Dipyridamole Decreases Renal Phosphate Leak and Augments Serum Phosphorus in Patients with Low Renal Phosphate Threshold

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Abstract. It has been shown that an acute infusion of dipyridamole increased renal phosphate reabsorption in rats and humans. A prospective study was performed to determine whether chronic treatment by dipyridamole given orally could decrease renal phosphate leak and increase serum phosphorus in patients with idiopathic low renal phosphate threshold (TmPO4/GFR < 0.77 mM). Sixty-four patients with low TmPO4/GFR were included and treated with dipyridamole (75 mg, 4 times daily) for more than 12 mo. Serum phosphorus, TmPO4/GFR, parathyroid hormone, serum calcium, and 1,25-dihydroxyvitamin D were measured sequentially before treatment, and after 3, 6 to 9, and 12 mo of treatment. Under chronic treatment with dipyridamole, TmPO4/GFR and serum phosphorus significantly increased in 80% of patients within 3 mo, with maximal values reached within 9 mo. This improvement persisted after 12 mo of treatment. In 28 patients, 1,25-dihydroxyvitamin D concentrations were above the normal range (>42 pg/ml) and normalized in parallel with the increase of serum phosphorus. The 24-h calcium excretion (which was initially increased in patients with high vitamin D concentrations) and urolithiasis decreased under treatment. Ionized serum calcium and parathyroid hormone remained unchanged. After 2 yr, treatment was discontinued in three patients; serum phosphorus and TmPO4/GFR decreased within 1 mo after discontinuation. Dipyridamole at a dose of 75 mg 4 times daily increases low TmPO4/GFR and improves hypophosphatemia in patients with renal phosphate losses and can be used to treat these patients.

Phosphate plays a critical role in cell metabolism and bone mineralization. Under physiologic conditions, serum phosphorus is strictly maintained above 0.8 mM. The kidney is the principal organ that regulates phosphate balance by reabsorbing, mainly in the proximal tubule, approximately 80% of the phosphate filtered by the glomerulus in adults, a process that is controlled by parathyroid hormone (PTH) (1,2). Increased urinary phosphate losses can be secondary to hormone hypersecretion (hyperparathyroidism, secretion of PTH-related peptide), can be due to inherited or acquired renal tubular dysfunction (Fanconi’s syndrome, tubular acidosis), or can be related to a circulating soluble factor (oncogenic osteomalacia), but urinary phosphate leak can also be idiopathic (3,4). This chronic renal phosphate loss can lead to hypophosphatemia, urolithiasis, or bone demineralization (4–8). The treatment of renal phosphate leak usually consists of increasing phosphate intake by oral phosphate and/or vitamin D therapy. However, this treatment may induce nephrolithiasis. It has been demonstrated that dipyridamole, a well known antiaggregant and vasodilatory drug, lowered urinary phosphate excretion in rats (9) and in humans (10). The aim of this prospective study was to determine whether treatment by dipyridamole increases serum phosphorus in patients with hypophosphatemia due to idiopathic increased urinary losses of phosphate.

Materials and Methods

This study was conducted in the department of clinical investigation at Hôpital Bichat and was approved by the center’s ethics committee and the scientific review committee of the Délégation à la Recherche Clinique Assistance Publique-Hôpitaux de Paris. Written informed consent was obtained from each subject. All measurements were made on site.

Inclusion Criteria

The patients included in this study were referred to our department by their physicians for suspicion of renal phosphate leak. Serum phosphorus and fractional excretion of phosphate (FEPO4) were measured in our laboratory, and the ratio between the maximal tubular reabsorption rate for phosphate and GFR (TmPO4/GFR) was calculated according to the nomogram of Bijvoet and Walton (11). Patients with low TmPO4/GFR (<0.77 mM) and hypophosphatemia (<0.8 mM) were included. A total of 64 patients was studied.
Table 1. Main characteristics of patients with or without renal lithiasis

