The Role of Activin Signaling in the Pathogenesis of Renal Osteodystrophy of CKD-MBD

FP406

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INTRODUCTION

- Renal osteodystrophy is an integral component of chronic kidney disease-mineral/bone disorder (CKD-MBD). CKD-MBD is strongly associated with unacceptable morbidity and mortality in end-stage kidney disease (ESKD).[1]
- Activin A is a transforming growth factor-β superfamily protein that is found at high levels in bone; its signaling is through the type II activin A receptor (ActRIIA).[2-4]
- Inhibin, an inhibitor of activin A signaling, is produced in the ovaries and stimulates bone growth.[2,5]
- Decreased inhibin expression is associated with post-menopausal bone loss.[5]
  - Non-clinical studies suggest that RAP-011 (the murine ActRIIA-IgG1 analog to the human investigational product sotatercept) modulates the balance between bone formation and bone resorption activity by blocking signaling through ActRIIA.[2,6,7]
  - In a 5/6 nephrectomy mouse model of CKD that exhibits bone loss, bone mass measurements were significantly improved with 8 weeks of RAP-011 treatment compared with the control mice.[6]
- A mouse model of *Idlr-/-* high-fat, 5/6 nephrectomy recapitulates many aspects of CKD-MBD, including vascular calcification, hyperphosphatemia, elevated FGF-23, and hyperparathyroidism.[8]
- Despite hyperparathyroidism, loss of bone mass is associated with adynamic bone disease assessed by histomorphometry in the CKD mice.[8]
- In the *Idlr-/-* high-fat fed, 5/6 nephrectomy model of vascular calcification, RAP-011 inhibited Smad-dependent signaling, blocked aortic osteoblastic transition, increased vascular smooth muscle protein levels, and decreased CKD-stimulated vascular calcification.[9]
- The goal of this study was to evaluate the role of activin signaling in the pathogenesis of renal osteodystrophy.

METHODS

- The 4 different groups of mice used in this study are described in **Table 1**.
- Sham-operated *Idlr-/-* high-fat fed mice (n=12) manifest diabetes and hypercholesterolemia. CKD with hyperphosphatemia, elevated FGF-23, and 60% reduction in glomerular filtration rate (CKD-3) was induced by 5/6 nephrectomy at 14 weeks of age in the *Idlr-/-* high-fat fed mice, and is a model of atherosclerotic vascular calcification.
- CKD-3 mice were injected intraperitoneally weekly beginning at 22 weeks of age with vehicle (CKD-3 V; n=13) or RAP-011 10 mg/kg (CKD-3 R; n=15) and studied at 28 weeks by skeletal histomorphometry. Results for CKD-3 mice were compared with wild type (WT) mice (n=5) and sham (n=12).

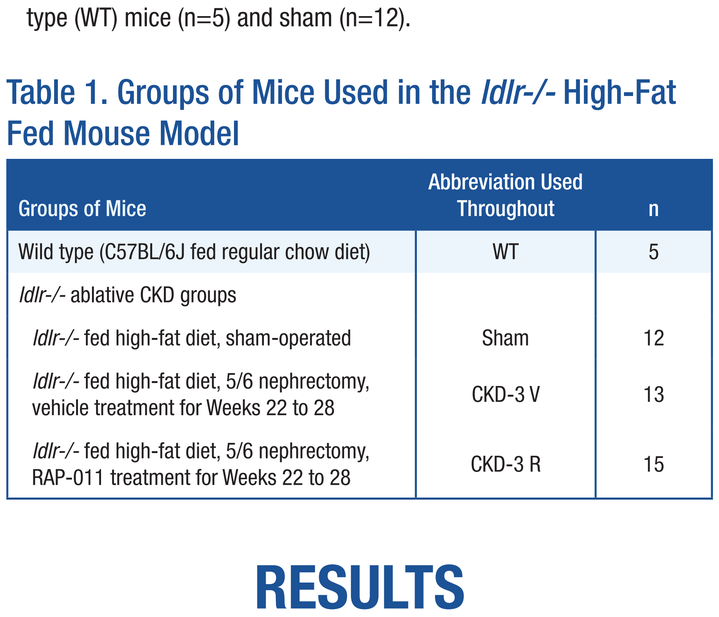
Table 1. Groups of Mice Used in the *Idlr-/-* High-Fat Fed Mouse Model

Groups of Mice	Abbreviation Used Throughout	n
Wild type (C57BL/6J fed regular chow diet)	WT	5
Idlr-/- ablative CKD groups		
Idlr-/- fed high-fat diet, sham-operated	Sham	12
Idlr-/- fed high-fat diet, 5/6 nephrectomy, vehicle treatment for Weeks 22 to 28	CKD-3 V	13
Idlr-/- fed high-fat diet, 5/6 nephrectomy, RAP-011 treatment for Weeks 22 to 28	CKD-3 R	15

RESULTS

- Activin A levels were increased in a model of CKD (**Figure 1**), making the use of RAP-011 as an activin inhibitor a viable therapeutic approach.[9]
- Kidney function was reduced to a degree that is analogous to human stage 3 CKD (CKD-3) in the 2 groups of *Idlr-/-* ablative CKD mice studied.

