In Vitro Analysis of Effects of Upacicalcet, Cinacalcet, and Etelcalcetide on the Calcium Sensing Receptor (CaSR)

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BACKGROUND

- Secondary hyperparathyroidism (SHPT) is a common complication of chronic kidney disease (CKD), particularly in patients receiving dialysis.[1,2]
- Progressive loss of kidney function disrupts calcium, phosphate, and vitamin D homeostasis, leading to increased parathyroid hormone (PTH) secretion and parathyroid gland hyperplasia.[1,2]
  - Persistent elevations in PTH contribute to CKD mineral and bone disorder, including high turnover bone disease, vascular calcification, and increased cardiovascular risk.[1,2]
- Upacicalcet, cinacalcet, and etelcalcetide, calcimimetic agents that target the calcium-sensing receptor (CaSR) on parathyroid cells, enhance receptor sensitivity to extracellular calcium, suppress PTH secretion, and are used in the management of SHPT.[1,3-6]
- While the clinical efficacy and safety profiles of cinacalcet and etelcalcetide are well characterized, clinical data for upacicalcet are newly emerging, and comparative in vitro data remain limited.[3-6]
- This analysis evaluated the differences in CaSR activation by examining the calcium set point across calcimimetic agents.
  - The calcium set point is defined as the extracellular calcium concentration required to achieve 50% inhibition of PTH secretion via CaSR activation.[7]
  - In untreated SHPT, the calcium set point curve is shifted towards the right, meaning higher calcium levels are required to suppress PTH secretion.

METHODS

- Two in vitro experiments used a live cell, non-imaging assay to detect CaSR activation. Activation of CaSR is detected through secondary messenger signaling that causes an increase in dye fluorescence.
- Experiment 1 determined the concentration required to elicit 50% of the maximal response (EC50) for upacicalcet, cinacalcet, and etelcalcetide at a constant calcium concentration, which was the concentration of calcium required to elicit 20% of the maximal response (EC20; 130 µM) in the absence of any allosteric modulator.
  - The range of concentrations evaluated for upacicalcet, cinacalcet, and etelcalcetide is shown in **Table 1**.

Table 1. Compound concentrations for EC50 determination.

Compound	Concentration Range
Upacicalcet	0.015 nM – 0.3 mM (10 concentration data points)
Cinacalcet	0.15 nM – 3 mM (10 concentration data points)
Etelcalcetide	1.5 nM – 30 mM (10 concentration data points)

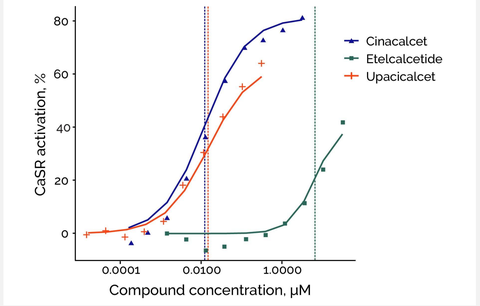
Abbreviation: EC50, concentration required to elicit 50% of the maximal response.

- Experiment 2 assessed the calcium concentration required for 50% maximal activation (RC50) in the presence of each compound at its EC50.
- Data were analyzed using a saturable maximal increase (Emax) model with a hill coefficient to the observed data.

RESULTS

- Upacicalcet, cinacalcet, and etelcalcetide all acted as positive allosteric modulators, causing a leftward shift in the calcium concentration-response profile. (**Figure 1**). The model-estimated EC50 values are displayed in **Table 2**.
  - Cinacalcet and upacicalcet have similar EC50 values when calcium levels are 130 µM.
  - Etelcalcetide is less potent than both cinacalcet and upacicalcet.

Figure 1. Mean observed data and Emax model fits for upacicalcet, cinacalcet, and etelcalcetide.



Symbols represent mean data from 2 samples. Solid lines represent the Emax model fit. Vertical lines represent the EC50 for each compound.
Abbreviations: EC50, concentration required to elicit 50% of the maximal response; Emax, maximal increase.

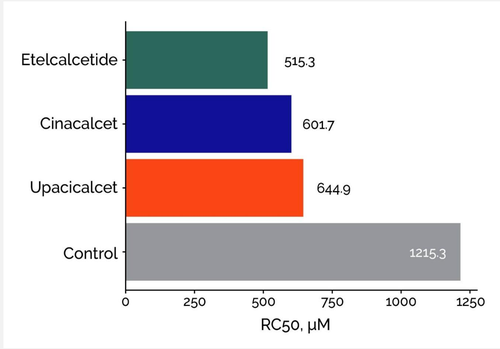
Table 2. Model-estimated EC50 values for upacicalcet, cinacalcet, and etelcalcetide.

Compound	EC50
Upacicalcet	14.2 nM
Cinacalcet	11.8 nM
Etelcalcetide	6.23 µM

Abbreviation: EC50, concentration required to elicit 50% of the maximal response.

- RC50 values for calcium were lowest for etelcalcetide (515.3 µM), then cinacalcet (601.7 µM), and highest for upacicalcet (644.9 µM), all lower than control (1215.3 µM) (**Figure 2**).

Figure 2. Model-estimated RC50 values for calcium chloride alone and with upacicalcet, cinacalcet, and etelcalcetide.



Abbreviations: RC50, calcium concentration required for 50% maximal activation.

LIMITATIONS

- The calcium concentrations in the assay ranged from 5 µM (0.02 mg/dL) to 10 mM (40.1 mg/dL) which are well outside of the physiologic range of 8.5 – 10.5 mg/dL; however, this cell-based assay is not intended to mimic human physiologic concentrations.

CONCLUSIONS

- Upacicalcet, cinacalcet, and etelcalcetide are effective positive allosteric modulators of CaSR in vitro.
- Upacicalcet has the highest calcium set point, suggesting less activation at low calcium levels compared with cinacalcet and etelcalcetide.
- The distinct in vitro activation profiles of upacicalcet, cinacalcet, and etelcalcetide may translate to differences in clinical outcomes, particularly regarding risk of hypocalcemia and potentially gastrointestinal adverse effects.
- Further clinical studies are warranted to confirm whether these in vitro differences in CaSR activation are associated with differentiated safety and efficacy profiles in hemodialysis patients with SHPT.

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