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>With Renal Lithiasis</th>
<th>Without Renal Lithiasis</th>
<th>Unpaired t Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>18</td>
<td>46</td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>46.1 ± 2.0</td>
<td>50.7 ± 1.6</td>
<td>NS</td>
</tr>
<tr>
<td>GFR (ml/min per 1.73 m²)</td>
<td>85 ± 3</td>
<td>89 ± 2</td>
<td>NS</td>
</tr>
<tr>
<td>Serum phosphorus (mM)</td>
<td>0.79 ± 0.02</td>
<td>0.77 ± 0.02</td>
<td>NS</td>
</tr>
<tr>
<td>TmPO4/GFR (mM)</td>
<td>0.60 ± 0.02</td>
<td>0.58 ± 0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Ionized serum calcium (mM)</td>
<td>1.20 ± 0.01</td>
<td>1.21 ± 0.02</td>
<td>NS</td>
</tr>
<tr>
<td>PTH (pg/ml)</td>
<td>33 ± 2</td>
<td>38 ± 2</td>
<td>NS</td>
</tr>
<tr>
<td>1,25(OH)₂vitamin D (pg/ml)</td>
<td>49.3 ± 2.8</td>
<td>37.6 ± 1.9</td>
<td>P &lt; 0.005</td>
</tr>
<tr>
<td>Calcium excretion (mmol/24 h)</td>
<td>6.0 ± 0.6</td>
<td>3.5 ± 0.4</td>
<td>P &lt; 0.005</td>
</tr>
</tbody>
</table>

Exclusion Criteria

The exclusion criteria were:
- Age >70 yr;
- Hypercalcemia (ionized Ca, >1.25 mM);
- Increased PTH in plasma >62 pg/ml;
- Chronic алкоголism, diabetes mellitus, intestinal malabsorption;
- Neoplasia, mesenchymal tumor;
- Familial hypophosphatemia;
- Renal tubular acidosis, decrease of GFR (<70 ml/min per 1.73 m²); and
- Previous history of cardiovascular disease (angina pectoris, myocardial infarction).

Management of Patients, Protocols

Initial Investigation. Patients were infused with inulin and p-aminohippurate to determine GFR and renal plasma flow (RPF), respectively. Urine was obtained by spontaneous voiding. After an initial bladder emptying, the subjects provided urine samples over four consecutive 30-min periods, and blood samples were collected at the same time. These periods were referred to as basal periods. Dipyridamole was then infused intravenously (1 mg/kg body wt, maximal dose of 60 mg) over a 30-min period. Thirty minutes after the end of the dipyridamole infusion, urine and blood samples were collected during three consecutive 30-min periods. These three periods were referred to as dipyridamole periods.

During each 30-min period, we measured GFR, RPF, serum calcium, serum phosphorus, calcium and phosphorus excretion, serum and urinary creatinine, urinary cAMP, and fractional excretion of phosphorus.

PTH and 1,25-dihydroxyvitamin D (1,25(OH)₂vitamin D) were measured on blood samples collected during the second 30-min period of the basal and dipyridamole periods. Serum PTH concentrations were determined by an immunoradiometric assay recognizing the intact 1,84 PTH (kit ELSA-PTH, CIS Biointernational, Gif-sur-Yvette, France). Normal values ranged from 11 to 62 pg/ml.

Serum 1,25(OH)₂vitamin D concentrations were measured by RIA (INClSTAR, Stillwater, MN). Values from 15 to 42 pg/ml were considered normal. Electrolyte concentrations were determined using routine standard methods. Urinary cAMP concentrations were measured by RIA (Amersham, Buckinghamshire, United Kingdom). The ratio between the maximal tubular reabsorption rate for phosphate and the GFR (TmPO4/GFR) was calculated according to the nomogram of Bijvoet and Walton (11).

Follow-Up of Patients. Patients were treated by dipyridamole given orally (75 mg, 4 times daily). Lower doses were tested in four patients (not included in this study) and shown to be ineffective. New investigations were performed during the first 3 mo of treatment and after 6 to 9 and 12 mo of treatment. These investigations were conducted as the initial one except there was no dipyridamole infusion and urinary samples were collected over five consecutive 30-min periods.

Statistical Analyses

Data from basal periods were compared with dipyridamole periods using a paired t test. Differences were considered significant when P < 0.05. Data collected during the successive investigations were compared with the data obtained during the initial basal period, using a 2-way ANOVA with repeated measures and a least significant difference test when P < 0.05 All results were expressed as mean ± SEM.
Table 2. Lack of effects of dipyridamole infusion on calcemia, serum parathyroid hormone (PTH) concentration, and cAMP/creatininuria

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before Dipyridamole Infusion</th>
<th>After Dipyridamole Infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum phosphorus (mmol/L)</td>
<td>0.78 ± 0.01</td>
<td>0.77 ± 0.02</td>
</tr>
<tr>
<td>Serum ionized calcium (mmol/L)</td>
<td>1.20 ± 0.01</td>
<td>1.21 ± 0.02</td>
</tr>
<tr>
<td>PTH (pg/ml)</td>
<td>36.9 ± 1.6</td>
<td>34.6 ± 1.8</td>
</tr>
<tr>
<td>cAMP/urinary creatinine (nmol/mmol)</td>
<td>348.9 ± 20.6</td>
<td>347.3 ± 20.7</td>
</tr>
</tbody>
</table>

Figure 2. Evolution of TmPO4/GFR during chronic treatment with dipyridamole. **P < 0.01; ***P < 0.001 compared with values before treatment. ●, patients with urolithiasis; ■, patients without urolithiasis; no symbol, both groups.

