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Calcimimetics inhibit renal pathology in rodent nephronophthisis

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The development and progression of renal cysts appears to be driven by reduced cellular calcium and increased cyclic adenosine monophosphate (cAMP) from G-protein-coupled receptors. To test whether treatment with a calcimimetic that stimulates the G-protein-coupled calcium-sensing receptor might normalize cystic epithelial cell intracellular calcium and cAMP, thereby inhibiting cyst progression, we used pcy mice. These animals develop cysts principally in the collecting duct, as do humans with nephronophthisis (NPHP). We administered the calcimimetic R-568 mixed in their food at early or late stages in the pathogenesis of cyst formation. The treatment reduced cyst enlargement, and the early treatment inhibited development of renal fibrosis. Although the effect of later treatment was more modest, both stages of the disease responded positively to treatment. Additionally, R-568 decreased total kidney cAMP in the pcy mice and, *in vitro*, decreased cAMP levels and cell proliferation, while increasing intracellular calcium in immortalized human autosomal recessive polycystic kidney disease renal epithelial cells. The latter two effects were unique to R-568 and not replicated by raising extracellular calcium. Thus, treating pcy mice with R-568 was effective in reducing cyst progression in this rodent model of NPHP. Direct studies will be needed to determine whether these results can be applied to the human disease.

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Cystic kidney diseases due to genetic disorders are a leading cause of kidney failure and death in children and adults. Two childhood forms of renal cystic disease, autosomal recessive polycystic kidney disease (ARPKD) and nephronophthisis (NPHP), are characterized by collecting-duct cysts.[1,2] Fibrocystin/polyductin, the protein encoded by the *PKHD1* gene mutated in ARPKD, and nephrocystin 3, from the *NPHP3* gene, are localized to primary cilia.[3] These cystic conditions are associated with abnormalities in cilia structure or function, altered regulation of cellular cyclic adenosine monophosphate (cAMP) and intracellular calcium, and increased epithelial cell proliferation and apoptosis.[4] Thus, interventions that reverse ciliary dysfunction, alter proliferation, or reverse the alterations in intracellular calcium/cAMP may impact the progression of the disease.

Calcium is regulated by several hormones, including parathyroid hormone (PTH) and calcitriol, acting on bone, kidney, and the intestine.[5] Extracellular calcium influences intracellular actions through its binding to the CaSR, a G-protein-linked receptor.[6] Activation of the CaSR increases intracellular calcium and decreases cAMP generation,[7] effects that would oppose the abnormalities in PKD. In the collecting duct, the CaSR is located apically and appears to sense increased luminal calcium and decreases arginine vasopressin-dependent aquaporin-2 expression, perhaps to dilute the urine and prevent hypercalciuria.[8] Type I agonists are inorganic polyvalent cations (for example, calcium and magnesium) and activate the CaSR, without the need for other agonists. In contrast, type II agonists, or calcimimetics, are positive allosteric modulators of the CaSR. The first generation type II CaSR agonist, R-568, was manufactured by NPS (Oread Labs, Lawrence, KS), and the second generation of calcimimetic, Cinacalcet, was developed by Amgen (Amgen Thousand Oaks, CA) and is approved by the FDA for use in the treatment of secondary hyperparathyroidism in patients on dialysis. The primary difference between R-568 and Cinacalcet are the P450 drug interactions.[9] We have previously demonstrated that treatment with R-568 inhibited cyst growth in the Cy/+ model of cystic kidney disease. Kidney size, cyst volume density, fibrosis, and renal function were all improved between 34 and 38 weeks of age, a time when there is cyst growth of all tubular segments.[10] In the

present study, we determined the effect of R-568 on an orthologous model of NPHP, the pcy mice, with cyst formation derived principally from the collecting duct. The results demonstrate that R-568 is effective in the prevention of renal cystic disease progression in this animal model.

RESULTS

The effect of R-568 on the prevention of cystic disease in pcy mice

NPHP (pcy) mice and normal CD1 mice were treated with control or R-568 for 11 weeks, starting from 4 weeks of age. There was no significant gender dimorphism in the cystic disease progression in the pcy mouse model; therefore, male and female data were combined. The observed changes in serum biochemistries are shown in Table 1. There were significant elevations in serum blood urea nitrogen, PTH, phosphorus, and calcium in pcy mice compared with CD1 control mice (Table 1). R-568 treatment, compared with no R-568, lowered serum PTH and blood urea nitrogen in pcy mice but had no significant effect in CD1 mice. However, there was no difference in serum calcium and phosphorus concentration in R-568-treated animals (CD1 or pcy mice) compared with non-treated animals.