Figure 1. CKD Increases Activin A in the Circulation



*P<0.005

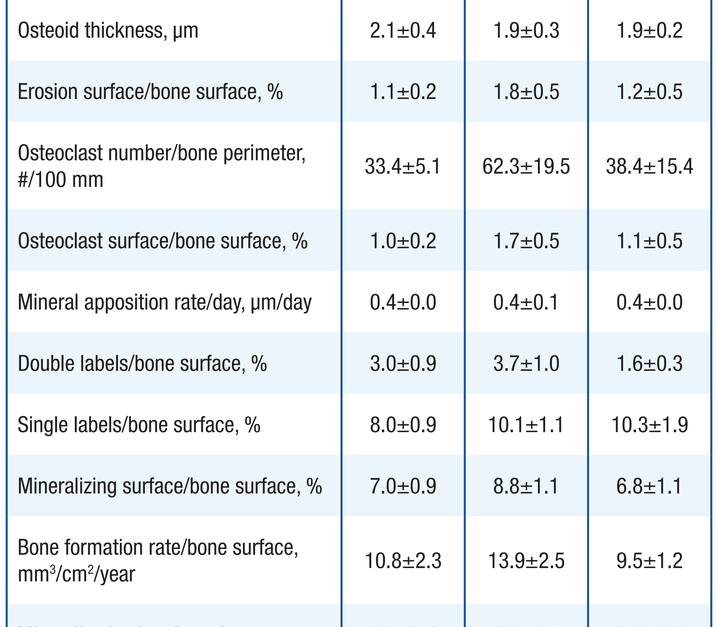
- Relative to WT mice (cancellous bone volume/tissue volume [BV/TV]: 12.90%), sham mice demonstrated reduced BV/TV (10.92%) associated with adynamic bone disease (**Table 2**). This effect of *Idlr-/-* and high-fat diet has been reported previously.[10]
- Induction of CKD-3 caused high turnover bone disease in CKD-3 V mice (unlike prior studies using this model with less severe CKD)[8] and lower BV/TV (11.22%) compared with WT (12.90%), and this was reversed by 6 weeks of RAP-011 treatment (BV/TV: 13.28%) in CKD-3 R (**Table 2**). Similar trends were noted in trabecular thickness.
- CKD-3 V-treated mice demonstrated higher erosion surface/bone surface and higher osteoclast number/100 mm bone length (1.83% and 62.32/100 mm, respectively) compared with sham (1.05% and 33.40/100 mm), which were mitigated by RAP-011 (1.23% and 38.37/100 mm) in CKD-3 R mice (**Table 2**).
- CKD-3 V-treated mice also demonstrated higher osteoblast surface/bone surface and higher osteoblast number/100 mm bone length (1.58% and 110.63/100 mm respectively; P<0.05) compared with WT (not shown) or sham (**Figures 2** and **3**).
- RAP-011 significantly reduced both osteoblast surface/bone surface and osteoblast number/100 mm bone length (0.68% and 43.17/100 mm, respectively; P<0.05) in CKD-3 R-treated mice compared with CKD-3 V-treated mice (**Figures 2** and **3**).
- Despite the significant reduction in the osteoblast number relative to CKD-3 V, the mineral apposition rate with RAP-011 treatment was maintained (0.42 and 0.40 µm/day, respectively), with a significantly higher bone formation rate/osteoblast (0.17 vs. 0.48 µm^3/100 cells/year, respectively; P<0.05 vs. vehicle), which was similar to WT (0.42 µm^3/100 cells/year) (**Figure 4**).
- RAP-011 did not affect hyperphosphatemia or FGF-23 levels.

