Cinacalcet HCl attenuates parathyroid hyperplasia in a rat model of secondary hyperparathyroidism

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Background. Secondary hyperparathyroidism (HPT) in chronic kidney disease (CKD) is a physiologic response to kidney failure characterized by elevated serum parathyroid hormone (PTH) levels and parathyroid gland enlargement. Calcimimetic agents acting through allosteric modification of the calcium-sensing receptor (CaR) can attenuate parathyroid hyperplasia in rats with secondary HPT. The present study explores the effects of the calcimimetic cinacalcet HCl on parathyroid hyperplasia, apoptosis, and PTH secretion in a rat model of secondary HPT.

Methods. Cinacalcet HCl was gavaged daily (1, 5, or 10 mg/kg) for 4 weeks starting 6 weeks post-5/6 nephrectomy. After dosing, hyperplasia was determined using parathyroid weight and proliferating cell nuclear antigen (PCNA) immunochemistry. Apoptosis was determined using in situ techniques. Serum PTH(1–34) and blood chemistries were determined throughout the course of the study.

Results. Administration of cinacalcet HCl (5 or 10 mg/kg) significantly reduced the number of PCNA-positive cells and decreased parathyroid weight compared with vehicle-treated 5/6 nephrectomized rats. There was no difference in apoptosis from cinacalcet HCl-treated or vehicle-treated animals. Serum PTH and blood ionized calcium levels decreased in cinacalcet HCl-treated animals compared with vehicle-treated controls.

Conclusion. The results confirm previous work demonstrating that calcimimetic agents attenuate the progression of parathyroid hyperplasia in subtotally nephrectomized rats, extending earlier observations to now include cinacalcet HCl. These results support a role for the CaR in regulating parathyroid cell proliferation. Therefore, cinacalcet HCl may represent a novel therapy for improving the management of secondary HPT.

Parathyroid cells secrete parathyroid hormone (PTH) and play a central role in maintaining circulating levels of ionized calcium. In contrast to normal adults, where proliferation and growth of the parathyroid glands is maintained at a low level, abnormally increased parathyroid cell proliferation (hyperplasia) is characteristic of hyperparathyroidism (HPT) secondary to chronic kidney disease (CKD) [1]. Although the mechanisms are not precisely defined, the onset of parathyroid hyperplasia due to CKD involves several factors, such as 1,25-dihydroxyvitamin D3 [vitamin D3 (calcitriol)] deficiency, phosphorus retention, and hypocalemia [2–4]. In addition, down-regulation of vitamin D receptor (VDR) and calcium-sensing receptor (CaR) expression may contribute to the overall pathogenesis of parathyroid hyperplasia [3, 4]. Since CKD results in impaired renal production of calcitriol, current therapies use exogenously administered calcitriol or other vitamin D sterols to repress both parathyroid cell proliferation and PTH synthesis [4, 5].

In CKD, phosphorus retention by the kidney results in high levels of serum phosphorus that worsen secondary HPT by enhancing parathyroid cell proliferation and PTH synthesis and secretion. Indirectly, high phosphate levels also mediate a reduction in serum calcitriol and blood ionized calcium levels [3, 4]. Although phosphorus restriction and/or the use of phosphate binders can ameliorate parathyroid hyperplasia [6, 7], potentially serious adverse clinical consequences have been associated with these types of therapeutic approaches to management of secondary HPT. These results have prompted investigators to search for alternative therapeutic targets mediating parathyroid hyperplasia and/or the control of PTH secretion/production. The main regulator of PTH secretion is serum calcium. Accordingly, one target is the CaR, a G protein–coupled receptor in the membrane of the parathyroid cells that modulates the minute-to-minute release of PTH into the circulation. Activation of the parathyroid cell receptor by calcium increases intracellular calcium concentration and results in abrupt decreases in PTH secretion [8, 9].
Calcimimetic compounds are low-molecular-weight phenylalkylamine derivatives that act as allosteric modulators of the CaR. These compounds enhance the sensitivity of the CaR to extracellular calcium, thus exerting a suppressive effect on PTH secretion [10]. In addition, calcimimetic agents can impede parathyroid gland hyperplasia in subtotally nephrectomized rats [11]. Cinacalcet HCl, an allosteric modulator of the CaR, has recently been approved for the treatment of secondary HPT in patients with CKD on dialysis, and has been shown to reduce PTH secretion both in vitro (bovine parathyroid cell assay) and in vivo (normal rats) [12]. Furthermore, in clinical studies, cinacalcet HCl has shown promise as a novel therapeutic for the treatment of secondary HPT [13–15]. The present report describes the effects of cinacalcet HCl on parathyroid gland hyperplasia, PTH secretion, and parathyroid apoptosis in an in vivo model of chronic renal insufficiency, 5/6 nephrectomized rats with secondary HPT.

