Discovery and Characterization of a Potent and Orally Bioavailable Parathyroid Hormone Receptor Type-1 (PTHR1) Antagonist for the Treatment of Hypercalcemia

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Hypercalcemia is a common disorder defined as a serum calcium concentration higher than the normal range of 8.5 - 10.5 mg/dL. The most common causes of hypercalcemia are over-secretion of parathyroid hormone (PTH) from one or more enlarged parathyroid glands, which can lead to primary hyperparathyroidism (PHPT), or over-secretion of parathyroid hormone related protein (PTHrP) from a cancerous tumor, which leads to humoral hypercalcemia of malignancy (HHM). PTH is an 84 amino acid peptide that regulates calcium and phosphate homeostasis through activation of its receptor, PTHR1. PTHrP has close homology to the N-terminal region of PTH and also activates PTHR1, inducing similar biological actions. Activation of PTHR1, a class B G-protein coupled receptor expressed in bone and kidney, leads to an increase in cAMP and PKA activation, inducing gene expression changes of important modulators of bone homeostasis. In the kidney, it increases renal phosphate excretion and calcium reabsorption. Hyperactivation of PTHR1 due to high levels of either PTH or PTHrP results in increased calcium release from the bone matrix, as well as increased calcium reabsorption in the kidney, causing hypercalcemia. Surgery is the first line therapy for PHPT, but patients that cannot or choose not to have surgery are prescribed calcimimetics and/or bisphosphonates. Calcimimetics decrease circulating calcium levels but have no effect on bone homeostasis, while bisphosphonates improve bone homeostasis but have little effect on circulating calcium levels. HHM patients are prescribed bisphosphonates or denosumab, both of which possess undesirable side effects. We hypothesize that blocking PTH/PTHrP action via a PTHR1 antagonist may provide an improved therapeutic mechanism to treat PHPT and HHM, and potentially other diseases of hypercalcemia.

Using an iterative medicinal chemistry approach, Crinetics has identified several nonpeptide PTHR1 antagonists via both binding and functional in vitro assays. One of these compounds, Antagonist 1 (ANT-1), has low nanomolar binding affinity for both human and rat PTHR1 and is potent in human, monkey, rat, mouse and canine PTHR1 functional antagonist assays in vitro. ANT-1 has good oral exposure in preclinical species and desirable drug-like properties, including lack of inhibition of cytochromes P450 and the hERG ion channel, and stability in liver microsomes. In rat models of PTH- and PTHrP-induced hypercalcemia, ANT-1 dose-dependently suppresses ionized blood calcium, providing support that a nonpeptide PTH antagonist has potential use as an effective therapeutic for hypercalcemia caused by PHPT and HHM. Currently, ANT-1 and other potential candidate molecules are being evaluated in a battery of safety studies to select the optimal molecule(s) suitable for evaluation in human clinical trials.

Targeting PTHR1 should decrease hypercalcemia associated with PTH and PTHrP over-secretion

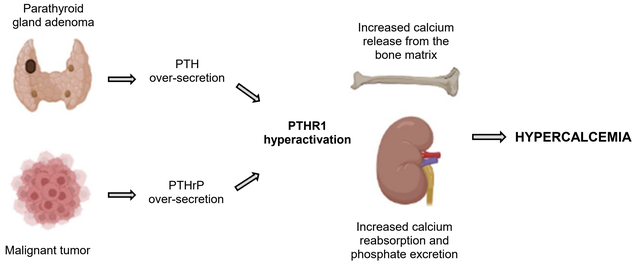

Figure 1. Hypercalcemia associated with PTH and PTHrP over-secretion. PTH and PTHrP over-secretion due to a parathyroid gland adenoma or a malignant tumor will hyperactivate PTH1R expressed in the bone and kidney inducing changes of important modulators of calcium homeostasis, ultimately resulting in hypercalcemia.

ANT-1 potently inhibits PTH binding to the human and rat PTH type-1 receptors

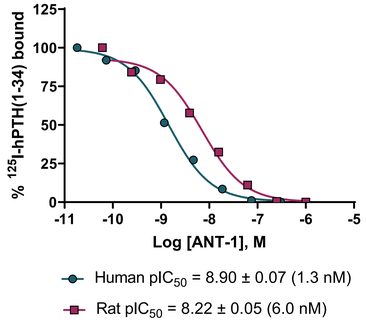

Figure 2. Radioligand displacement assays using human and rat PTHR1. Representative dose-response curves for ANT-1 in assays measuring displacement of ^{125}I-hPTH(1-34) binding to membranes from CHO-K1 cells heterologously expressing human or rat PTHR1. Concentration of ^{125}I-hPTH(1-34) used in the assays was less than its K_d (human K_d = 0.6 nM, rat K_d = 1.5 nM). pIC$_{50}$ values reported with SEM, n ≥ 3.

