Urinary Prostaglandins and the Effect of Indomethacin on Phosphate Excretion in Children With Hypophosphatemic Rickets

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ABSTRACT: We recently reported the urinary prostaglandin E_2 /creatinine ratio (PGE_2/Cr) was markedly elevated in *Hyp* mice, the animal model for X-linked hypophosphatemia, compared with control mice. We provided evidence for altered prostaglandin production mediating the phosphaturia and that indomethacin decreases urinary phosphate excretion in *Hyp* mice but not control mice. To determine the levels of urinary PGE_2/Cr , the safety and efficacy of indomethacin on phosphate excretion in children with hypophosphatemic rickets (HPR), a prospective clinical trial was performed in 16 children with HPR and 16 age- and gender-matched healthy controls. Urinary PGE_2/Cr excretion was determined on a 24 h timed urine collection. A randomized cross over, placebo *versus* indomethacin, clinical trial was performed in the 16 children with HPR. There was no difference in urinary PGE_2/Cr excretion between controls and patients with HPR. In children with HPR, indomethacin treatment for 3 mo had no significant effect on serum phosphorus or urinary phosphate excretion. In conclusion, urinary prostaglandin excretion is similar in children with HPR compared with controls. Indomethacin had no significant effect on serum phosphorus or urinary phosphate excretion in children with HPR. (*Pediatr Res* 64: 210–212, 2008)

Hypophosphatemic rickets (HPR) is characterized by hypophosphatemia because of impaired proximal tubular reabsorption of phosphate. HPR is associated with X-linked hypophosphatemia (XLH), autosomal dominant hypophosphatemic rickets, oncogenic rickets, and Fanconi's syndrome. XLH is the most common inherited defect in tubular phosphate transport. The resulting hypophosphatemia leads to defective bone mineralization (1). XLH is secondary to a mutation in a phosphate-regulating gene with endopeptidase activity located on the X chromosome (2). Most patients with XLH have elevated serum fibroblast growth factor-23 (FGF-23) levels (3), as do patients with tumor induced osteomalacia and autosomal dominant hypophosphatemic rickets (4,5). We have recently shown that *Hyp* mice have elevated urinary prostaglandin E_2 /creatinine levels (PGE_2/Cr) compared with controls (6) and that administration of FGF-23 to control mice increases urinary PGE_2/Cr excretion and renal tubular production of PGE_2 (7). In addition, administration of indomethacin to *Hyp* mice results in an increase in proximal tubule phos-

phate transport, a reduction in fractional excretion of phosphate and an increase in serum phosphorus levels (6). The aim of the present study was to determine urinary PGE_2/Cr excretion levels in patients with HPR compared with controls and whether treatment with indomethacin for 3 mo will decrease phosphate excretion in children with HPR.

MATERIALS AND METHODS

We prospectively measured urinary PGE_2/Cr in 24-h urine collections in seven male and nine female children with HPR and 16 age and sex matched controls. Fifteen of the patients with HPR had a family history consistent with an X-linked mode of inheritance. The diagnosis of HPR was established by the clinical and biochemical criteria we have described previously (8). The average age of the patients with XLH was 11.8 ± 0.9 y (range 5–17 y). Sixteen healthy volunteer children were recruited to serve as controls 12.1 ± 1.0 y (range 5–18 y).

We also performed a prospective double-blind crossover study using indomethacin (Qualitest Pharmaceuticals, Inc., Huntsville, Alabama) as the treatment drug and a placebo as control drug in the above children with HPR. The dose of indomethacin was 2 mg/kg/d rounded to the next higher multiple of 25 mg (maximum 150 mg/d). The indomethacin was administered three times daily. The indomethacin dose once determined was not altered during the study. Indomethacin was pulverized and placed in 25 mg capsules to match placebo capsules; the latter were compounded using sodium bicarbonate fillers (Pharmacy Compounding Specialties, Dallas, Texas). Patients were randomized to receive either placebo or drug for 3 mo then crossed over to receive either drug or placebo for additional 3 mo. Data from only 11 of the above patients who completed the 6-mo study were analyzed. Five patients exited the study because of either drug intolerance or they did not adhere to the study protocol. There were four males and seven females who completed the study. All patients who entered the drug study had XLH.