METHODS

Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA, USA) weighing 300 to 350 g were used in these studies. One week after arrival, animals were rendered uremic using a two-step partial ligation procedure or were sham-operated. All animals received standard lab chow (Harlan Teklad, Madison, WI, USA) containing 1.17% calcium and 1.00% phosphorus and water ad libitum. The animal protocol was approved by the Institutional Animal Care and Use Committee of Amgen, Inc. (Thousand Oaks, CA, USA).

5/6 nephrectomy model of renal insufficiency

CKD accompanied by secondary HPT was induced in male Sprague-Dawley rats by 5/6 nephrectomy, a procedure that reduces the original functional renal mass by five sixths. In the first surgery, animals were anesthetized by an intramuscular injection (0.1 mL) of a combination of two parts ketamine (100 mg/mL), one part xylazine (100 mg/mL), and 0.3 parts acepromazine (10 mg/mL). The left kidney was exposed, after which the renal artery was located and the uppermost bifurcation (two branches) sutured. Following a 1-week recovery period, animals were again anesthetized and the right kidney was removed. Control animals (shams) were similarly anesthetized and subjected to surgical procedures without suturing the renal artery branches or removing the kidney. Following the surgical procedures, animals were returned to their home cages. Animals were weighed throughout the course of treatment to assess whether drug treatment caused any weight loss.

Cinacalcet HCl dosing for 4 weeks

Starting 6 weeks postsurgery, 5/6 nephrectomized (N = 35) and sham (N = 18) animals received orally either vehicle (20% capisalt in water) (mL/kg) or cinacalcet HCl (1, 5, or 10 mg/kg) for 4 weeks. Sampling for the determination of serum PTH and serum chemistries after the initiation of cinacalcet HCl treatment began at the 8-week time point (see Figs. 4 and 5).

Blood collection for PTH and serum chemistry profile

Blood for the determination of serum PTH, phosphorus, and blood ionized calcium levels was collected at 0 (1 week postsurgery), 2, 4, and 6 weeks. At week 6, vehicle or drug treatment was initiated; therefore, the first posttreatment blood sample to be collected was at week 8. At weeks 8 and 10, blood was collected 2 hours after drug/vehicle treatment. For measurement of blood ionized calcium levels, blood was collected from the orbital sinus of anesthetized rats (2% isofluorane in O2) with heparinized capillary tubes and analyzed using a Ciba-Corning 634 ISE Ca++/pH Analyzer (Ciba-Corning Diagnostics Corp., Medfield, MA, USA). Separately, blood was collected for PTH, blood urea nitrogen (BUN), creatinine, and serum phosphorus levels into SST (clot activator) brand blood tubes (BD, Franklin Lakes, NJ, USA) and allowed to clot. Serum was removed and stored at −70°C until assayed. PTH levels were quantified according to the vendor’s instructions using a rat PTH[1–34]+ immunoradiometric assay kit (Immutopics, San Clemente, CA, USA). BUN, creatinine, and phosphorus levels were determined using a blood chemistry analyzer (Olympus AU 400) (Melville, NY, USA).

