Effect Of Uric Acid On Plasma Levels of 1,25(OH)₂D in Renal Failure

Raymond Vanholder, Sanjeev Patel, and Chen H. Hsu

R. Vanholder, Nephrology Division, Department of Medicine, University Hospital, Ghent, Belgium
S. Patel, C.H. Hsu, Nephrology Division, Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI

(AJN; 1993; 4:1035-1038)

ABSTRACT
Previous studies from these laboratories have demonstrated that uric biologic fluids contain substances that suppress 1,25(OH)₂D metabolism. Among these substances, it was found that uric acid suppresses 1α-hydroxylase activity and synthesis of 1,25(OH)₂D in rats. In this study, the effect of uric acid on plasma concentrations of 1,25(OH)₂D in patients with renal failure was examined. Nine patients with stable chronic renal failure (serum creatinine, 1.9 to 6.4 mg/dL) were studied. None of the patients received vitamin D supplementation. Plasma concentrations of Ca, P, parathyroid hormone, creatinine, uric acid, 1,25(OH)₂D, and 25(OH)D were measured before and after the patients received allopurinol, 300 mg daily. Plasma creatinine, Ca, P, parathyroid hormone, and 25(OH)D did not change before and after allopurinol treatment. However, plasma uric acid decreased significantly from 7.3 ± 0.4 to 4.0 ± 0.4 mg/dL (P < 0.01) and plasma concentration of 1,25(OH)₂D rose from 30.8 ± 2.7 to 38.2 ± 4.8 pg/mL (P < 0.01) after the ingestion of allopurinol. Allopurinol itself did not appear to directly enhance 1α-hydroxylase activity in rats. It was concluded that short-term administration of allopurinol suppresses plasma uric acid and increases plasma 1,25(OH)₂D in patients with chronic mild to moderate renal failure.

Key Words: 1,25(OH)₂D, allopurinol, uric acid, 1,25(OH)₂D production rate

The classic biologic role of 1,25(OH)₂D is the regulation of mineral homeostasis and parathyroid hormone (PTH) secretion. Thus, abnormal 1,25(OH)₂D metabolism plays an important role in renal osteodystrophy and secondary hyperparathyroidism in chronic renal failure (1). Recent studies have suggested a wider biologic effect of 1,25(OH)₂D. These biologic actions include cellular differentiation, immunoregulatory properties (1), and proper functions of skeletal muscle (2) and myocardium (3). Therefore, abnormal vitamin D metabolism may lead to impaired monocyte function (4), neuromuscular disorder (5), cardiovascular dysfunction, and myopathy (2.3).

Recently, we have demonstrated that purine derivatives suppress 1,25(OH)₂D synthesis (6). Thus, raising plasma uric acid from 1.1 to 4.2 mg/dL in rats reduces the 1,25(OH)₂D production rate by 42% (6). Because plasma uric acid increases frequently in renal failure, we studied the effect of uric acid on plasma 1,25(OH)₂D levels in patients with chronic renal failure.

METHODS
Nine patients (six women and three men, ages 35 to 70 yr) with stable chronic renal failure were selected from the renal outpatient clinic of the Ghent University Hospital, Ghent, Belgium. Six patients had chronic interstitial nephritis, two had polycystic kidney disease, and one had lupus nephritis. None of the subjects had a history of gout. They were not taking calcium or vitamin D supplements before the study. They were eating regular diets and were on various outpatient medication regimens, including antihypertensive medications and diuretics, which were maintained throughout the duration of study. We used allopurinol to lower plasma uric acid and studied its effect on plasma 1,25(OH)₂D. In addition, 25(OH)D, creatinine, uric acid, calcium, phosphorus, and PTH were measured before and after the patients received 300 mg of allopurinol daily for 1 wk. All subjects were studied with the approval of the Human Use Committee at the Ghent University Hospital.