At the time of enrollment in the study, all patients with XLH were maintained on calcitriol (Rocaltrol, Hoffmann-LaRoche, Basel Switzerland) at 17.8 ± 2.3 ng/kg body wt/d, and oral phosphate at 10.3 ± 2.4 mg/kg body wt/d. Conventional therapy with calcitriol and oral phosphate was adjusted to maintain clinical remission of rickets, normal PTH levels, and a urinary calcium excretion of less than 1 mmol/kg body wt/d. The phosphate and calcitriol doses were not altered after initiation of the study. Four patients were treated with hydrochlorothiazide (0.53 ± 0.24 mg/kg/d) to control their hypercalcaemia. Evaluation of study subjects with XLH included: a) physical exam; b) blood analysis for a creatinine, electrolytes, calcium, phosphorus, alkaline phosphatase, PTH, 1,25 (OH) $_2$ vitamin D, osteocalcin; c) timed urine for creatinine, calcium and PGE_2 excretion. Tubular maximum of phosphate reabsorption (TmP/GFR) was computed using the formula:

$$TmP/GFR = S_p - \left[\frac{U_p \cdot S_{cr}}{U_{cr}} \right]$$
 as previously described, where S_p and S_{cr} are serum phosphorus and creatinine, respectively, and U_p and U_{cr} are urine phosphate and creatinine, respectively (9). Control subjects had a 24 h timed urine collection for creatinine, calcium, and PGE_2 . Urinary PGE_2 levels were measured by RIA (Core Endocrine Laboratories, Hershey, PA). All timed

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Abbreviations: ADHR, autosomal dominant hypophosphatemic rickets; HPR, hypophosphatemic rickets; PGE_2/Cr , prostaglandin divided by urine creatinine; TmP/GFR , tubular maximum for the reabsorption of phosphate; XLH, X-linked hypophosphatemia

Table 1. Serum indices and urinary indices in children with XLH (prior to treatment with indomethacin) and age matched controls

	Reference range	XLH (n = 16)	Control (n = 16)	p
Serum phosphorus (mmol/L)	0.80–1.60	0.81 ± 0.05	N/A	N/A
Serum calcium (mmol/L)	2.20–2.58	2.21 ± 0.02	N/A	N/A
Serum osteocalcin (ng/mL)	16.3–68.7	87.81 ± 9.42	N/A	N/A
Alkaline phosphatase (IU/L)	Age dependent references (10)	414.63 ± 42.01	N/A	N/A
Intact-PTH (pmol/L)	9–78	70.4 ± 8.2	N/A	N/A
Serum 1,25 (OH) ₂ Vit D (pg/mL)	27–71	57.6 ± 4.6	N/A	N/A
Urine				
TmP/GFR (mg/dL)	Age dependent references (9)	1.95 ± 0.13	N/A	N/A
Urinary Ca (mmol/kg/d)	<1.0	0.36 ± 0.08	N/A	N/A
Urinary PGE ₂ (ng/24 h)	Age dependent reference values	379.4 ± 83.3	318.1 ± 103.4	p = 0.163
Urinary PGE ₂ /Cr (ng/mg)	—	0.36 ± 0.04	0.32 ± 0.07	p = 0.052

N/A, not available.

urine collections were refrigerated during collection period and transferred to clinic on ice. When received in the clinic, urine samples were frozen at -20°C and shipped to the reference laboratory.

