

Original Article

Npt2b plays a major role in phosphorus transportation and homeostasis in colon and small intestine of uremia patients

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Abstract: Objective: To explore the mechanism about how phosphorus in colon was excreted specifically. Methods: Male rats were divided into 3 groups: control, sham and uremia. Uremia rat models were constructed by 5/6 renal excision method to raise the content of serum phosphorus. After construction successfully, the content of serum and excrement phosphorus were determined. The mRNA level of Npt2b in the epithelial cells of colon and small intestine was determined by qRT-PCR. The protein expression of Npt2b in colon and small intestine epithelial cells was determined by western blot assay. Results: The content of serum and excrement phosphorus in uremia group was significantly higher than that in the control and sham group. The relative mRNA level of Npt2b in the epithelial cells of colon was significantly lower than that in small intestine, and the value in model group was markedly decreased compared with the control and sham group. Conclusion: Phosphorus in colon of uremia rats can be specifically excreted via Npt2b pathway.

Keywords: Npt2b, phosphorus, uremia, colon, small intestine

Introduction

Chronic kidney disease (CKD) is a general term for heterogeneous disorders affecting kidney structure and function [1]. Outcomes of CKD include not only kidney failure but also complications of decreased kidney function [2]. Hyperphosphatemia, calcitriol deficiency, and secondary hyperparathyroidism (SHPT) are common complications of CKD [3]. Hyperphosphatemia is a predictable consequence of chronic renal failure and is present in most patients on dialysis [4]. Elevated serum phosphorus has been identified as a cardiovascular risk factor in CKD patients and a clear understanding of phosphorus homeostasis is very important for practicing nephrologists [5]. Dysregulation of calcium (Ca) and phosphate (P) metabolism is common in CKD patients [6]. High plasma phosphate is a risk factor for decline in renal function. Dietary restrictions to control serum phosphorus, which are routinely recommended to persons with chronic kidney disease, are usually associated with a reduction in protein intake [7].

The intestine and kidney play important roles in the absorption of phosphorus (in the form of Pi) from the diet and in the excretion of phosphorus (in the form of Pi) in the urine, respectively [8]. In states of neutral phosphorus balance, the amount of phosphorus absorbed in the intestine (13 mg/kg/day~1-1.5 g/24 h) is equivalent to the amount excreted in the urine. Various hormones and factors involved in the regulation of phosphorus homeostasis alter the efficiency of Pi absorption in the intestine or the reabsorption of Pi in the proximal tubule of the kidney. In the kidney, Pi is reabsorbed along the proximal convoluted and proximal straight tubule, and Pi reabsorption is influenced by numerous factors. Serum Pi concentrations can be altered significantly without changes in the absorption of Pi in the intestine or changes in excretion in the kidney. The small intestine is an important site for phosphate absorption. Early studies showed that Pi transport through the apical membrane of small intestinal epithelial cells is coupled with sodium [9]. Intestinal phosphate sensing increases the fractional excretion of phosphorus in the kidney [10]. Intestinal

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Table 1. Comparison of serum creatinine and blood urea nitrogen in the 3 groups

Group	N	C (serum creatinine)/(umol/L)	C (blood urea nitrogen)/(mmol/L)
Control	18	26.40 ± 6.75	2.75 ± 0.48
Sham	18	27.96 ± 6.41	3.04 ± 0.61
Uremia	18	66.75 ± 8.67*	10.32 ± 2.36*

* $P < 0.05$.

Npt2b plays a major role in phosphate absorption and homeostasis [11]. Maintaining phosphate homeostasis is essential and any deviation can lead to several acute and chronic disease states [12].

In this study, we aimed to study the role of Npt2b in mediating the transportation of phosphorus from kidney to intestine.

Material and methods

Experimental groups

Male Wistar rats (approximately 10 weeks old) weighing 180 to 200 g were purchased from the Center of Experiment and Animal in Shanghai and used for the study. They were maintained on a 12-hour light/dark cycle with continuous access to food and water. All animals were treated according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All animal procedures were conducted after gaining the approval of the animal care committee of Shanghai resident standardization training base.