The effect of allopurinol on 1,25(OH)₂D synthesis was studied in rats. Because plasma levels of uric acid are very low in rats (1.10 ± 0.07 mg/dL) (6), we were able to test the effect of allopurinol itself on 1,25(OH)₂D metabolism without further significantly lowering plasma concentrations of uric acid. Normal
Sprague Dawley rats weighing approximately 200 g were used for this study. Rats were fed Purina rat chow (Ralston Purina, St. Louis, MO) containing 1.0% Ca, 0.86% P, and 4.5 IU/g of vitamin D. On the day of experimentation, the femoral artery and vein were cannulated with the rats under ether anesthesia with polyethylene tubing (PE 10) for blood sampling and fluid infusion. The animals were placed in individual cages and divided into two groups. One group of rats ($N = 5$) was infused iv for 20 h with a 20-mL saline solution containing 60 mg/kg of allopurinol and radiolabeled 1,25(OH)2D. Control rats ($N = 5$) received 20 mL of vehicle and radiolabeled 1,25(OH)2D. Metabolic clearance rate and production rate of 1,25(OH)2D were measured at the end of the 20-h infusion as described previously (7). In addition, plasma concentrations of 1,25(OH)2D, calcium, and phosphorus, creatinine clearance, and urinary excretions of calcium and phosphorus were determined. Animal studies were approved by the University Committee On Use And Care Of Animals at the University of Michigan.

**Analytical Methods**

1,25(OH)2D concentration was determined in duplicate by radioreceptor assay. Our interassay coefficients of variation were 7.0% for low control (20 pg/mL; $N = 12$) and 4.1% for high control (100 pg/mL; $N = 12$). The intra-assay coefficients of variation were 5.4% for low control ($N = 6$) and 4.7% for high control.$^1$ 1,25(OH)2D recovery averaged 65%. 25(OH)D was measured by competitive binding assay, and intact PTH was measured by the Allegro immunoradiometric assay (Nichols Institute, San Juan Capistrano, CA). Plasma calcium, phosphorus, creatinine, and uric acid were measured by standard laboratory methods.

All data are expressed as mean ± SE. Statistical analysis was performed by use of the t test and paired t test where appropriate. A $P$ value of less than 0.05 was considered significant.

**RESULTS**

All patients had chronic renal failure with serum creatinine ranging from 1.9 to 6.4 mg/dL and plasma uric acid levels varying from 5.6 to 9.7 mg/dL (normal values: men, 3.5 to 7.5 mg/dL; women, 2.3 to 6.0 mg/dL). Three female and one male patients had hyperuricemia. Seven patients had plasma uric acid greater than 6.0 mg/dL. Plasma concentrations of creatinine, calcium, phosphorus, PTH (normal values, 10 to 65 pg/mL), and 25(OH)D (normal values, 9 to 62 ng/dL) did not change after the administration of allopurinol (Table 1). Plasma uric acid decreased in each patient (all below 6.0 mg/dL), and plasma 1,25(OH)2D (normal values, 15 to 65 pg/mL) increased in all patients except one, who remained unchanged after allopurinol treatment (Figure 1). None of the patients developed side effects or intolerance to allopurinol.

Allopurinol did not change the 1,25(OH)2D clearance rate or its production rate in normal rats (Table 2). Therefore, it had no direct effect on 1α-hydroxylase activity. Plasma concentrations of calcium and phosphorus and urinary excretions of calcium and phosphorus were not different between allopurinol and vehicle-infused rats (Table 2). Plasma uric acid was not measured in rats receiving allopurinol, because the concentration was already very low before the allopurinol treatment. It appears that further reduction of plasma uric acid did not alter calcitriol synthesis in normal rats.

