in severe hypomagnesemia. Cetuximab is a monoclonal antibody against the EGFR and is prescribed for the treatment of colorectal or head and neck cancer [32].

The calcineurin inhibitors (CNI) cyclosporin A (CsA) and tacrolimus (FK506) are immunosuppressant drugs of choice after transplantation. The use of CNIs has been associated with hypertension and renal Mg^{2+} wasting. The mechanism of Mg^{2+} wasting is not fully understood, but results of some experiments suggested that CsA may interfere with the EGF signaling pathway in DCT cells [35].

Hypomagnesemia is a frequent complication of cisplatin treatment as its effect on electrolyte wasting is highly specific for Mg^{2+}. Cisplatin causes hypomagnesemia in 40% - 80% of treated patients [30]. Recently, two animal studies have examined the effects of cisplatin treatment in detail. Both observe significant downregulation of TRPM6 mRNA levels [20].

Several classes of antimicrobials may cause hypomagnesemia, the underlying mechanisms leading to Mg^{2+} wasting differ greatly. Aminoglycoside antibiotics (AGA) including gentamycin, neomycin, tobramycin, and amikacin may induce renal Mg^{2+} wasting by reducing the expression of NKCC2 that provides the driving force for TAL Mg^2 transport [10]. The use of pentamidine (an antimicrobial against Pneumocystis jirovecii infections), has been associated with severe hypomagnesemia due to renal Mg^{2+} wasting, but the exact mechanism of reduced Mg^{2+} reabsorption

remains unresolved [4]. Rapamycin (an antibiotic that is frequently used to prevent organ rejection after transplantation) causes hypomagnesemia in 10–25% of patients [1]. Amphotericin B (an antifungal agent) is also associated with hypomagnesemia and hypokalemia [3].

CONCLUSION

The maintaining of the Mg^{2+} concentrations within normal limits is of vital importance. The kidney plays a crucial role in this regulation. The renal excretion of the filtered load varies from 0.5 to 70%. The kidney is able to conserve magnesium during magnesium deprivation by reducing its excretion or rapidly increase Mg^{2+} excretion in cases of excess intake. Several inherited disorders associated with perturbations in renal magnesium reabsorption lead to severe hypomagnesemia. Some drugs such as thiazide diuretics, cisplatin, aminoglycoside antibiotics, calcineurin inhibitors or antibodies against epidermal growth factor receptor are also associated with hypomagnesemia due to the disturbances of renal magnesium transport.

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