Rats were divided at random into the following 3 groups: (1) the control group: rats were treated with nothing; (2) the sham group: only the renal capsule of both kidneys in rats were stripped; (3) the uremia group: models were constructed by 5/6 renal excision method and penicillin was injected for 3 days after surgery to resist infection. After 6 weeks, the renal function was determined and the level of serum creatinine was 2~3 times the level of the normal rats means models were constructed successfully. All rats in the 3 groups were intramuscular injected penicillin and gentamycin to prevent peritonitis. The content of phosphate in blood and excrement was determined by two-point method.

qRT-PCR

The mRNA level of Npt2b in epithelial cells of small intestine and colon was determined by

qRT-PCR. For RNA isolation, total RNA was extracted and isolated from tissue samples or cell lines using either the mirVana miRNA isolation kit (Ambion, Austin, TX) or the TRIzol method. Trizol of 1 mL was added and the solution

was mixed homogeneously for 10 min. The mixture was then transferred into eppendorf tubes (EP, 1.5 mL) with 200 μ L chloroform. After 15 min shake, the EP tubes were centrifuged at 4°C for 15 min (12000 \times g). The supernatant was transferred into other EP tubes and mixed with isopyknic isopropanol for 15 s. The centrifugation (4°C, 10 min, 12000 \times g) was carried out again and the supernatant was discarded. The precipitate was washed by 75% ethanol twice and dissolved into 30 μ L diethylpyrocarbonate (DEPC) after dried to obtain RNA stock solution. After isolation, the concentration of RNA was assessed using a NanoDrop 1000 spectrophotometer (NanoDrop Technologies, Wilmington, Delaware, USA) and the RAN solution was stored at -80°C for further use. Genes were amplified by specific oligonucleotide primer, and human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was used as an endogenous control. The detection and quantification contained the following steps: first, reverse transcription was performed at 55°C for 30 min, initial activation for 15 min at 95°C, next 40 cycles of denaturation were conducted at 94°C for 15 s, then annealing for 30 s at 55°C, extension for 30 s at 72°C. The expression level was normalized using U6 small nuclear RNA by the 2- Δ Ct method. The Δ Ct values were normalized to GAPDH level.

Western blot

Cells were washed thrice with PBS and transferred to buffer containing 25 mM HEPES, 2.5 mM EDTA, 0.1% Triton X-100, 1 mM PMSF and 5 μ g/mL leupeptin. The mixture was centrifuged at 3000 r/min for 10 min and the supernatant was stored at 4°C. Total protein concentrations were determined with a UV spectrophotometer using a modified Bradford assay (Beckman Coulter, Fullerton, CA, USA). Protein (15 μ L) were separated on 5% stacking/15% SDS-polyacrylamide gel electrophoresis gels and transferred to nitrocellulose membranes (Millipore, Billerica, MA), which had been infiltrated

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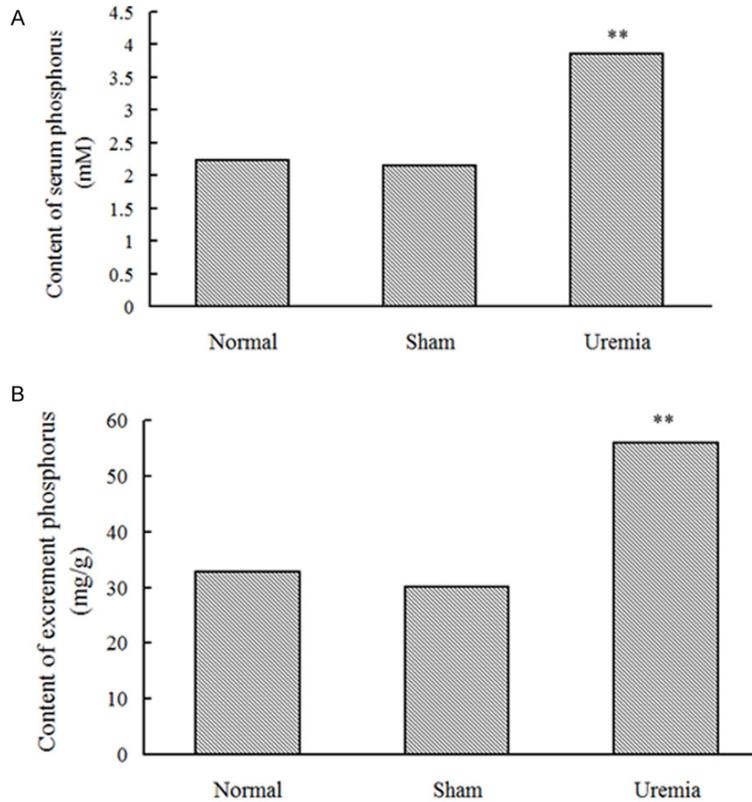