The effect of R-568 on the kidneys is shown in Table 1 and Figure 1. The pcy mice had significant renal pathology, with enlarged kidneys, increased weight of kidneys, and fibrocystic histopathology. R-568 treatment prevented much of the renal histopathology in the early stage in NPHP in the pcy mouse. For example, R-568 significantly decreased the total kidney weight (0.66 ± 0.21 versus 0.93 ± 0.21 g, $P < 0.05$) in pcy mice but had no effect in CD1 mice. R-568 also reduced the KW%BW in pcy mice (3.3 ± 0.8 versus 4.8 ± 1.1, Table 1, $P < 0.05$). There was also a significant reduction in cystic change and fibrosis with R-568 treatment. R-568 significantly decreased cyst volume density (%) in pcy mice (Table 1 and Figure 1, $P < 0.05$) and cyst volume (0.20 ± 0.11 with R-568 versus 0.38 ± 0.16 ml without R-568, $P < 0.05$, Table 1). Furthermore, R-568 treatment reduced renal fibrosis (Table 1). These results demonstrate the efficacy of R-568 treatment in slowing early-stage renal disease in this animal model of NPHP.

The effect of R-568 on late-stage NPHP in pcy mice

As shown in Table 2, all three treatment groups (R-568, Ca^{2+}, and R-568 + Ca^{2+}) reduced serum PTH levels compared with control ($P < 0.05$), with no difference between the three treatment groups. Calcium (with or without R-568) significantly decreased serum phosphorus and increased calcium levels versus control and R-568 alone. In contrast, R-568 alone decreased serum calcium (Table 2). None of the treatments significantly reduced blood urea nitrogen levels, but there was a trend in reduction in the R-568-treated group.

Among the three treatment groups, only R-568 alone reduced total kidney weight (R-568: 0.75 ± 0.15 versus control: 0.94 ± 0.30 g, $P < 0.05$; Table 2 and Figure 2), whereas the addition of calcium negated the effects of R-568.

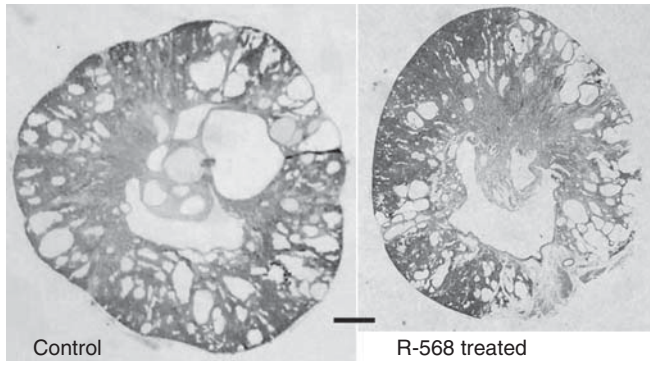

Figure 1 | Renal histopathology in pcy mice treated with R-568 from 5 to 15 weeks of age. At 15 weeks of age, pcy kidney demonstrates diffuse cystic change and near-maximal cystic enlargement (left panel). Treatment with 0.05% R-568 causes a significant delay in the development of cystic pathology (right panel). Scale bar = 1 mm.

Table 1 | Effect of R-568 on the early-stage renal disease in pcy and normal CD1 mice

	pcy		CD1	
Treatment group (4–15 weeks)	Control (*n*=20)	R-568 (*n*=20)	Control (*n*=22)	R-568 (*n*=24)
BUN (mg/dl)	29.9 ± 10.5*	24.8 ± 3.0*#	20.7 ± 3.2	19.7 ± 2.7
PTH* (pg/ml)	201 ± 136*	88 ± 62*#	48 ± 23	26 ± 11
Phosphate (mg/dl)	9.9 ± 2.1*	10.7 ± 2.2*	6.4 ± 1.3	7.0 ± 1.4
Calcium (mg/dl)	8.8 ± 1.2*	8.2 ± 1.3*	5.9 ± 0.7	6.7 ± 1.2#
BW (g)	19.8 ± 2.4*	19.9 ± 1.9*	40.0 ± 8.9	41.8 ± 7.2
KW (g)	0.93 ± 0.21*	0.66 ± 0.21#	0.65 ± 0.15	0.58 ± 0.16
KW%BW	4.8 ± 1.1*	3.3 ± 0.8*#	1.6 ± 0.2	1.4 ± 0.2
Cyst vol density (%)	40.0 ± 9.1	27.7 ± 10#		
Cyst vol (ml)	0.40 ± 0.16	0.21 ± 0.12#		
Fibrosis score	4.0 ± 0.0	3.0 ± 0.3#		

Abbreviations: BUN, blood urea nitrogen; BW, body weight; KW, kidney weight; PTH, parathyroid hormone.
KW%BW is KW as a percent of the total BW; cyst vol density is the cyst volume (assuming 1 g/cc of cyst) expressed as a percent of the total BW; cyst vol is the cyst volume in cubic centimeters (cc) determined from the cyst volume density × KW; and fibrosis score is based on a qualitative, 1+ to 4+ scale.
Data presented as mean ± s.d.
*$P < 0.05$, pcy versus CD1 mice, with or without R-568; #$P < 0.05$, R-568 versus control, pcy, or CD1 mice.