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The effect of calcitriol, paricalcitol, and a calcimimetic on extraosseous calcifications in uremic rats

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Vitamin D derivatives and calcimimetics are used to treat secondary hyperparathyroidism in patients with chronic renal failure. We investigated the effect of calcitriol, paricalcitol, and the calcimimetic AMG 641 on soft-tissue calcification in uremic rats with secondary hyperparathyroidism. Control and uremic rats were treated with vehicle, calcitriol, paricalcitol, AMG 641, or a combination of AMG 641 plus calcitriol or paricalcitol. Parathyroid hormone levels were reduced by all treatments but were better controlled by the combination of paricalcitol and AMG 641. The calcimimetic alone did not induce extraosseous calcification but co-administration of AMG 641 reduced soft-tissue calcification and aortic mineralization in both calcitriol- and paricalcitol-treated rats. Survival was significantly reduced in rats treated with calcitriol and this mortality was attenuated by co-treatment with AMG 641. Our study shows that extraskeletal calcification was present in animals treated with calcitriol and paricalcitol but not with AMG 641. When used in combination with paricalcitol, AMG 641 provided excellent control of secondary hyperparathyroidism and prevented mortality associated with the use of vitamin D derivatives without causing tissue calcification.

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Secondary hyperparathyroidism (HPT) and vascular calcification (VC) are common complications of stage 5 chronic kidney disease. The elevations in calcium, phosphorus, and Ca × P product observed in patients with secondary HPT have been associated with VC and increased risk of cardiovascular morbidity and mortality.[1–4]

Vitamin D derivatives, such as calcitriol and paricalcitol, are commonly used to treat the elevated parathyroid hormone (PTH) associated with secondary HPT;[5–10] however, they have been shown to induce VC *in vivo* and *in vitro* animal models.[11–13] Vitamin D derivatives increase expression of several proteins involved in calcification and decrease expression of proteins that inhibit calcification.[14,15] In addition, they potentially lead to hypercalcemia and hyperphosphatemia,[6,7,10] although paricalcitol has been reported to have less calcemic effects than calcitriol.[16,17] Together, these data suggest the need for alternative treatment strategies.

One such strategy is the use of calcimimetics. These compounds bind to the calcium-sensing receptor and increase its sensitivity to extracellular calcium, thereby suppressing PTH synthesis[18] and secretion[19] without inducing hypercalcemia.[20,21] We have recently demonstrated that, when administered to uremic rats, the calcimimetic R-568 reduces PTH levels without inducing VC, attenuates the calcitriol-induced calcifying effects on vascular tissue, and decreases mortality associated with calcitriol.[22] AMG 641 is a research calcimimetic that has a more sustained action than R-568, allowing administration every 48 h.

In this study, we investigate the effect of calcitriol, paricalcitol, and the research calcimimetic AMG 641, alone or in combination, on the development of vascular and other soft-tissue calcifications in a rat model of uremia-associated secondary HPT.

RESULTS

Animal survival

During the first 14 days following nephrectomy and the change in diet, survival was >90% in all treatment groups, except in rats treated with calcitriol, in which survival was 80%. However, marked differences in survival were found between treatment groups from days 15 through 28. Survival

was not different in the five-sixth (5/6) Nx + vehicle (100%) and in the AMG 641 groups (86%). However, treatment with vitamin D derivatives resulted in a significant reduction in survival that was more marked with calcitriol (18%, $P<0.001$ vs 5/6 Nx + vehicle) than with paricalcitol (50%, $P=0.02$ vs 5/6 Nx + vehicle). The addition of AMG 641 to the vitamin D derivatives improved survival both in calcitriol (40%)- and in paricalcitol (70%, NS vs 5/6 Nx + vehicle)-treated rats (Figure 1).

Serum biochemical parameters

At day 14, mean plasma creatinine concentration in sham-operated rats was 0.57 ± 0.01 mg per 100 ml. As expected, all 5/6 Nx rats had significantly ($P<0.05$) higher creatinine levels, but significant differences were found between treatment groups. Plasma creatinine concentrations in rats treated with either calcitriol (1.86 ± 0.68 mg per 100 ml) or paricalcitol (1.83 ± 0.85 mg per 100 ml) were significantly higher ($P<0.05$) than in rats treated with vehicle (1.16 ± 0.05 mg per 100 ml) or AMG 641 (0.94 ± 0.14 mg per 100 ml). Addition of AMG 641 to vitamin D derivatives significantly ($P=0.001$) reduced plasma creatinine in paricalcitol-treated rats (1.15 ± 0.27 mg per 100 ml) but not in rats treated with calcitriol (1.58 ± 0.51 mg per 100 ml).