ANT-1 is potent in functional antagonist assays targeting human and rat PTHR1

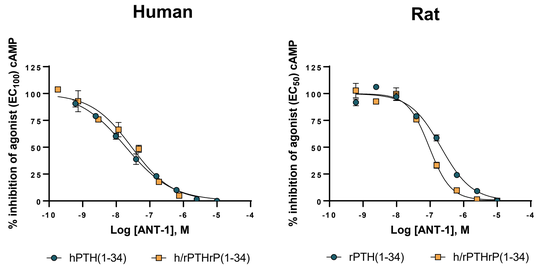

Figure 3. Functional antagonist assay at human and rat PTHR1. Cells heterologously expressing human or rat PTHR1 were treated with a constant concentration of agonist in the presence of eight concentrations of ANT-1. Agonist concentrations were EC$_{100}$ hPTH(1-34) or h/rPTHrP for hPTHR1 and EC$_{50}$ rPTH(1-34) or h/rPTHrP(1-34) for rPTHR1. cAMP production was quantified after 30 minute incubation and IC$_{50}$s were calculated from concentration-response curves. Points represent mean ± SEM of three technical replicates. Figures are representative of two independent experiments.

ANT-1 is also potent in functional antagonist assays targeting PTHR1 from other species

	Agonist	
	PTH(1-34)	PTHrP(1-34)
PTHR1	pIC$_{50}$ ± SEM (IC$_{50}$, nM)	
Human	7.60 ± 0.13 (25 nM)	7.43 ± 0.06 (38 nM)
Monkey	6.96 ± 0.04 (110 nM)	6.93 ± 0.03 (118 nM)
Rat	6.81 ± 0.09 (150 nM)	7.12 ± 0.12 (80 nM)
Mouse	6.96 ± 0.04 (110 nM)	6.37 ± 0.06 (436 nM)
Dog	7.46 ± 0.16 (35 nM)	6.68 ± 0.05 (213 nM)

Table 1. ANT-1 potency at functional antagonist assay. Cells overexpressing the specific PTHR1 were treated as in Figure 3 and the ability of ANT-1 to suppress cAMP production was calculated and expressed as pIC$_{50}$ ±SEM of 2-3 independent experiments.

ANT-1 has good drug-like properties and is orally bioavailable

	CYP450 Inhibition (μM)		hERG (μM)	LM stability $t_{1/2}$ (min)		Rat PK		Dog PK	
	2D6	3A4		Human	Rat	F%	$t_{½}$ (h)	F%	$t_{½}$ (h)
ANT-1	>10	>10	8.6	>600	>600	49	17	52	64

Table 2. Drug-like characteristics of ANT-1. ANT-1 was screened for CYP and hERG inhibition and liver microsomal (LM) stability. Bioavailability was measured in rat and dog as described in the figure below.

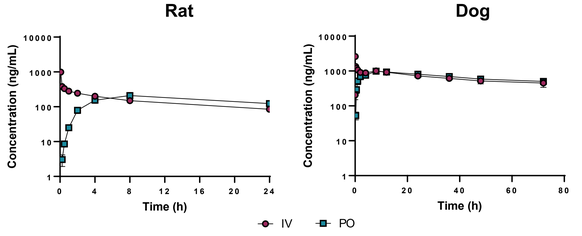

Figure 4. PTHR1 antagonists' pharmacokinetic plasma profile in preclinical species. Sprague Dawley rats were administered ANT-1 at 5 mg/kg (IV-intravenous) and 10 mg/kg (PO-oral). Male Beagle dogs were administered ANT-1 at 2.5 mg/kg (IV-intravenous) and 5 mg/kg (PO-oral). ANT-1 concentration was measured in plasma at specific time points. Points represent mean ± SEM (n=3).

ANT-1 suppresses ionized calcium in rat models of PTH- and PTHrP-induced hypercalcemia

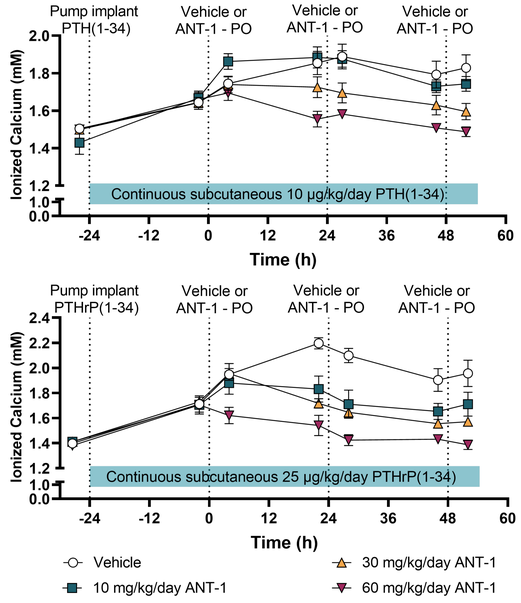

Figure 5. Effect of oral administration of ANT-1 on ionized calcium levels in rat PTH- and PTHrP-dependent models of hypercalcemia. Adult male Sprague Dawley rats received continuous subcutaneous infusion of 10 μg/kg/day rat PTH(1-34) (top panel) or 25 μg/kg/day rat/human PTHrP(1-34) (bottom panel) via osmotic minipump to induce a rise in blood ionized calcium. Starting at 24 hours post pump implant, ANT-1 was administered once daily by oral gavage for 3 days and blood ionized calcium was measured at -2h and 4h post dose. Points represent mean ± SEM (n=7-8 rats/group).

Conclusion

Crinetics has developed potent, drug-like PTHR1 antagonists that inhibit hypercalcemia in a rat model and could be a viable treatment for hypercalcemia in humans.

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