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Effect of cinacalcet on urine calcium excretion and supersaturation in genetic hypercalciuric stone-forming rats

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Idiopathic hypercalciuria is the most common metabolic abnormality in patients with nephrolithiasis. Through successive inbreeding, we have developed a strain of rats whose urine calcium (UCa) excretion is ~8–10-fold greater than that of control rats and who spontaneously form kidney stones. We have termed these rats genetic hypercalciuric stone-forming (GHS) rats. The physiology of the hypercalciuria in the GHS rats closely parallels that of man. We have recently shown that the GHS rat kidneys have an increased number of receptors for calcium (CaR) compared to Sprague–Dawley rats, the strain of rats originally bred to develop the GHS rats. Calcimimetics, such as cinacalcet (Cin), increase the sensitivity of the CaR to Ca. The effects of Cin on UCa are complex and difficult to predict. We tested the hypothesis that Cin would alter urinary (U) Ca and supersaturation with respect to calcium hydrogen phosphate ($CaHPO_4$) and calcium oxalate (CaOx). GHS or control rats were fed a normal Ca diet (0.6% Ca) for 28 days with Cin (30 mg/kg/24 h) added to the diet of half of each group for the last 14 days. The protocol was then repeated while the rats were fed a low Ca (0.02% Ca) diet. We found that Cin led to a marked reduction in circulating parathyroid hormone and a modest reduction in serum Ca. Cin did not alter UCa when the GHS rats were fed the normal Ca diet but lowered UCa when they were fed the low Ca diet. However, Cin did not alter U supersaturation with respect to either CaOx or $CaHPO_4$ on either diet. If these findings in GHS rats can be confirmed in man, it suggests that Cin would not be an effective agent in the treatment of human idiopathic hypercalciuria and resultant stone formation.

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Hypercalciuria is the most common metabolic abnormality found in humans with nephrolithiasis.[1–5] Hypercalciuria raises urine saturation with respect to the solid phases of calcium hydrogen phosphate ($CaHPO_4$, brushite) and calcium oxalate (CaOx), enhancing the probability of nucleation and growth of crystals into clinically significant kidney stones.[1,2,4]

We have established a model of hypercalciuria and nephrolithiasis by successively inbreeding 67 generations of the most hypercalciuric progeny of the most hypercalciuric Sprague–Dawley rats found on an initial screen. Each rat now excretes 8–10 times as much urinary calcium (UCa) as similarly fed controls.[6–23] The hypercalciuria is due to increased intestinal Ca absorption[6,7] coupled to a defect in renal tubular Ca reabsorption[7,14] and enhanced bone mineral resorption,[11] suggesting a systemic dysregulation of Ca homeostasis.[8] Human stone formers and these rats share many metabolic features in common; many human stone formers also have increased intestinal Ca absorption, increased bone resorption, and decreased renal tubule Ca reabsorption.[1,3] Virtually all of these hypercalciuric rats form kidney stones while there was no evidence of stone formation in controls.[10] We have termed the rats genetic hypercalciuric stone-forming (GHS) rats.[10,12,13,15,17–20] The stones formed by the GHS rats fed standard rat chow contain only Ca and phosphorus (P).[10,13,18,19] The dietary addition of hydroxyproline, a common amino acid and an oxalate precursor,[24] results in the formation of CaOx kidney stones.[17,20]

The Ca receptor (CaR) is a seven membrane-spanning protein that is part of the G-coupled protein family of plasma membrane receptors.[25] The CaR is expressed in a wide variety of tissues including parathyroids, kidney, and gastrointestinal tract.[26–28] There is marked homology between the parathyroid and kidney CaR in a variety of animals including man and rat.[25,28] In the thick ascending limb of the loop of Henle, the secretion of potassium into the lumen, through the potassium channel ROMK, increases the lumen positive voltage and drives Ca reabsorption through the paracellular space.[26] At this tubular site, elevation of the blood Ca level is detected by the CaR, located on the plasma (anti-luminal) membrane, decreasing potassium traffic through this channel

resulting in decreased luminal positivity, decreased Ca reabsorption, increased UCa, and a reduction in the concentration of serum Ca.

The GHS rats have been found to have elevated levels of vitamin D receptors (VDRs) in the intestinal mucosa, bone, and renal cortex.[8,11,16,23] Analogously, human stone formers have also been shown to have an elevated number of VDRs in their circulating monocytes.[29] CaR contains vitamin D response elements in its promoter region.[30] We found that there was increased CaR mRNA and protein in the GHS rat kidney and that $1,25(OH)_2D_3$ increased CaR levels through both elevated CaR gene expression and prolonged tissue half-life.[21]

The calcimimetics, such as cinacalcet (Cin), are small organic molecules that act as allosteric activators of the CaR, increasing the sensitivity of the CaR to serum Ca and substantially lowering parathyroid hormone (PTH) levels.[31–33] In patients with secondary hyperparathyroidism treated with Cin, there is a marked reduction of PTH ($\sim$50%) and a modest reduction of serum Ca ($\sim$10%).[31,33] The effect of Cin on human UCa and supersaturations with respect to common solid phases responsible for kidney stones has not been reported.