Figure 1. The content of serum (A) And excrement phosphorus (B) Determined in the control, sham and uremia group. ** $P < 0.01$, compared with the control and sham group, the content of serum or excrement phosphorus in uremia group had significant difference.

by methanol and transfer membrane liquid. The membrane was washed by TBST (50 mM Tris, 150 mM NaCl and 2% Tween-20; pH 7.5) for 3 times and each for 10 min at room temperature, and incubated at 4°C overnight with a polyclonal antibody against THARP1 (Santa Cruz, SC12013). Then incubation with the secondary antibody was performed at room temperature for 1 h. Membrane was washed again with TBST and incubated in SuperECL Plus detection reagent (Nanjing KeyGEN Biotech, KGP1123, China), which produced a chemiluminescence signal that was detected by exposure to X-ray film. Images were scanned and analyzed semiquantitatively using Image (National Institutes of Health, Bethesda, MD) and Image Gauge software (Fujifilm, Tokyo, Japan). The samples were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Statistical analysis

Statistical analysis was performed by SPSS 19.0 statistical software. All data were expressed

as means \pm SD from at least three independent experiments. P values were determined using one-way ANOVA. Significance was defined as $P < 0.05$.

Results

Construction of uremia models

The construction results were shown in **Table 1**. As shown, the levels of serum creatinine and blood urea nitrogen in uremia group were markedly increased compared with the control and sham group.

Level of phosphorus in serum and excrement

After uremia rat models were constructed successfully, the level of phosphorus in serum and excrement was determined by fully automatic biochemical analyzer. The results were shown in **Figure 1**. As shown, the phosphorus level in serum in uremia group was significantly higher than that in the control and sham group, while the level in control and sham group was almost the same (**Figure 1A**). The results on phosphorus in excrement shown the level of phosphorus in excrement in uremia group was markedly higher than that in the control and sham group (**Figure 1B**).

Level of Npt2b was upregulated in uremia

To explore the role of Npt2b in small intestine and kidney, we determined the mRNA level of Npt2b in small intestine and kidney in the control, sham and uremia group. Results showed the mRNA level of Npt2b in kidney was much lower than that in small intestine. Moreover, Npt2b level in uremia group was much lower than the control and sham group (**Figure 2**). To further verify the change, we also determined the protein expression of Npt2b in small intestine and kidney by western blot. Results showed the protein expression of Npt2b in uremia group was decreased compared with the control and sham group. Moreover, the expression

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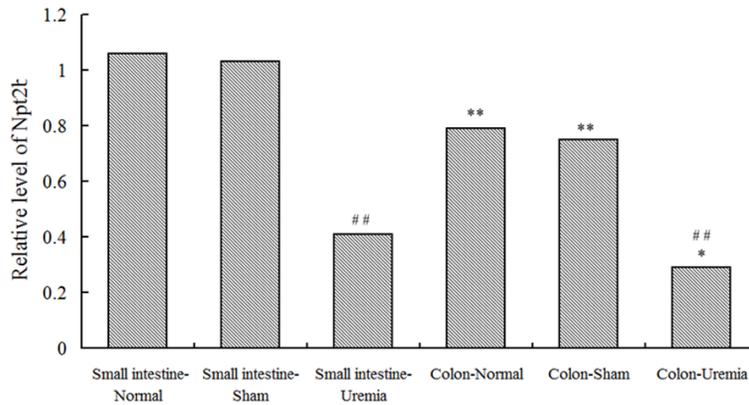


Figure 2. Relative mRNA levels of Npt2b in small intestine and colon in the control, sham and uremia group by qRT-PCR. * $P < 0.05$ or ** $P < 0.01$, compared with the small intestine group, the relative mRNA levels of Npt2b in colon group had significant difference. ## $P < 0.01$, compared with the control and sham group, the relative mRNA levels of Npt2b in uremia group had significant difference.