**DISCUSSION**

The biologic actions of 1,25(OH)2D are diminished in renal failure primarily because of decreased production of 1,25(OH)2D. Decreased biologic action of 1,25(OH)2D is partly responsible for the development of renal osteodystrophy and secondary hyperparathyroidism (1). Recent studies provide evidence that end-organ resistance to 1,25(OH)2D could also account for secondary hyperparathyroidism in renal

---

**TABLE 1. Plasma concentrations of creatinine, calcium, phosphorus, 25(OH)D, 1,25(OH)2D, and PTH before and after the administration of allopurinol in patients with chronic renal failure**

<table>
<thead>
<tr>
<th></th>
<th>Pcr (mg/dL)</th>
<th>Pca (mg/dL)</th>
<th>Pp (mg/dL)</th>
<th>Pu (ng/mL)</th>
<th>25(OH)D (ng/mL)</th>
<th>1,25(OH)2D (ng/mL)</th>
<th>PTH (pg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before Allopurinol ($N = 9$)</td>
<td>3.7 ± 0.17</td>
<td>9.3 ± 0.03</td>
<td>3.1 ± 0.1</td>
<td>7.3 ± 0.4</td>
<td>56 ± 4.3</td>
<td>30.8 ± 2.7</td>
<td>186 ± 79</td>
</tr>
<tr>
<td>After Allopurinol ($N = 9$)</td>
<td>3.5 ± 0.17</td>
<td>9.5 ± 0.07</td>
<td>3.4 ± 0.1</td>
<td>4.0 ± 0.4</td>
<td>58 ± 4.3</td>
<td>38.2 ± 4.8</td>
<td>199 ± 85</td>
</tr>
<tr>
<td>$P$ Value</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.01</td>
<td>NS</td>
<td>0.01</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Abbreviations: Pcr, plasma creatinine; Pca, plasma calcium; Pp, plasma phosphorus; Pu, plasma uric acid; NS, not significant. Data are expressed as mean ± SE.
failure (8). We have previously demonstrated that uremic plasma contains substances that inhibit 1,25(OH)₂D synthesis (9). Subsequently, we have identified uric acid and xanthine, which are often elevated in renal failure, as inhibitors of 1α-hydroxylase (6). In this study, the plasma concentration of 1,25(OH)₂D increased more than 20% after plasma uric acid was lowered 45% by allopurinol. The increase in plasma 1,25(OH)₂D was unlikely due to a direct effect of allopurinol, because it did not alter 1,25(OH)₂D metabolism in normal rats. It should be noted, however, that allopurinol inhibits xanthine oxidase and raises plasma levels of xanthine, which could offset the effect of decreased uric acid levels on 1,25(OH)₂D synthesis (6). Because allopurinol increased plasma 1,25(OH)₂D levels, either the elevation of xanthine was insufficient to change 1,25(OH)₂D metabolism or uric acid was a more potent factor inhibiting 1α-hydroxylase.

Although we did not measure the production rate of 1,25(OH)₂D in these patients, allopurinol could increase 1,25(OH)₂D production by more than the 20% increase reflected in the plasma 1,25(OH)₂D concentration. Uric acid not only inhibits the 1,25(OH)₂D production rate but also suppresses its clearance rate (6). Thus, the metabolic clearance rate of 1,25(OH)₂D increases when uric acid synthesis is decreased. Furthermore, the increase in 1,25(OH)₂D production also accelerates its own metabolic clearance rate in renal failure (10). Therefore, the plasma concentration of 1,25(OH)₂D may not reflect the true increase in 1,25(OH)₂D production, because the increased clearance rate could reduce the elevation of plasma concentration.

In this study, the elevation of plasma levels of 1,25(OH)₂D was not associated with any changes in plasma levels of calcium, phosphorus, or PTH. Plasma concentrations of calcium and phosphorus are tightly regulated by PTH, vitamin D metabolites, and the kidneys. It is difficult to detect any subtle changes of these minerals in patients with only moderate impairment of renal functions. Further, parathyroid glands are often hyperplastic and hypertrophic in patients with chronic renal failure; therefore, changes in PTH levels could not be detected in this short-term treatment of allopurinol.

The biologic actions of 1,25(OH)₂D appear to be mediated through a hormone-receptor complex interacting with nuclear chromatin to regulate gene expression in a manner analogous to the mechanism of action of steroid and thyroid hormones (11). The hormone-receptor complex binds to its DNA-responsive element in the promoter region of a target. The binding to DNA results in the genomic synthesis of bioactive proteins (11), that carry out the biologic actions of 1,25(OH)₂D. The binding of the hormone-receptor complex to DNA is, therefore, the primary effector pathway orchestrating the biologic action of 1,25(OH)₂D.