Proliferating cell nuclear antigen (PCNA) immunohistochemistry

The laryngo-tracheal complex was removed at sacrifice and stored 2 to 3 days in zinc-buffered formalin, then transferred to 70% alcohol and trimmed. At trimming, the parathyroids were dissected away from the thyroid and blotted dry on a lint-free Kim wipe (Kimberly Clark Corp., Roswell, GA, USA) prior to being individually weighed on a Sartorius BP211D balance (Gottingen, Germany). Parathyroids were then processed for paraffin embedment. After embedding, 5 μm sections were cut and placed onto charged slides (VWR Scientific, West Chester, PA, USA). Immunostaining was performed on the sections according to the vendor’s instructions using a PCNA staining kit (Zymed Laboratories, Inc., South San Francisco, CA, USA). Ileum tissue from the 5/6 nephrectomized animals were also processed for PCNA immunostaining and used as a measure of general toxemia.

Parathyroid area was determined through the use of an area-measurement graticle containing a series of 0.01 mm² grids (area initially determined using a calibrated graticle) overlaying the central region of a parathyroid section. Sections were taken from approximately the same level of individual parathyroids. Tissue...
samples were visualized at 100× on a Leitz Laborlux microscope (Wetzlar, Germany), and the number of grids overlaying the parathyroid tissue was counted. The total area of the parathyroid was thus determined by multiplying the number of grids by 0.01 mm², after which the number of PCNA-positive cells in the gridded sectional area were counted and expressed as the number of PCNA-positive cells/mm². Slides were coded and an observer who was unaware of treatment group assignment performed quantification of parathyroid proliferation.

**Apoptosis**

To identify apoptosis in parathyroid glands from 5/6 nephrectomized or sham rats treated with vehicle [phosphate-buffered saline (PBS)] or cinacalcet HCl (10 mg/kg), nuclear DNA fragmentation was measured in situ using the Apoptag System (Serologicals Corporation, Atlanta, GA, USA). Briefly, parathyroid gland sections from animals treated with vehicle or cinacalcet HCl were digested with 20 μg/mL proteinase K (Sigma Chemical Company, St Louis, MO, USA) in 0.1 mol/L PBS at room temperature for 15 minutes and incubated with 3% hydrogen peroxide/methanol for 5 minutes to block endogenous peroxidase. Sections were incubated for 1 hour at 37°C with terminal deoxynucleotidyl transferase (TdT) to label exposed 3′-OH DNA ends with digoxigenin-tagged nucleotides. Digoxigenin-labeled DNA was detected by the immunoperoxidase method. Sections were developed with 3,3'-diaminobenzidine (DAB), and the nuclei of apoptotic cells were stained brown. The specificity for apoptosis was verified by negative staining when distilled water was substituted for TdT. Positive control samples were provided by Serologicals Corporation.

**Statistical analysis**

Results were expressed as the mean ± standard error. All data were initially subjected to a two-way analysis of variance (ANOVA), followed by post hoc analysis using Fisher protected least square difference (PLSD) test. The Mann-Whitney test or unpaired Student t test was utilized for two-group comparisons. Significance was set a priori at α < 0.05 for all comparisons. Grubb’s outlier test was also used (GraphPad Software Inc., San Diego, CA, USA). The correlations between serum parameters (PTH, ionized calcium, and phosphorus) vs. parathyroid weight or PCNA-positive cell count were analyzed using Pearson’s correlation test.