Results

Sixty-four patients with low TmPO4/GFR (27 women, 37 men; mean age, 49.4 ± 1.3) were included in this study. These patients exhibited two types of clinical features: 46 were referred to our department for investigation of arthralgia, myalgia, muscle weakness, and/or bone demineralization and 18 for a history of renal lithiasis (calcium oxalate and/or calcium phosphate exclusively). These two subgroups differed only in the serum concentration of 1,25(OH)2vitamin D and calcium excretion, which were significantly higher in patients with renal lithiasis (Table 1).

As a first step, we showed that an acute infusion of dipyridamole did increase renal phosphate reabsorption in these patients, as reported previously in an other group of subjects (10). Patients were infused with dipyridamole during the first investigation, and we compared TmPO4/GFR before and after the infusion. As shown in Figure 1, dipyridamole significantly improved TmPO4/GFR in all patients by 24%. Serum phosphorus, serum PTH concentration, serum ionized calcium, and cAMP excretion remained unchanged (Table 2). The response to dipyridamole infusion was similar in patients with and without renal stones (Figure 1).

To determine whether a long-term treatment by dipyridamole increases serum phosphorus, the patients were treated with dipyridamole given orally (300 mg divided in four daily doses). Serum phosphorus and TmPO4/GFR were sequentially measured. Under these conditions, dipyridamole still significantly increased TmPO4/GFR with a maximal effect within 9 mo (Figure 2). The maximal values of TmPO4/GFR, however, were below those observed after the acute infusion of dipyridamole (compare Figures 1 and 2). Dipyridamole treatment was similarly effective on TmPO4/GFR in both patient groups (Figure 2). In parallel, the increase of renal phosphate reabsorption led to a significant improvement of serum phosphorus within 3 mo (Figure 3). Serum phosphorus reached a plateau after 9 mo of treatment. The improvement (serum phosphorus after 3 mo of treatment > serum phosphorus during the first investigation) was observed in 51 of 64 patients, persisted beyond 12 mo of treatment, and was similar in patients with or without urolithiasis (Figure 3). Patients who initially complained of bone pain or muscle weakness reported an improvement of their physical condition under treatment. Serum PTH concentration and serum calcium remained unchanged (Table 3).
Table 3. Evolution of serum calcium and serum PTH concentration during the treatment by dipyridamolea

<table>
<thead>
<tr>
<th>Variable</th>
<th>0</th>
<th>3</th>
<th>9</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ionized serum calcium (mmol/L)</td>
<td>1.20 ± 0.01</td>
<td>1.21 ± 0.01</td>
<td>1.20 ± 0.01</td>
<td>1.21 ± 0.01</td>
</tr>
<tr>
<td>PTH (pg/ml)</td>
<td>36.9 ± 1.6</td>
<td>37.9 ± 1.9</td>
<td>34.8 ± 2.3</td>
<td>36.8 ± 3.1</td>
</tr>
</tbody>
</table>

a PTH and serum calcium concentrations were unchanged.

Under dipyridamole treatment, 24-h calcium excretion decreased only in patients with initially high vitamin D levels (baseline: 5.9 ± 0.6; 12 mo: 4.5 ± 0.5 mmol/24 h; P < 0.05) and in patients with renal stones (baseline: 6.0 ± 0.6; 12 mo: 4.0 ± 0.3 mmol/24 h; P < 0.05).

After 2 yr, the treatment was discontinued in three consenting patients for 1 mo, and the serum phosphorus and the TmPO4/GFR were measured. As shown in Table 4, serum phosphorus and TmPO4/GFR decreased in these three patients after discontinuation of the treatment.

Discussion

This prospective study indicates that in patients with hypophosphatemia due to renal phosphate leak, treatment by dipyridamole improves serum phosphorus by increasing renal phosphate reabsorption.

We examined patients with hypophosphatemia and low TmPO4/GFR. These patients had no other proximal tubular dysfunction, and the sensitivity of the kidney to PTH was normal as assessed by urinary cAMP to urinary creatinine ratios, which were not different from those of control subjects (data not shown). An increase of phosphate intake could not explain low TmPO4/GFR because serum phosphorus concentrations were below normal values.

