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Bone growth during daily or intermittent calcitriol treatment during renal failure with advanced secondary hyperparathyroidism

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Calcitriol is a standard therapy for secondary hyperparathyroidism in chronic renal failure. We evaluated whether the effect of daily or intermittent calcitriol administration is more efficient in enhancing bone growth in renal failure with advanced secondary hyperparathyroidism in weanling 5/6 nephrectomized rats loaded with phosphorus to induce severe secondary hyperparathyroidism. The animals were treated daily or three times weekly with calcitriol for 4 weeks but the total weekly dose of calcitriol was the same. Although calcitriol increased the serum calcium, it did not lower parathyroid hormone (PTH) or improve tibia and body length. Animals with renal failure and advanced secondary hyperparathyroidism had decreased PTH/PTHrP, which was accompanied by an increase in the cyclin kinase inhibitor $p57^{Kip2}$. Calcitriol treatment upregulated the PTH/PTHrP receptor but also increased inhibitors of cell proliferation such as $p21^{Waf1/Cip1}$, IGFBP3, and FGFR3. Calcitriol also enhanced markers of chondrocyte differentiation, such as IGF1, Vitamin D receptor, FGF23, and bone morphogenetic protein-7. Receptor activator of nuclear factor-$\kappa\beta$ ligand levels improved with calcitriol treatment but without changes in osteoprotegerin suggesting an enhancement of osteo/chondroclastogenesis and mineralization. Overall, both daily and intermittent calcitriol had similar effects on endochondral bone growth in phosphorus-loaded rats with renal failure.

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Calcitriol is a standard treatment for secondary hyperparathyroidism and renal bone disease. Whether daily calcitriol administration is more effective than intermittent therapy in improving growth in children with chronic renal failure and advanced secondary hyperparathyroidism remains to be defined. In pediatric patients with Stage 3–4 chronic renal failure with mild elevations of parathyroid hormone (PTH), daily calcitriol was equally effective as intermittent therapy in the reduction of PTH and enhancement of growth.[1,2] A greater improvement of osteitis fibrosa was demonstrated during intermittent calcitriol therapy in children maintained on peritoneal dialysis with refractory secondary hyperparathyroidism, although 30% of the patients developed low turnover bone.[3] Conversely, Mehls *et al.* reported that only daily calcitriol and not intermittent administration enhanced growth in rats with renal failure after 2 weeks of treatment.[4] The divergence of growth findings in these studies suggests that daily and intermittent calcitriol may have different effects on endochondral bone growth. In the growth plate, calcitriol has dose-dependent inhibitory effects on chondrocyte proliferation and matrix synthesis.[5,6] The aim of the current study is to assess the effects of daily or intermittent calcitriol on chondrocyte proliferation and differentiation in young rats with renal failure and advanced secondary hyperparathyroidism.

RESULTS

Anthromorphic measurements

Weight gain did not differ in nephrectomized and control animals (Table 1). At the end of the study, body length was 16–19% less in Nx-Phos and in both calcitriol groups compared with Control (Table 1). Tibial length was 6–9% shorter in calcitriol and Nx-Phos groups (Table 1). Food efficiency ratio was used to evaluate growth under conditions of controlled food intake;[7] the values did not differ in all groups (Table 1).

Serum biochemical parameters

Bioactive PTH levels increased more than 40-fold in all nephrectomized animals and did not change after 4 weeks of daily or intermittent calcitriol (Table 2). Serum phosphorus, creatinine, and urea nitrogen were much higher in nephrec-

tomized groups (Table 2). Calcitriol increased serum calcium in Nx-Phos animals comparable with Control (Table 2). There was a positive correlation between calcium and tibial length, $R = 0.4$, $P < 0.03$, but serum phosphorus and PTH negatively correlated with body and tibial length, $R = -0.7$, $P < 0.007$.

Growth plate morphometry

The width of the growth plate was 20–30% shorter in phosphorus-loaded rats (Figure 1). There was no difference in the ratio between the proliferative zone and the growth plate, 0.5 ± 0.005, $P =$ NS, and between the hypertrophic zone and the total width in all groups, 0.4 ± 0.05, $P =$ NS (Figure 1). The columnar architecture of the growth plate was nearly restored after 4 weeks of treatment with either daily or intermittent calcitriol (Figure 1).