**RESULTS**

**Confirmation of secondary HPT induction**

Six weeks after surgery, statistically significant increases in serum BUN (P < 0.001) and creatinine (P < 0.001) were observed, indicating CKD was successfully induced (Table 1). Serum PTH (P < 0.001) and phosphorus (P < 0.01) levels were significantly higher in 5/6 nephrectomized animals (N = 35) when compared to sham controls (N = 18), and Ca²⁺ levels were significantly lower (P < 0.05) (Table 1). These alterations are consistent with the pathophysiology of secondary HPT in rodents that has been previously described in detail [2, 11]. There were no significant differences in body weights among the treatment groups [e.g., at 10 weeks postnephrectomy body weights (mean ± SEM) were 5/6 nephrectomy 10 mg/kg = 495 ± 8 g; 5/6 nephrectomy vehicle = 466 ± 17 g; sham 10 mg/kg = 476 ± 12 g; sham vehicle = 491 ± 17 g].

**Effect of cinacalcet HCl on parathyroid gland weight, area, and hyperplasia**

Administration of cinacalcet HCl at 5 and 10 mg/kg doses for 4 weeks starting 6 weeks post-5/6 nephrectomy resulted in a significant (P < 0.001) reduction in the number of PCNA-positive cells compared with vehicle-treated 5/6 nephrectomy animals (Figs. 1 and 2) (Table 2).
Similarly, cinacalcet HCl resulted in a significant reduction in parathyroid gland weight (P = 0.022) in 5/6 nephrectomy animals, as determined by ANOVA. Additional post hoc analysis revealed a significant (P < 0.05) reduction in parathyroid weight in the cinacalcet HCl (10 mg/kg)-treated 5/6 nephrectomy animals compared with vehicle-treated 5/6 nephrectomy animals (Table 2). A 25% reduction in parathyroid weight was also observed for those animals treated with 5 mg/kg cinacalcet HCl, compared with vehicle treated 5/6 nephrectomy animals. Mean parathyroid gland area was significantly greater (P < 0.01) in 5/6 nephrectomized rats (1.14 ± 0.12 mm²) when compared to rats that underwent the sham procedure (0.47 ± 0.05 mm²). 5/6 nephrectomized rats treated with cinacalcet HCl at 10 mg/kg showed a 25% reduction in parathyroid gland area (0.87 ± 0.09 mm²) compared to 5/6 nephrectomized rats treated with vehicle.

In sham animals, cinacalcet HCl had no effect on parathyroid gland cell proliferation or parathyroid weight compared with vehicle treatment (Table 2). Furthermore, cinacalcet HCl had no effect on cell proliferation from ileum tissue (Fig. 3), which was used as a measure of general toxemia.

In addition, cinacalcet HCl treatment had a significant effect (P = 0.0001) on blood ionized calcium compared with vehicle treatment (Fig. 4B). Decreases in blood ionized calcium were significant in the 5 and 10 mg/kg dose groups at all time points. Although the 1 mg/kg cinacalcet HCl dose reduced blood ionized calcium, the reductions were not significantly different from vehicle controls.

No differences in serum phosphorus levels were observed in cinacalcet HCl (10, 5, or 1 mg/kg) treated 5/6 nephrectomized animals (6.6 ± 0.4, 7.8 ± 1.0, and 9.4 ± 2.7 mg/dL, respectively) compared to vehicle-treated 5/6 nephrectomized animals (7.8 ± 0.8 mg/dL).

In sham animals, cinacalcet HCl treatment resulted in a significant reduction in serum PTH levels (P < 0.001) (Fig. 5A). Additional analysis revealed significant (P < 0.05) decreases in serum PTH levels occurred after cinacalcet HCl treatment in sham animals in the 10 mg/kg-dose group following 2 and 4 weeks of dosing (8 and 10 weeks) and were observed in the 5 mg/kg dose group after 4 weeks of dosing (10 weeks).

Cinacalcet HCl treatment significantly reduced blood ionized calcium levels (P < 0.0001) (Fig. 5B) in sham animals. All doses of cinacalcet HCl resulted in significant (P < 0.05) decreases in blood ionized calcium at the 8- and 10-week time points, corresponding to 2 and 4 weeks of drug administration, respectively.