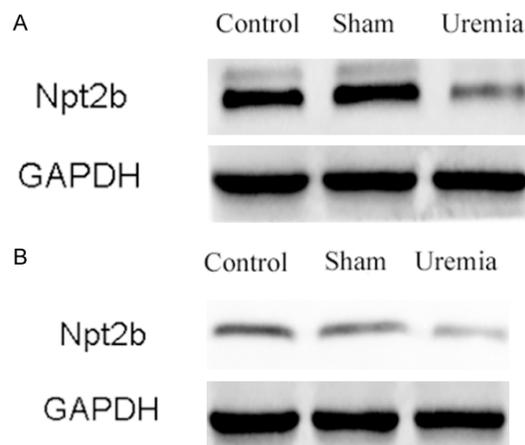


Figure 3. Protein expression values of Npt2b in small intestine and colon in the control, sham and uremia group by western blot assay. ** $P < 0.01$, compared with the control and sham group, the protein expression of Npt2b in uremia group had significant difference.

value in kidney was much lower than that in small intestine (**Figure 3**).

Discussion

Chronic kidney disease is a worldwide public health problem with an increasing incidence and prevalence, poor outcomes, and high cost [2]. Hyperphosphatemia is a predictable consequence of chronic renal failure and is present in most patients on dialysis [4]. Phosphorus plays a critical role in diverse biological processes. The regulation of phosphorus balance and ho-

meostasis are critical to the well being of the organism. Changes in environmental, dietary and serum concentrations of inorganic phosphorus are detected by sensors that elicit changes in cellular function and alter the efficiency by which phosphorus is conserved [13]. Inorganic phosphorus participates in the composition of cellular structure and many important biological functions [14]. Inorganic phosphorus actively performs inverse chemical and electric potential gradient across a cell membrane transport [15]. In mammalian cells, this task is completed by Npt which use free energy as driving force to increase the intake of phosphorus.

Npt2 belongs to SLC34 phosphorus transport protein family, mainly transports HPO_4^{2-} and plays a key role in maintaining the balance of phosphorus metabolism in the body [16]. Able-bodied person maintain the normal of blood phosphorus by adjusting the function of intestine, kidney and bone. Intestinal tongue adjusts the role of kidney on phosphorus excretion by influencing the absorption of phosphorus and shedding [17]. Rapid adjustment of intestines phosphorus on the excretion of renal phosphate relies on Npt2b to mediated phosphorus transport across the membrane. The phosphorus in food is mainly absorbed in small intestine and Npt2b in apical membrane of intestinal epithelial cells transports phosphorus to the body by sodium and phosphorus co-transportation [18]. Npt2b is mainly expressed in intestinal epithelial cells of intestinal and also has a small amount of expression in colon epithelial cells also has a small amount of expression [19]. In this study, we aimed to study the role of Npt2b in adjusting phosphorus content in colon and intestines.

Male rats were purchased and divided into 3 groups: control, sham and uremia group. In uremia group, uremia rat models were constructed by 5/6 renal excision method. After uremia rat models were successfully constructed, the contents of serum and excrement phosphorus were determined. Results showed the content of serum or excrement phosphorus in uremia group was significantly higher than that in the control and sham group. The determination on

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mRNA level of Npt2b in small intestine and colon by qRT-PCR showed the value in colon was significantly lower than in small intestine. Moreover, the value in uremia group was much lower than in the control and sham group in both small intestine and colon experiments. The results on protein expression of Npt2b in small intestine and colon by western blot were kept consistent with those of qRT-PCR. Hyperphosphatemia is a common complication of CKD and is closely related to the high mortality of cardiovascular disease in CKD population [20]. Phosphorus distribution and transfer in both inside and outside of the cells are regulated by Npt. Npt2b participates in the reabsorption of phosphorus in primary liver bile [21]. In salivary gland, Npt2b also takes part in the secretion of salivary phosphorus [22]. All those indicated Npt2b could promote the excretion of phosphorus.

In conclusion, in high phosphorus environment, the expression of Npt2b gene mediated the transport of phosphorus. Prevention on hyperphosphatemia mainly included restrictions on the intake of phosphorus and promotion on phosphorus excretion. Therefore, in the research and treatment of hyperphosphatemia, Npt2b is a preferable breakthrough and need further in-depth study.

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Disclosure of conflict of interest

None.

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