Data are reported as the mean $\pm$ standard error of the mean. Paired two tailed *t* test was used to evaluate the before and after periods. Urinary PGE₂/Cr compared in controls and patients with HPR using the Mann-Whitney Rank Sum Test whereas urinary PGE₂ was compared in controls and patients with HPR using a two tailed unpaired *t* test. Determination of whether there was a correlation between urinary PGE₂/Cr and urine TmP/GFR was made using linear regression. When placebo was given for the first 3 mo, we used data obtained at the 3-mo visit for baseline values. If indomethacin was given for the first 3 mo, we used data obtained at the baseline (or 0 mo) visit for baseline values. The study protocol was approved by the Institutional Review Board of the University of Texas Southwestern Medical Center. The legal guardian of every eligible patient and volunteer control consented to participation in this study.

RESULTS

Serum and urinary indices of children with XLH before treatment with indomethacin and their age-matched controls are shown in Table 1. All children with HPR were in clinical and radiologic remission of their rickets at the time of enrollment into the study. Serum alkaline phosphatase, osteocalcin, and PTH were within ranges normal for age (10). TmP/GFR was low while urinary calcium excretion was normal in all enrolled children with HPR. PGE₂/Cr was 0.43 ± 0.07 ng/mg compared with 0.31 ± 0.03 ng/mg, in male and female patients with HPR, respectively ($p = 0.11$). There was no difference in urinary PGE₂/Cr excretion between controls (0.32 ± 0.07 ng/mg) and patients with XLH (0.36 ± 0.04 ng/mg) ($p = 0.052$) (Fig. 1). There were two very high values for PGE₂/Cr in the control group. The reason for the discordance from the other controls is not clear but they were not ill during the time of study. There was no correlation between urinary PGE₂/Cr levels and TmP/GFR ($R = 0.023$, $p > 0.05$).

The serum and urine biochemical data of patients with HPR before and after treatment with indomethacin are shown in Table 2. Treatment with indomethacin was well tolerated in the children with HPR. Indomethacin treatment for 3 mo decreased PGE₂/Cr excretion (0.34 ± 0.11 versus 0.26 ± 0.14 ng/mg, $p < 0.05$). However, indomethacin had no effect on serum phosphorus (0.79 ± 0.15 versus 0.81 ± 0.10 mM) or urinary phosphate excretion (1.84 ± 0.49 versus 1.94 ± 0.39 mg/dL).

DISCUSSION

Current therapy for HPR includes oral vitamin D and phosphate. Although this therapy is effective in healing rickets

Figure 1. Urinary Prostaglandin excretion (PGE₂/Cr) in controls and patients with XLH (* $p = 0.052$).

and decreasing bone pain, it does not significantly improve growth (8). In addition, this therapy is associated with morbidity including nephrocalcinosis and hyperparathyroidism (11,12). Thus, finding a safer therapy would be of benefit to patients with HPR.

Our previous studies provided several lines of evidence that there is altered prostaglandin production in *Hyp* mice that is mediated by FGF-23 (6,7). Urinary PGE₂ excretion is about 2-fold greater in *Hyp* mice compared with control mice (6). Incubation of a tubule suspension enriched for proximal tubules had a higher rate of production of PGE₂ in *Hyp* mouse tubules than that of control tubules (7). Injection of FGF-23 into C57/B6 mice resulted in an increase in urinary PGE₂/Cr excretion compared with vehicle-treated mice and incubation of a control mouse tubule preparation with FGF-23 resulted in an increase in PGE₂ production compared with vehicle (7). Finally, we demonstrated that PGE₂ could inhibit phosphate transport in the perfused control mouse proximal tubule and incubation of control mouse proximal tubules with PGE₂ results in a decrease brush border membrane vesicle NaPi-2A expression (7).