No significant differences in mean serum phosphorus levels were observed in the cinacalcet HCl (10 mg/kg)-treated sham animals (5.8 ± 0.3 mg/dL) compared to vehicle-treated sham animals (5.4 ± 0.2 mg/dL).

### Table 2. Effect of cinacalcet HCl or vehicle treatments on parathyroid weight and parathyroid proliferation in 5/6 nephrectomy and sham rats

<table>
<thead>
<tr>
<th>5/6 nephrectomy or sham</th>
<th>Treatment</th>
<th>Parathyroid weight mg</th>
<th>No. of parathyroids</th>
<th>PCNA-positive cells/mm²</th>
<th>No. of parathyroid sections</th>
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</thead>
<tbody>
<tr>
<td>5/6 nephrectomy</td>
<td>Vehicle</td>
<td>1.95 ± 0.18</td>
<td>17</td>
<td>58.1 ± 8.9</td>
<td>17</td>
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<tr>
<td>5/6 nephrectomy</td>
<td>Cinacalcet HCl (1 mg/kg)</td>
<td>2.17 ± 0.38</td>
<td>8</td>
<td>57.5 ± 10.1</td>
<td>8</td>
</tr>
<tr>
<td>5/6 nephrectomy</td>
<td>Cinacalcet HCl (5 mg/kg)</td>
<td>1.53 ± 0.22</td>
<td>15</td>
<td>18.3 ± 3.4</td>
<td>15</td>
</tr>
<tr>
<td>5/6 nephrectomy</td>
<td>Cinacalcet HCl (10 mg/kg)</td>
<td>1.05 ± 0.13</td>
<td>20</td>
<td>19.0 ± 6.4</td>
<td>15</td>
</tr>
<tr>
<td>Sham</td>
<td>Vehicle</td>
<td>0.46 ± 0.03</td>
<td>6</td>
<td>17.9 ± 2.7</td>
<td>6</td>
</tr>
<tr>
<td>Sham</td>
<td>Cinacalcet HCl (1 mg/kg)</td>
<td>0.37 ± 0.04</td>
<td>6</td>
<td>17.7 ± 2.7</td>
<td>5</td>
</tr>
<tr>
<td>Sham</td>
<td>Cinacalcet HCl (5 mg/kg)</td>
<td>0.49 ± 0.04</td>
<td>8</td>
<td>18.0 ± 3.9</td>
<td>8</td>
</tr>
<tr>
<td>Sham</td>
<td>Cinacalcet HCl (10 mg/kg)</td>
<td>0.52 ± 0.07</td>
<td>8</td>
<td>16.3 ± 5.2</td>
<td>8</td>
</tr>
<tr>
<td>Normal (control) rats</td>
<td></td>
<td>0.39 ± 0.06</td>
<td>9</td>
<td>18.7 ± 3.7</td>
<td>9</td>
</tr>
</tbody>
</table>

5/6 nephrectomy and sham rats were treated with oral administration of cinacalcet HCl or vehicle daily for 4 weeks beginning 6 weeks postsurgery. At 10 weeks postsurgery, animals were sacrificed, parathyroid glands removed for determination of parathyroid weight and parathyroid hyperplasia by proliferating cell nuclear antigen (PCNA). Data shown as mean ± SEM.

5/6 nephrectomy cinacalcet HCl (1 mg/kg) 1.53 ± 0.22
5/6 nephrectomy Cinacalcet HCl (5 mg/kg) 1.05 ± 0.13
5/6 nephrectomy Cinacalcet HCl (10 mg/kg) 0.52 ± 0.07
Sham Cinacalcet HCl (1 mg/kg) 0.37 ± 0.04
Sham Cinacalcet HCl (5 mg/kg) 0.49 ± 0.04
Sham Cinacalcet HCl (10 mg/kg) 0.52 ± 0.07
— Normal (control) rats 0.39 ± 0.06