Plasma levels of ionized calcium, phosphorus, and PTH at day 14 are depicted in Figure 2. Plasma ionized calcium levels were significantly ($P<0.001$) reduced in all 5/6 Nx groups (range 0.92–1.05 mmol l^{-1}) when compared with sham-operated rats (1.18 ± 0.02 mmol l^{-1}). A higher ionized calcium concentration ($P<0.05$) was identified in rats treated with calcitriol (1.05 ± 0.03 mmol l^{-1}) than in groups treated with AMG 641 (0.94 ± 0.02 mmol l^{-1}) and paricalcitol (0.92 ± 0.03 mmol l^{-1}) (Figure 2a). Plasma phosphorus levels (Figure 2b) were consistently ($P<0.01$) elevated in 5/6 Nx rats, except in groups treated with AMG 641. Plasma phosphorus was higher in rats treated with calcitriol (19.7 ± 1.6 mg per 100 ml), and lower in rats treated with AMG 641 alone (9.1 ± 0.7 mg per 100 ml). Paricalcitol treated rats had significantly higher ($P=0.002$) plasma phosphorus (16.0 ± 2.1 mg per 100 ml) than rats treated with AMG 641 (9.1 ± 0.7 mg per 100 ml). It is also interesting to note that addition of AMG 641 achieved a reduction in plasma phosphorus both in rats treated with paricalcitol (10.2 ± 0.8 mg per 100 ml) and in rats treated with calcitriol (14.8 ± 1.8 mg per 100 ml). Plasma PTH concentration was significantly ($P<0.001$) increased in 5/6 Nx rats (450.1 ± 45.9 pg ml^{-1}) when compared with sham-operated animals (44.4 ± 16.2 pg ml^{-1}). Treatment with AMG 641, alone or in combination with vitamin D derivatives, reduced plasma PTH concentrations to levels that were not significantly different from the sham-operated rats. However, PTH concentrations were higher in rats treated with calcitriol (284.1 ± 44.0 pg ml^{-1}, $P<0.001$) and with paricalcitol

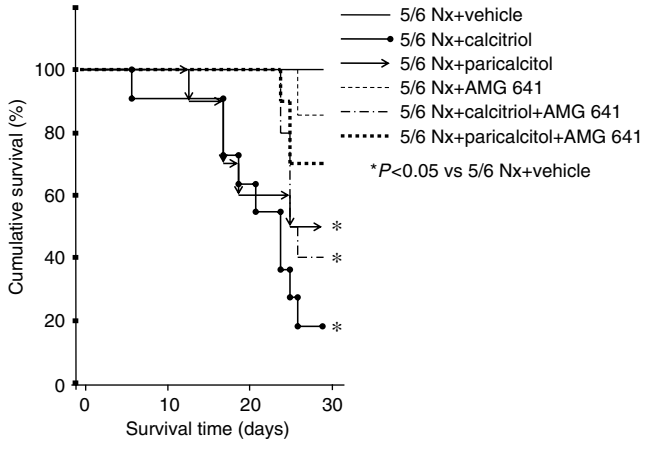

Figure 1 | Kaplan-Meier survival curve in nephrectomized rats (5/6 Nx) treated with vehicle (saline) ($n=8$), calcitriol 80 ng kg^{-1} i.p. ($n=11$), paricalcitol 240 ng kg^{-1} i.p. ($n=10$), AMG 641 1.5 mg kg^{-1} subcutaneously ($n=7$), the combination calcitriol 80 ng kg^{-1} and AMG 641 1.5 mg kg^{-1} ($n=10$), or the combination paricalcitol 240 ng kg^{-1} and AMG 641 1.5 mg kg^{-1} ($n=10$).

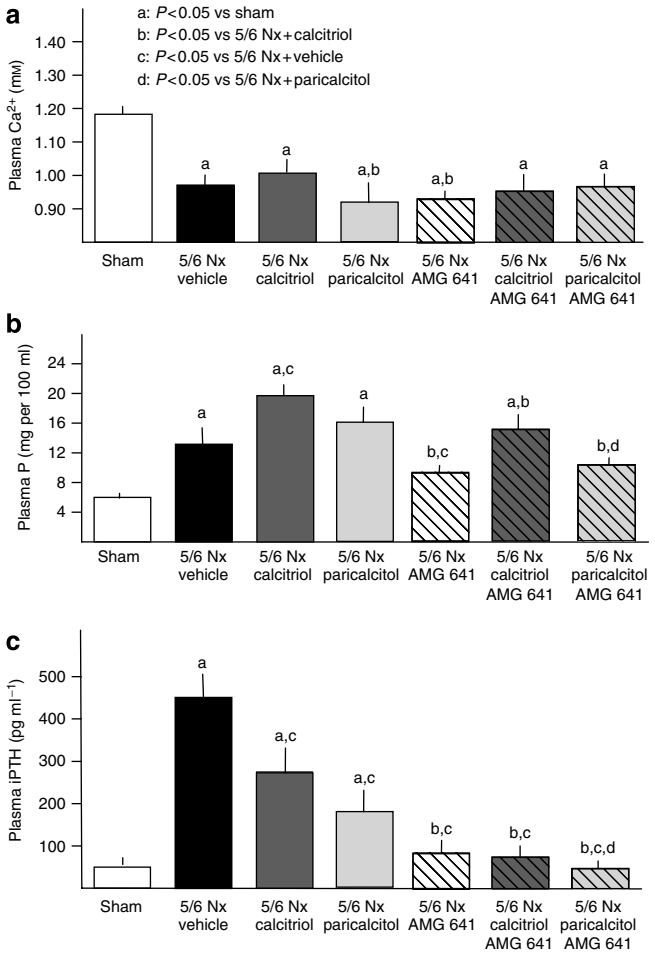

Figure 2 | Blood biochemistry. Plasma levels of (**a**) ionized calcium, (**b**) phosphorus, and (**c**) PTH in sham-operated rats ($n=7$), or 5/6 Nx rats treated for 14 days (qod) with vehicle ($n=18$), calcitriol (80 ng kg^{-1}) ($n=20$), paricalcitol (240 ng kg^{-1}) ($n=12$), AMG 641 1.5 mg kg^{-1} ($n=16$), calcitriol + AMG 641 ($n=15$), or paricalcitol + AMG 641 ($n=10$).