Dysregulation of renal vitamin D metabolism in the uremic rat

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The progressive decline in kidney function and concomitant loss of renal 1α-hydroxylase (CYP27B1) in chronic kidney disease (CKD) are associated with a gradual loss of circulating 25-hydroxyvitamin D_3 ($25(OH)D_3$) and 1α,25-dihydroxyvitamin D_3 ($1\alpha,25(OH)_2D_3$). However, only the decrease in $1\alpha,25(OH)_2D_3$ can be explained by the decline of CYP27B1, suggesting that insufficiency of both metabolites may reflect their accelerated degradation by the key catabolic enzyme 24-hydroxylase (CYP24). To determine whether CYP24 is involved in causing vitamin D insufficiency and/or resistance to vitamin D therapy in CKD, we determined the regulation of CYP24 and CYP27B1 in normal rats and rats treated with adenine to induce CKD. As expected, CYP24 decreased whereas CYP27B1 increased when normal animals were rendered vitamin D deficient. Unexpectedly, renal CYP24 mRNA and protein expression were markedly elevated, irrespective of the vitamin D status of the rats. A significant decrease in serum $1\alpha,25(OH)_2D_3$ levels was found in uremic rats; however, we did not find a coincident decline in CYP27B1. Analysis in human kidney biopsies confirmed the association of elevated CYP24 with kidney disease. Thus, our findings suggest that dysregulation of CYP24 may be a significant mechanism contributing to vitamin D insufficiency and resistance to vitamin D therapy in CKD.

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Vitamin D insufficiency is commonly observed in patients with chronic kidney disease (CKD) and is causally related to secondary hyperparathyroidism, a disorder characterized by elevated serum-intact parathyroid hormone (iPTH) levels, parathyroid gland hyperplasia and imbalances in bone and mineral metabolism.[1–3] Low vitamin D levels have also been linked to the pathogenesis of other diseases related to CKD, including diabetes,[4] hypertension,[5] and obesity.[6,7] External factors, such as lack of sunlight and inadequate vitamin D intake, are recognized as important factors contributing to vitamin D insufficiency in CKD patients;[8] however, disturbances in the regulation of key cytochrome P450 enzymes involved in the synthesis (1α-hydroxylase; CYP27B1) and catabolism (24-hydroxylase; CYP24) of vitamin D metabolites may also be implicated.

Vitamin D_3 is synthesized in human skin from 7-dehydrocholesterol after ultraviolet light exposure and is metabolized in the liver to form the prohormone, 25-hydroxyvitamin D_3 ($25(OH)D_3$). Circulating $25(OH)D_3$ provides substrate for conversion to the biologically active hormone 1α,25-dihydroxyvitamin D_3 ($1\alpha,25(OH)_2D_3$) by 1α-hydroxylase CYP27B1 primarily expressed in renal proximal and distal convoluted tubules.[9,10] Although kidneys produce the bulk of circulating hormones, extra-renal expression of CYP27B1 is thought to be important for localized production of $1\alpha,25(OH)_2D_3$.[11,12] The effects of $1\alpha,25(OH)_2D_3$ are mediated by the vitamin D receptor expressed in target organs, including those involved in the maintenance of calcium/phosphate homeostasis and normal bone mineralization, immunomodulation, as well as the regulation of cell growth and differentiation, insulin secretion, cardiovascular function, and blood pressure regulation.[13] Vitamin D insufficiency observed in CKD is associated with morbidity, which extends well beyond compromised bone and mineral metabolism,[14–16] and contributes to increased mortality.[17–20]

Declining renal mass and concomitant loss of renal CYP27B1 capacity in CKD are commonly associated with reductions in circulating levels of both $1\alpha,25(OH)_2D_3$ and $25(OH)D_3$.[21,22] However, observations of low serum

$1\alpha,25(OH)_2D_3$ have not been consistently linked with decreases in renal CYP27B1 expression, as levels of CYP27B1 mRNA may in some cases remain unchanged in CKD patients deficient in $1\alpha,25(OH)_2D_3$.[23] Moreover, diminishing CYP27B1 expression levels cannot directly account for the progressive loss of $25(OH)D_3$. These findings suggest that additional intrinsic mechanisms may underlie declining vitamin D metabolites, namely, $25(OH)D_3$ and $1\alpha,25D(OH)_2D_3$, in renal disease.

Apart from disturbances in $1\alpha,25(OH)_2D_3$ synthesis, accelerated catabolism may also have a role in lowering circulating $1\alpha,25(OH)_2D_3$ and $25(OH)D_3$ levels in CKD patients. The mitochondrial cytochrome P450 enzyme CYP24 has a unique role in the catabolism of both $1\alpha,25(OH)_2D_3$ and $25(OH)D_3$.[24,25] Deletion of the *CYP24* gene significantly increases the half-lives of circulating $1\alpha,25(OH)_2D_3$ and $25(OH)D_3$ and renders CYP24-null animals hypersensitive to vitamin D, thus confirming the importance of CYP24 in vitamin D homeostasis.[26,27] Normally, CYP24 protein seems to be most abundant in the proximal tubule of the kidney, with lower expression observed in distal segments.[28,29] However, CYP24 is also ubiquitously expressed in vitamin D target tissues external to the kidney.[30] In some disease states, such as genetically linked hypophosphatemia[31–33] and certain types of cancer,[34–39] CYP24 expression and activity is enhanced and may be linked to both vitamin D insufficiency, as well as increased resistance to vitamin D treatment often associated with these pathologies. Given the important functional role of CYP24 in tightly regulating the biological activity of $1\alpha,25(OH)_2D_3$ and $25(OH)D_3$, overexpression of this enzyme in kidney can also have a significant impact on vitamin D status.