Effect of cinacalcet HCl on serum PTH, phosphorus, and blood ionized calcium levels

Administration of cinacalcet HCl to 5/6 nephrectomized animals for 4 weeks starting 6 weeks after surgery resulted in a significant reduction in (P = 0.0001) on serum PTH (Fig. 4A). The 8-week time point represented the first blood collection for PTH determinations after cinacalcet HCl administration had begun. Significant decreases in serum PTH levels were observed in both the 5 and 10 mg/kg dose groups at the 8-week and 10-week time points (P < 0.05 for each). The 1 mg/kg dose of cinacalcet HCl caused no significant alterations in serum PTH levels at the times studied.

In sham animals, cinacalcet HCl treatment resulted in a significant reduction (P = 0.0001) on serum PTH levels (Fig. 4B). Decreases in serum PTH levels were highly correlated with parathyroid weight and PCNA-positive cells with PTH, blood ionized calcium, and phosphorus levels. Serum PTH, blood ionized calcium, and phosphorus levels were highly correlated with parathyroid weight.
Fig. 2. Sections from rat parathyroid glands (400×) (A) 5/6 nephrectomy vehicle, (B) 5/6 nephrectomy cinacalcet HCl 10 mg/kg, (C) sham vehicle, and (D) sham cinacalcet HCl 10 mg/kg. Proliferating cell nuclear antigen (PCNA)-positive cells are shown as brown staining. Arrows indicate positive cells (400×) (A to D). Cinacalcet HCl (B) treatment for 4 weeks in 5/6 nephrectomized animals decreases parathyroid gland proliferation when compared to vehicle-treated 5/6 nephrectomized animals (A). Cinacalcet HCl has no effect on parathyroid gland proliferation in sham-operated animals (C and D).

Fig. 3. Ileum taken from a rat receiving either 5/6 nephrectomy treated with vehicle (A) or cinacalcet HCl 10 mg/kg (B). Proliferating cell nuclear antigen (PCNA)-positive cells are shown as brown staining. Arrows indicate positive cells (400×). There is no apparent difference in cell proliferation in drug versus vehicle-treated animals.

DISCUSSION

The results of this study clearly demonstrate that cinacalcet HCl, an allosteric modulator of the CaR [12], reduces parathyroid cell proliferation in rats with secondary HPT, a conclusion consistent with that of Wada.

Similarly, serum PTH ($r = 0.61, P = 0.01$) and blood ionized calcium ($r = 0.47, P = 0.036$) correlated with number of PCNA-positive cells. No correlation existed between phosphorus ($r = 0.069, P = 0.7$) levels and number of PCNA-positive cells.

Apoptosis

Very few or no apoptotic cells were found in parathyroid glands from either treatment group (data not shown). The positive controls for each run, regressing rat mammary gland, showed positive staining for apoptotic cells.

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DISCUSSION

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et al [11] linking the CaR and the development of parathyroid hyperplasia. This is the first study, however, that has demonstrated reduced parathyroid cell proliferation with cinacalcet HCl in a rodent model of secondary HPT. Although the data generated do not allow us to explicitly state that delayed cinacalcet HCl treatment can mediate regression of parathyroid hyperplasia, recent preliminary data suggest that this is possible [16]. The present data, coupled with previous findings with NPS R-568 [11, 17, 18], offer encouraging support for efficient targeting of the CaR with cinacalcet HCl in secondary HPT.

Based on pharmacokinetic exposure data in rats for a 10 mg/kg dose, the area under the curve (AUC) was 225 ng/hour * mL (data on file, Amgen, Inc.) comparable to a patient receiving the 60 mg dose of cinacalcet HCl (AUC 215 ng/hour * mL) (data on file, Amgen, Inc.), which is used in the clinical setting [15]. Although the effect of this dose on parathyroid hyperplasia in the clinic has not yet been confirmed, these data suggest that the antiproliferative effect described in this report may be reproducible in humans at clinically achievable concentrations of drug.