Table 2. Histomorphometric Results (Mean+SEM)

	Group		
	Sham	CKD-3 V	CKD-3 R
BV/TV, %	10.9±1.3	11.2±0.8	13.3±1.2
Trabecular thickness (plate), µm	30.9±2.1	31.8±1.7	33.2±1.9
Trabecular separation (plate), µm	269.4±17.3	261.9±16.6	238.1±24.9
Osteoid volume/BV, %	0.3±0.1	0.4±0.1	0.2±0.1
Osteoid surface/bone surface, %	2.5±0.6	2.9±0.7	1.7±0.4
Osteoid thickness, µm	2.1±0.4	1.9±0.3	1.9±0.2
Erosion surface/bone surface, %	1.1±0.2	1.8±0.5	1.2±0.5
Osteoclast number/bone perimeter, #/100 mm	33.4±5.1	62.3±19.5	38.4±15.4
Osteoclast surface/bone surface, %	1.0±0.2	1.7±0.5	1.1±0.5
Mineral apposition rate/day, µm/day	0.4±0.0	0.4±0.1	0.4±0.0
Double labels/bone surface, %	3.0±0.9	3.7±1.0	1.6±0.3
Single labels/bone surface, %	8.0±0.9	10.1±1.1	10.3±1.9
Mineralizing surface/bone surface, %	7.0±0.9	8.8±1.1	6.8±1.1
Bone formation rate/bone surface, mm^3/cm^2/year	10.8±2.3	13.9±2.5	9.5±1.2
Mineralization lag time, days	1.9±0.4	1.9±0.5	1.3±0.3
Osteoid maturation time, days	6.3±1.4	5.4±0.9	4.9±0.5

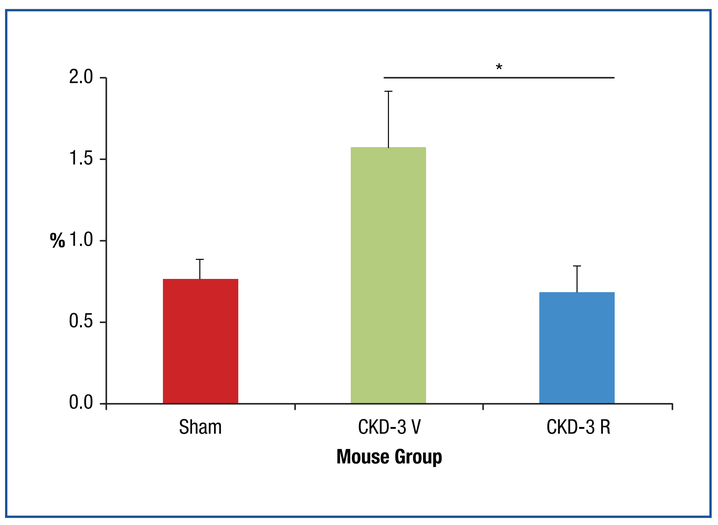
SEM=standard error of the mean.

Figure 2. Osteoblast Number/Bone Perimeter Number/100 mm



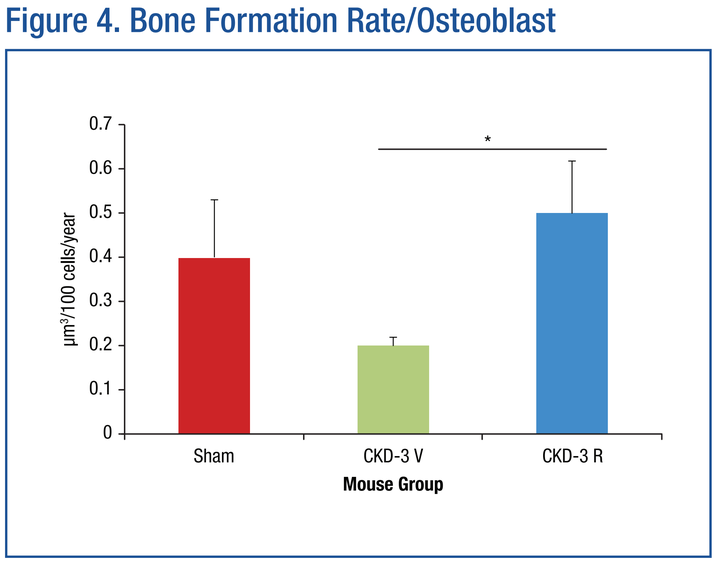
*P<0.05

Figure 3. Percentage of Osteoblast Surface/Bone Surface



*P<0.05

Figure 4. Bone Formation Rate/Osteoblast



*P<0.05

CONCLUSIONS

- Increased circulating activin contributes to the high turnover osteodystrophy associated with CKD-3 in mice.
- Activin inhibition with RAP-011, an ActRIIA ligand trap, increased BV in CKD-3 by inhibiting bone resorption and bone formation rate/osteoblast, counteracting the negative effects of CKD.

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