To determine whether CYP24 and CYP27B1 expression is altered in uremia, we investigated the regulation of these enzymes in normal and adenine-induced uremic rats, as well as in renal biopsy tissue from patients with kidney disease. Our findings suggest that dysregulation of CYP24 may be a significant mechanism contributing to vitamin D insufficiency and resistance to vitamin D therapy in CKD.

RESULTS

Renal *CYP24* and *CYP27B1* gene and protein expression in uremic vs normal rats

The effects of uremia on the expression of renal CYP24 and CYP27B1 mRNA and protein were examined using the adenine rat model of CKD. Previous studies using adenine-treated rats have shown that this model exhibits all key features of CKD pathology, including elevated creatinine, iPTH and fibroblast growth factor 23 (FGF23), hypocalcemia, hyperphosphatemia, and reduced serum $1\alpha,25(OH)_2D_3$.[40–42] Uremia in adenine-treated rats was evident from elevated mean serum creatinine levels of 1.86 ± 0.20 mg/dl compared with 0.39 ± 0.17 mg/dl in normal rats ($P < 0.001$). Plasma iPTH and serum FGF23 levels were elevated in uremic rats, serum calcium was decreased, serum phosphorus was increased (Table 1), and serum $1\alpha,25(OH)_2D_3$ declined (59.20 ± 9.80 pg/ml nonuremic vs 15.20 ± 3.16 pg/ml uremic; $P < 0.01$; Figure 1d). Although serum $25(OH)D_3$ levels remained unchanged (23.90 ± 2.09 ng/ml nonuremic vs 25.90 ± 1.90 ng/ml uremic; Figure 1e) 1 week after adenine treatment, a decline of about 20% in $25(OH)D_3$ levels was observed at 6 and 8 weeks after treatment compared with normal control animals (Figure 1f). These findings are consistent with the accelerated elimination of $25(OH)D_3$, raising the possibility that elevated CYP24 may have a role in declining vitamin D status in CKD patients.

Examination of renal mRNA revealed a greater than fivefold increase in CYP24 expression after adenine treatment ($P < 0.001$; Figure 1a). Consistent with this finding, an increased CYP24 protein expression was also observed in uremic kidneys (Figure 1b). CYP27B1 mRNA expression increased nearly twofold in uremic kidney (Figure 1a; $P < 0.01$). Concordant with mRNA, CYP27B1 protein expression was clearly elevated in uremic kidney (Figure 1b), indicating that translation of CYP27B1 mRNA was not impaired in this model.[43]

Renal CYP24 and CYP27B1 mRNA and protein expression in uremic rats treated with $1\alpha,25(OH)_2D_3$

We next investigated the regulatory effect of $1\alpha,25(OH)_2D_3$ on these enzymes in uremia. It is well established that $1\alpha,25(OH)_2D_3$ treatment induces CYP24 and attenuates CYP27B1 expression in vitamin D target tissues, including kidney.[9,28,44] In the uremic kidney, CYP24 mRNA levels were approximately threefold greater than levels of CYP27B1 (Figure 1c). Administration of $1\alpha,25(OH)_2D_3$ markedly increased the expression of CYP24 by approximately 12-fold relative to CYP27B1 expression, which increased only slightly (Figure 1c). Administration of $1\alpha,25(OH)_2D_3$ (0.50 μg/kg) to uremic rats increased mean serum

Table 1 | Biochemical parameters measured in normal vs uremic rats

	Nonuremic vehicle	Uremic vehicle	Uremic +$1\alpha,25(OH)_2D_3$
Creatinine (mg/dl)	0.39 ± 0.17 (8)	1.86 ± 0.20 (10)***	1.38 ± 0.06 (5)**
iPTH (pg/ml)	199 ± 91.3 (10)	521 ± 158.4 (10)	29.6 ± 17.3 (7)†
FGF23 (ng/ml)	0.41 ± 0.02 (10)	21.80 ± 10.3 (8)	138 ± 39.2 (7)***††
Calcium (mg/dl)	11.04 ± 0.24 (10)	9.72 ± 0.10 (10)**	13.96 ± 0.41 (7)***†††
Phosphorus (mg/dl)	10.41 ± 0.16 (10)	14.24 ± 0.98 (10)*	19.04 ± 2.01 (7)***†

Abbreviations: FGF23, fibroblast growth factor 23; iPTH, intact parathyroid hormone; $1\alpha,25(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3.
Significant from nonuremic vehicle *($P < 0.05$) **($P < 0.01$) ***($P < 0.001$); significant from uremic vehicle †($P < 0.05$) ††($P < 0.01$) †††($P < 0.001$).
Data are presented as mean ± s.e.m.; (*n*) denotes sample size.