While hyperplasia correlated well with serum PTH and ionized calcium as previously reported [17, 18], this was not the case with serum phosphorus. The lack of correlation between hyperplasia and circulating phosphorus levels was surprising, considering studies indicating a role for phosphorus in parathyroid hyperplasia [3, 4]. However, the CaR, rather than serum phosphorus, is probably the dominant factor involved in parathyroid gland enlargement and regulation of PTH secretion in vivo [19].

Although low serum vitamin D₃, low calcium, and elevated phosphorus are thought to contribute to development of parathyroid gland hyperplasia [2, 3, 20–23], increasing evidence suggests the CaR also plays an important role [11, 16, 17]. Parathyroid hyperplasia is a characteristic of familial benign hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism arising from inactivating CaR mutations [24]. These clinical observations were confirmed in the CaR knockout mouse, a genetic model for neonatal severe hyperparathyroidism [25]. Furthermore, CaR expression in parathyroid tissue is down-regulated in uremic rats, in patients with secondary HPT, and in adenomas from patients with primary HPT [22, 26–28].

Attenuation of parathyroid hyperplasia by cinacalcet HCl was independent of serum creatinine levels, thereby ruling out improved kidney function. Although serum
Parathyroid gland hyperplasia is an important contributor to excess PTH secretion in patients with secondary HPT and often accounts for disease progression. It has been demonstrated clinically that vitamin D₃ pulse therapy can be effective in reducing hyperplasia in patients with mild to moderate secondary HPT, although patients have been refractory to vitamin D sterol therapy [30, 31]. PTH synthesis and secretion and parathyroid cell proliferation are inhibited by high concentrations of extracellular calcium in vivo [11]. This suggests that calcimimetics like cinacalcet HCl might be a more effective alternative, and the attenuation of parathyroid gland hyperplasia by cinacalcet HCl observed in this study may have important clinical relevance.

High dietary phosphorus will exacerbate uremia-induced parathyroid gland hyperplasia and PTH synthesis and secretion. Once secondary HPT develops, dietary P restriction or phosphate binders can return high PTH
levels toward normal, however, parathyroid hyperplasia persists and unwanted side effects can occur [4, 3, 32]. Although the present studies demonstrated no increases in serum phosphorus with cinacalcet HCl, there are reports demonstrating that calcimimetics can increase serum phosphorus in normal and uremic rats [11, 12, 17, 18]. Inconsistency between reports may reflect sampling time relative to cinacalcet HCl administration and/or the calcimimetic utilized. While hyperphosphatemia in chronic renal insufficiency has been proposed as the major driving force for parathyroid cell hyperplasia [4], elevations in serum phosphorus do not appear to hamper the pronounced calcimimetic-mediated suppressive action on parathyroid proliferation [11, 17]. Inhibition of cellular hyperplasia following calcitriol administration has been associated with an increase of both serum calcium and phosphorus levels, suggesting that increases in serum calcium can overpower the proliferative actions of serum phosphorus [2]. Recent clinical studies in end-stage renal disease (ESRD) patients on dialysis demonstrate reductions in plasma intact PTH and serum calcium, phosphorus, and calcium-phosphorus product following treatment with cinacalcet HCl [13–15].

Parathyroid cell proliferation likely involves a complex cascade of biochemical signaling pathways which may be mediated, at least in part, through CaR activation. Although the second-messenger pathways involved in CaR signaling and their roles in parathyroid proliferation have not been fully delineated, they have been shown to include phospholipases, several mitogen-activated protein kinase (MAPK) cascades, and adenylate cyclase inhibition through multiple mechanisms [33–35]. Inhibition of parathyroid hyperplasia by cinacalcet HCl may reflect a specific CaR signaling pathway associated with growth mechanisms in these cells.