To evaluate whether daily or intermittent calcitriol has divergent effects on chondrocyte proliferation, several markers of cell proliferation were evaluated: *PTH/PTHrP receptor*, *cyclin* D_1, *histone-4*, *mTOR* (mammalian target of rapamycin), *Col2a1*, and β_1 integrin. In the growth plate, β_1 *integrin* is critical in the maintenance of the columnar architecture, cell-to-extracellular matrix attachment and may

Table 1 | Anthropometric measurements and food efficiency ratio in all groups

	Nx-Phos *N*=8	Nx-Daily D *N*=9	Nx-Int D *N*=9	Control *N*=10
Change in body weight[a] (g)	138 ± 34	139 ± 20	140 ± 33	151 ± 20
Change in body length[a] (cm)	12.1 ± 1.1[b]	11.8 ± 1.1[b]	12.1 ± 1.0[b]	14 ± 1.1
Tibial length[c] (cm)	3.5 ± 0.1[d]	3.6 ± 0.1[d]	3.6 ± 0.1[d]	3.8 ± 0.09
Food efficiency ratio[e]	3.3 ± 1.2	3.3 ± 1.0	3.3 ± 0.8	3.0 ± 0.3

[a]Difference between final and baseline measurements.
[b]$P < 0.05$ versus Control.
[c]Obtained at the time of killing.
[d]$P < 0.01$ versus Control.
[e]Total food consumed/weight gain during the study period.

Table 2 | Serum biochemical measurements in all groups

	Nx-Phos *N*=8	Nx-Daily *N*=9	Nx-Int D *N*=9	Control *N*=10
Bioactive 1-84PTH (ng/l)	1192 ± 403[a]	1362 ± 685[a]	1347 ± 286[a]	29 ± 10
Calcium (mmol/l)	2.0 ± 0.3[b]	2.4 ± 0.1	2.3 ± 0.1	2.5 ± 0.06
Phosphorus (mmol/l)	4.4 ± 1.0[a]	4.0 ± 1.1[a]	4.1 ± 0.9[a]	3.1 ± 0.3
Urea nitrogen (mmol/l)	23 ± 5[a]	29 ± 12[a]	27 ± 10[a]	8 ± 2
Creatinine (μmol/l)	88 ± 35[a]	97 ± 32[a]	88 ± 34[a]	27 ± 9

[a]$P < 0.0001$ versus Control.
[b]$P < 0.0002$ versus all groups.

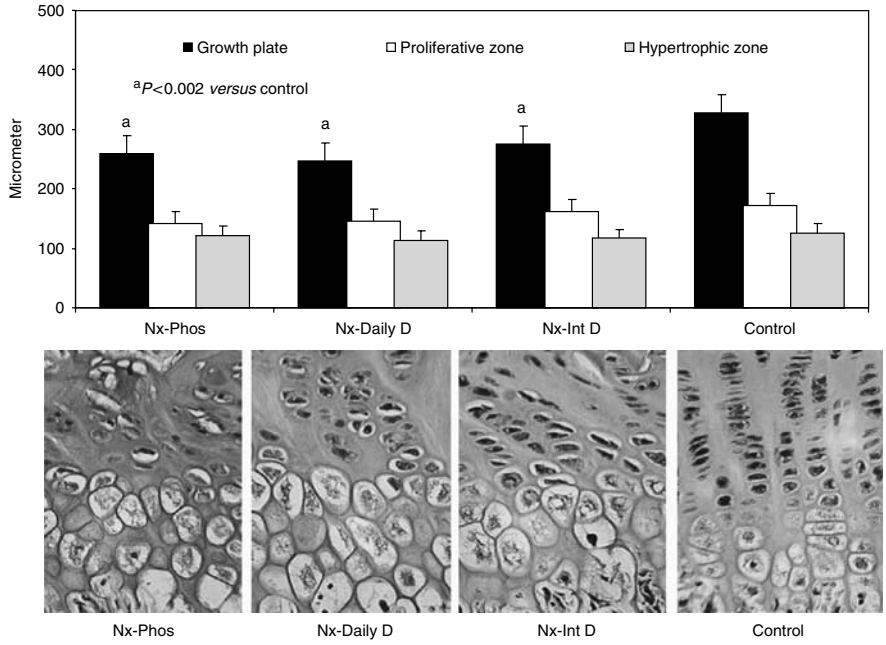

Figure 1 | Measurements of the width of the growth plate, proliferative zone, and hypertrophic zone in all groups (upper panel) and the corresponding photomicrographs in the lower panel, $\times$ **65**. Note the disorganized growth plate architecture in the Nx-Phos group.