The potential importance of the above findings was demonstrated by the effect of indomethacin treatment in *Hyp* mice (6). *Hyp* mice treated with indomethacin for 4 d had a decrease of their fractional excretion of phosphate, an increase in their serum phosphorus level, and an increase in proximal tubule

Table 2. Effect of 3 mo of indomethacin on serum and urinary biochemical indices in children with XLH

	Reference range (SI)	Baseline (n = 11)	Indomethacin (n = 11)	p
Indomethacin (mg/kg/d)	2–3	2.8 ± 0.7	2.7 ± 0.8	NS
Serum phosphorus (mmol/L)	0.80–1.60	0.79 ± 0.15	0.81 ± 0.10	NS
Serum calcium (mmol/L)	2.20–2.28	2.21 ± 0.09	2.21 ± 0.12	NS
Serum creatinine (mmol/L)	50–110 µmol/L	42 ± 15	45 ± 15	NS
Alkaline phosphatase (IU/L)	Age dependent references (18)	419 ± 171	415 ± 167	NS
Serum intact-PTH (pmol/L)	9–78	83 ± 35	79 ± 31	NS
Serum osteocalcin (ng/mL)	16.3–68.7	99.3 ± 29.7	106.2 ± 34.5	NS
1,25 (OH) ₂ Vit D (pg/mL)	27–71	60 ± 20	48 ± 14	NS
Urine				
Urinary calcium (mmol/kg/d)	0.12–0.23 gender dependent	0.38 ± 0.29	0.30 ± 0.30	NS
TmP/GFR (mg/dL)	Age dependent references (9)	1.84 ± 0.49	1.94 ± 0.39	NS
Urinary PGE ₂ /Cr (ng/mg)		0.34 ± 0.11	0.26 ± 0.14	p < 0.05

brush border membrane vesicle NaPi-2A protein expression compared with vehicle-treated mice. Control mice treated with indomethacin did not have a difference in any of these parameters. Thus, in *Hyp* mice, indomethacin may have potential benefit, but it was never determined if it improved their rickets with long-term therapy or if long-term therapy with indomethacin resulted in a chronic increase in serum phosphate levels.

Our current study showed that urinary PGE₂/Cr was slightly higher than age- and gender-matched healthy control subjects. Although the difference neared statistical significance ($p = 0.052$), there is a significant amount of overlap between the two groups. A lack of significance may be the result from the number of patients in these groups and the two-outlier controls. In addition, there are several shortcomings to the present study. Alterations in dietary sodium and potassium intake can affect urinary prostaglandin excretion (13). Thus, the fact that our patients were not on a controlled diet could have affected their urinary prostaglandin excretion. In addition, PGE₂ is produced in many nephron segments and urinary PGE₂ reflects the prostaglandin production of all sites of production (14–17). Thus, this study does not address if there is increased proximal tubule prostaglandin production in HPR as we have shown in *Hyp* mice. Although we found a significant reduction in urinary PGE₂/Cr ratios in patients with XLH treated with indomethacin, the reduction was quite small and there was no increase in serum phosphorus levels. In contradistinction to mice, indomethacin treatment in children did not affect serum phosphorus or its urinary phosphate excretion. It is possible that concomitant treatment with calcitriol and phosphorus will have minimized our ability to demonstrate a significant effect of indomethacin on TmP/GFR. In our previous study, *Hyp* mice were calcitriol and phosphorus naïve. Recent evidence has shown that calcitriol increases FGF-23 production and thus could minimize the effect of indomethacin because of higher FGF-23 levels (18). In addition, there was a significant drop out rate in our study that could have affected the overall results. Finally, our study design was a crossover rather than a randomized double blind placebo *versus* indomethacin trial. This study design was used, because of the limited number of patients, but is not ideal. The limitations cited above may have contributed to the negative results of indomethacin therapy in our patients. Further studies are needed to determine whether

nonsteroidal antiinflammatory agents affect urinary phosphate excretion given the limitations of this study. In conclusion, urinary PGE₂ is comparable in children with HPR as in controls. In addition, treatment with indomethacin in the current clinical setting does not appear to be beneficial in children with XLH as it is in *Hyp* mice.

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