Several reports have demonstrated that few parathyroid cells undergo apoptosis in normal, uremic, or uremic rats treated with vitamin D₃ or with a phosphorus-restricted diet [3, 36, 37]. Calcimimetics did not induce parathyroid cell apoptosis in 5/6 nephrectomy or sham rats in either the present or a previous study by Wada et al [11]. However, if apoptosis was occurring, it was unlikely that the present study would reveal this phenomenon because sampling occurred several weeks after cinacalcet HCl administration and the process of apoptosis occurs very early. Thus, additional apoptotic studies are warranted at earlier time points (24 to 72 hours) post-cinacalcet HCl administration in uremic rats.

Parathyroid cells are quiescent under normal physiologic conditions [1, 4]; the normal physiologic state was not perturbed in sham-operated animals receiving cinacalcet HCl or vehicle, in agreement with previous studies using NPS R-568 [11, 17, 18]. Our in vivo rat studies are consistent with previous in vitro data demonstrating that changes in extracellular calcium had no effect on proliferation of parathyroid cells derived from normal chick, rat, or bovine tissues [38–40], but did in cells derived from uremic animals or patients [41]. Although the mechanism of action behind the difference in responses has not yet been elucidated, it could result from alterations in postreceptor signal transduction associated with hyperparathyroid states. Alternatively, parathyroid cells could express multiple CaR-like proteins. Evidence indicates that CaR is down-regulated in hyperplastic tissue; therefore, parathyroid proliferation
by calcium might occur through a CaR isoform [41]. However, cinacalcet HCl–treated sham animals do mediate a decrease in serum PTH, as expected by lowering the threshold for activation of the CaR by extracellular calcium ions. This difference in detecting a decrease in PTH secretion versus no change on parathyroid cell proliferation in sham animals could simply reflect the sensitivity of the assay systems available to assess serum PTH levels versus the histomorphometry required to detect changes in parathyroid gland proliferation in normal animals.

The CaR is present on numerous cell/tissue types; therefore, it was important to demonstrate that the antiproliferative actions of cinacalcet HCl were specific to parathyroid tissue and not the result of general cytotoxicity. Cinacalcet HCl had no effect on intestinal epithelial cells. These results agree with a previous finding that NPS R-568 had no antiproliferative actions on intestinal epithelial cells and thyroidal C cells [11]. This is in contrast to vitamin D3, which, like cinacalcet HCl, is a very effective inhibitor of parathyroid hyperplasia, but, unlike cinacalcet HCl, vitamin D3 also inhibits cell proliferation in a variety of cell types, including intestinal mucosa [2].

In addition to attenuating parathyroid hyperplasia, cinacalcet HCl caused rapid dose-dependent decreases in serum PTH and blood ionized calcium in 5/6 nectomized rats. These findings agree with studies with cinacalcet HCl in normal rats [12, 42]. Reduction in blood-ionized calcium has also been observed using NPS R-568 in acutely nephrectomized animals, suggesting that the mechanism for the observed serum calcium reductions in response to calcimimetic treatment does not involve the kidney CaR [43]. Calcimimetics in rats cause a transient increase in serum calcitonin that contributes to the rate of onset of the observed decrease in serum calcium levels [12, 43]. As pointed out by Nemeth et al [12], calcimimetics depress serum PTH levels at doses that are at least to 10 times lower than those that increase plasma calcitonin levels.

CONCLUSION

Cinacalcet HCl treatment attenuated parathyroid gland hyperplasia and reduced serum PTH levels in CKD rats with elevated PTH, most likely through direct actions on the CaR. Since parathyroid hyperplasia is initiated at an early stage of CKD and is often refractory to current medical treatment, the CaR is a potentially useful target for therapeutic agents like cinacalcet HCl to reduce PTH secretion, decrease calcium × phosphorus levels and attenuate parathyroid gland hyperplasia in ESRD patients with secondary HPT.

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