The effects of Cin on renal tubular Ca reabsorption and resulting UCa are complex. A reduction in PTH should increase UCa; in addition, increasing the sensitivity of the renal CaR to Ca should lead to an increase in UCa. However, the lowered filtered load of Ca from Cin-induced hypocalcemia should lower UCa. In this study, we utilized the GHS rats to test the hypothesis that Cin would alter urine Ca excretion and supersaturation with respect to $CaHPO_4$ and CaOx.

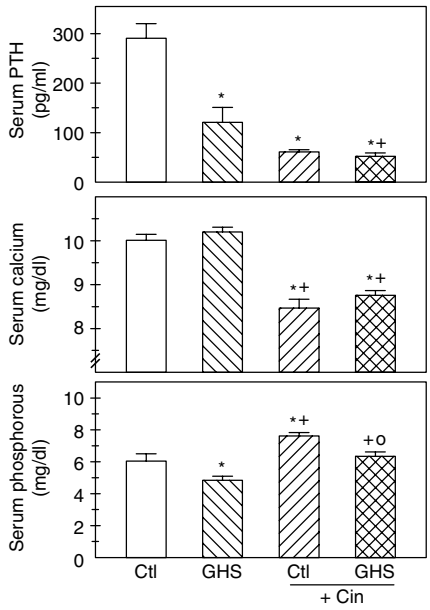

Figure 1 | Serum PTH, Ca, and P at the conclusion of the experiment. Fourteen 67th generation female GHS and 14 Ctl rats were placed in metabolic cages. From days 1–14, each rat in each group was fed NCD. From days 15 to 28, half of each group (seven GHS and seven Ctl rats) was continued on NCD and the other half (seven GHS and seven Ctl rats) were fed NCD supplemented with Cin. From days 29 to 42, all GHS and Ctl rats were fed LCD. No rat received Cin. From days 43 to 56, half of each group was continued on LCD without modification and the other half (the same rats that had previously received Cin) was fed LCD supplemented with Cin. Blood was then drawn at the conclusion of day 56. Abbreviations: Ctl, Sprague–Dawley rats; GHS, genetic hypercalciuric stone-forming rats; NCD, 0.6% Ca and 0.65% P; LCD, 0.02% Ca and 0.65% P; Cin, cinacalcet; *, $P<0.05$ vs Ctl; +$P<0.05$ vs GHS; o, $P<0.05$ vs Ctl + Cin.

RESULTS

Serum PTH, Ca, and P

At the conclusion of the experiment, when all rats were being fed low Ca diet (LCD), the serum PTH was significantly lower in the GHS rats compared to the control (Ctl) rats (Figure 1, top). The addition of Cin led to a significant fall in PTH both in the Ctl and in the GHS rats compared to respective non-Cin-fed rats. There was no difference in serum PTH between the two Cin-fed groups. There was no difference in serum Ca between the GHS and the Ctl rats not fed Cin; however, the addition of Cin led to a significant reduction in Ca both in the Ctl and in the GHS rats (Figure 1, middle). There was no difference in serum Ca between the two Cin-fed groups. Compared to the Ctl rats, serum P was significantly lower in the GHS rats; the addition of Cin led to an increase in serum P both in the Ctl and in the GHS rats (Figure 1, bottom). Serum P was lower in the GHS rats fed Cin compared to the Ctl rats fed Cin.

Urine Ca excretion

While being fed the normal Ca diet (NCD, 0.6% Ca), the GHS rats excreted significantly more UCa compared to the Ctl rats (Figure 2, top). With the addition of Cin to half of each group, UCa remained significantly elevated in the GHS rats compared to the Ctl rats whether or not they received Cin. Cin did not alter UCa in either group. On the LCD (0.02% Ca) without Cin, the GHS rats continued to excrete significantly more UCa than the Ctl rats (Figure 2, bottom). With the addition of Cin to the same rats that had received Cin previously, UCa remained significantly elevated in the GHS rats compared to the Ctl rats whether or not they received Cin. Cin significantly lowered UCa in the GHS, but not in the Ctl rats.

Urine P excretion

While being fed NCD the GHS rats excreted slightly, but significantly, more P compared to the Ctl rats (Figure 3, top). With the addition of Cin to half of each group, urine phosphorus (UP) remained significantly greater in the GHS rats compared to the Ctl rats whether or not they received Cin. Cin did not alter UP in the Ctl or in the GHS rats. On the LCD without Cin, the GHS rats continued to excrete significantly more UP than the Ctl rats and there was a significant increase in the P excretion for both Ctl and GHS rats compared to NCD (Figure 3, bottom). With the addition of Cin, UP was not different between the GHS and the Ctl rats. Cin did not alter UP in the Ctl or in the GHS rats.