The DNA-binding domain has a two-loop structure stabilized by cysteine-zinc bonding, the so-called zinc fingers (12). It is sensitive to sulphydryl-blocking agents (13) and uremic toxins (14,15), which inhibit

![Figure 1. Plasma uric acid and 1,25(OH)₂D before and after allopurinol treatment. Each symbol represents a single patient.](image)

**TABLE 2.** Renal function, plasma concentrations of calcium and phosphorus, urinary excretions of calcium and phosphorus, and metabolic clearance and production of 1,25(OH)₂D in rats infused for 20 h with allopurinol and vehicleα

<table>
<thead>
<tr>
<th></th>
<th>Ccr (mL/min · 100 g)</th>
<th>Pca (mg/dL)</th>
<th>Pp (mg/min)</th>
<th>UVca (µg/min)</th>
<th>UVp (µg/mL)</th>
<th>P1,25(OH)₂D (pg/mL)</th>
<th>MCR (µL/min·kg)</th>
<th>PR (ng·kg·day)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Allopurinol</strong> (N = 5)</td>
<td>0.53 ± 0.02</td>
<td>9.86 ± 0.09</td>
<td>7.92 ± 0.17</td>
<td>0.16 ± 0.02</td>
<td>19.5 ± 3.27</td>
<td>86.2 ± 2.9</td>
<td>268.1 ± 5.7</td>
<td>33.3 ± 1.35</td>
</tr>
<tr>
<td><strong>Control</strong> (N = 5)</td>
<td>0.50 ± 0.03</td>
<td>9.84 ± 0.25</td>
<td>7.89 ± 0.18</td>
<td>0.20 ± 0.03</td>
<td>20.4 ± 1.92</td>
<td>82.3 ± 5.3</td>
<td>266.7 ± 2.6</td>
<td>31.7 ± 2.24</td>
</tr>
<tr>
<td><strong>P Value</strong></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

α Abbreviations: Pcr, plasma creatinine; Pca, plasma calcium; Pp, plasma phosphorus; P1,25(OH)₂D, plasma concentration of 1,25(OH)₂D; MCR, metabolic clearance rate of 1,25(OH)₂D; PR, production rate of 1,25(OH)₂D.
the binding of the 1,25(OH)2D receptor to DNA cellulose. Uric acid also reduces the hormone-receptor complex-binding affinity for DNA. The receptor-binding affinity for DNA was partially inhibited when the receptor was incubated with 10 mg/dL of sodium urate for 18 h at 4°C (6). Although diminished biologic action of calcitriol in clinical hyperuricemia has not been reported, our in vitro study (6) suggests that hyperuricemia itself could reduce the biologic action of 1,25(OH)2D. A functional defect of the DNA-binding domain and its consequence are best illustrated by type II vitamin D–dependent rickets, a syndrome characterized by rickets, osteomalacia, hypocalcemia, secondary hyperparathyroidism, and high plasma concentrations of 1,25(OH)2D. Genomic DNA analysis of affected family members has identified a single nucleotide mutation in the DNA-binding domain encoding the receptor protein (16). This molecular defect produces a defective 1,25(OH)2D receptor that is unable to bind to DNA cellulose and to activate gene transcription. This functional defect accounts for end-organ resistance to 1,25(OH)2D in these patients (17).

In summary, we have previously shown that uric acid suppresses the enzymatic activity of 1α-hydroxylase in normal rats (6). In this study, we have demonstrated that a short-term administration of allopurinol increases plasma concentrations of 1,25(OH)2D in mild to moderate renal failure with or without hyperuricemia. However, the effect of uric acid on calcitriol metabolism in patients with normal renal function and hyperuricemia remains to be investigated.

ACKNOWLEDGMENTS

This work was supported by a grant-in-aid from the American Heart Association and the Extramural Grant Program, Baxter Health Care Corporation. The vitamin D metabolites used in this study were kindly provided by Dr. M. Uskokovic, Hoffman-LaRoche, Nutley, NJ.

REFERENCES