Chronic renal phosphate leak is known to induce hypophosphatemia and osteopenia or urolithiasis (4–8,13), but the frequency of low TmPO4/GFR among these patients remains to be studied. We reported previously that dipyridamole increased phosphate uptake in proximal tubular cells in culture and renal phosphate reabsorption in rats and in humans (9,10). Dipyridamole may increase phosphate reabsorption by at least two mechanisms. First, dipyridamole is known to inhibit adenosine uptake (14,15). In the proximal tubule, adenosine is generated from cAMP, which is metabolized in AMP and then adenosine by phosphodiesterases and the ecto-5′-nucleotidase, respec-

Table 4. Modifications of serum phosphorus and renal phosphate threshold before and after discontinuation of treatment for 1 moa

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before Treatment</th>
<th>After 2 yr of Dipyridamole Treatment</th>
<th>After 1 mo Discontinuation of Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum phosphorus (mmol/L)</td>
<td>0.77 ± 0.01</td>
<td>0.93 ± 0.05b</td>
<td>0.81 ± 0.04</td>
</tr>
<tr>
<td>TmPO4/GFR (mmol/L)</td>
<td>0.56 ± 0.04</td>
<td>0.67 ± 0.02b</td>
<td>0.54 ± 0.05</td>
</tr>
</tbody>
</table>

a Results are given as mean ± SEM. n = 3.
b P < 0.05 compared with basal values.
centrations of TmPO4/OFR resistance
adenosine

enough after

prevent not

during transport (19). Furthermore, dipyridamole, by decreasing adenosine intracellular levels, might hamper cAMP formation.

Second, dipyridamole is a potent inhibitor of the multidrug resistance P-glycoprotein activity (20). It is a member of the ATP binding cassette family (like cystic fibrosis transmembrane conductance regulator) that is expressed in the renal proximal tubule (21) and regulates cell volume-activated chloride channels (22). We can hypothesize that the P-glycoprotein may downregulate NaPi cotransporter activity in the proximal tubule. These two hypotheses are currently under investigation in the laboratory. However, the mechanism whereby dipyridamole increases phosphate uptake is probably not related to the cause of renal phosphate leak because acute infusion of dipyridamole is effective in subjects with low and normal TmPO4/GFR as well (reference 10 and our unpublished data). We have also tested the effect of dipyridamole in two patients, not reported here, with Fanconi’s syndrome. Dipyridamole infusion was ineffective on TmPO4/GFR in both patients and was not given orally.

Under chronic dipyridamole treatment, the maximal values of TmPO4/GFR were reached within 9 mo and remained below those observed after a single infusion of dipyridamole. During acute infusion of dipyridamole, serum phosphorus concentration did not change (Table 2). This was not the case during chronic treatment. Under physiologic conditions, an increase in serum phosphorus induces a decrease in TmPO4/GFR (23). This may slow down the improvement of TmPO4/GFR and prevent TmPO4/GFR from reaching values obtained after dipyridamole infusion. Furthermore, serum dipyridamole concentrations were probably lower during oral treatment than after intravenous infusion.

In 13 patients (20%, three with renal lithiasis), dipyridamole failed to increase TmPO4/GFR and serum phosphorus despite a significant decrease of phosphate excretion during the initial investigation. We were unable to correlate this lack of effect with any biological or clinical parameters among those recorded. We did not try higher doses of dipyridamole (>300 mg/d) in these patients.

In 51 patients, the increase of TmPO4/GFR was important enough to improve serum phosphorus. To our knowledge, this is the first time that a drug is reported to increase serum phosphorus without altering PTH concentration or serum calcium. Although dipyridamole has been widely used for a long time in patients, there are no data available regarding its effect on renal phosphate excretion.

Serum phosphorus is one of the main determinants of renal 1α-hydroxylase activity in humans: the lower the serum phosphorus, the higher 1α-hydroxylase activity and 1,25(OH)2vitamin D synthesis (12). We did observe that the effect of dipyridamole on serum phosphorus led to a significant decrease of 1,25(OH)2vitamin D concentration in serum.

The normalization of calcium excretion and the decrease of phosphaturia under dipyridamole treatment in patients with urolithiasis and low TmPO4/GFR suggest that stone recurrence might be reduced, but this point requires further study. Similarly, additional studies with a longer follow-up of patients are needed to assess the effect of dipyridamole on the bone mass.

In conclusion, in this prospective study we reported that dipyridamole, given orally, increased renal phosphate reabsorption and serum phosphorus and can be used to treat patients with idiopathic renal phosphate leak.

Acknowledgment
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References
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