

Can calcimimetics inhibit parathyroid hyperplasia? Evidence from preclinical studies

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Introduction

The cells of the parathyroid gland secrete parathyroid hormone (PTH), which plays a pivotal role in maintaining circulating levels of ionized calcium (Ca^{2+}) within a narrow physiological range. The main actions of PTH include (i) releasing calcium and phosphorus from bone, (ii) decreasing renal calcium excretion, (iii) increasing urinary phosphorus excretion and (iv) stimulating renal production of calcitriol (1,25-dihydroxy vitamin D₃), the active form of vitamin D. Vitamin D and its receptors (VDRs) also play key roles in calcium homeostasis: vitamin D acts on VDRs in the intestine to increase calcium absorption, and on VDRs in parathyroid cells to inhibit PTH mRNA synthesis [1].

Secondary hyperparathyroidism (SHPT) represents an adaptive response to the progressively impaired control of calcium, phosphorus and vitamin D in chronic kidney disease (CKD). It is characterized by parathyroid hyperplasia and excessive synthesis and secretion of PTH, resulting in excessive bone resorption, soft-tissue and vascular calcification and significantly increased risk for cardiovascular morbidity and mortality [2,3].

Extracellular calcium is the primary physiological stimulus regulating secretion of PTH and there is an inverse, sigmoidal relationship between the levels of plasma PTH and calcium. A cell surface receptor located on parathyroid cells, the calcium-sensing receptor (CaR), has been recognized as the primary mechanism that mediates the effects of Ca^{2+} on PTH

secretion [4,5]. The CaR also appears to play a key role in the excessive cell proliferation that occurs in parathyroid hyperplasia [5]. Drugs that mimic or potentiate the action of Ca^{2+} at this receptor, calcimimetics, have become available for treatment of dialysis patients (CKD stage 5) with insufficient control of PTH and calcium and/or phosphate levels on traditional therapies [6].

This article overviews the key pathophysiological mechanisms that drive parathyroid hyperplasia in SHPT and examines the potential of calcimimetics for attenuating this condition, based on emerging data from animal models.

Pathophysiological aspects of parathyroid hyperplasia

CKD is associated with disturbed calcium and phosphorus homeostasis and decreased calcitriol production. PTH secretion is increased in an attempt to correct serum calcium and phosphate: however, as renal failure progresses, higher PTH levels are required to maintain calcium homeostasis, in association with an increased phosphate burden resulting from decreased glomerular filtration and insufficient renal production of calcitriol. Phosphate accumulation and calcitriol deficiency decrease serum calcium, which stimulates the parathyroid to produce PTH (Figure 1). Accumulation of phosphorus also stimulates parathyroid cell function directly and the decrease in circulating calcitriol leads to disinhibition of PTH synthesis [7–10].

Parathyroid cells are generally quiescent and rarely divide under normal physiological conditions [11,12], but can proliferate in response to mitogenic stimuli such as low levels of calcium and calcitriol and elevated phosphorus. Indeed, these are key factors in the development of parathyroid hyperplasia, as well as in excessive PTH synthesis and secretion, as summarized in Table 1. Although the parathyroid glands initially

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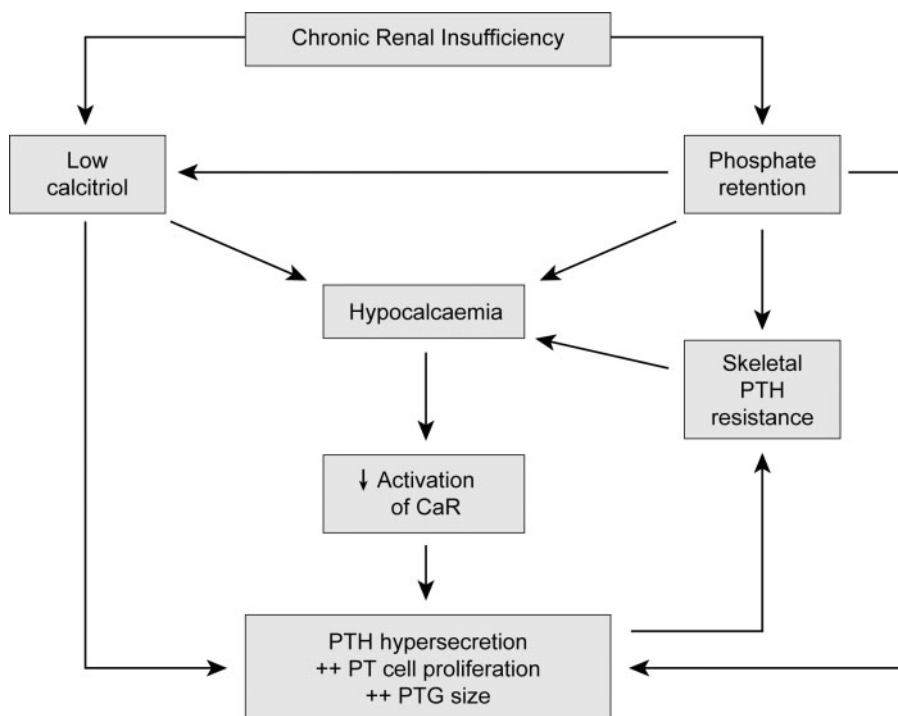


Fig. 1. Schematic representation of the key factors involved in secondary hyperparathyroidism and parathyroid (PT) hyperplasia. PTG, parathyroid gland; PTH, parathyroid hormone.

Table 1. Overview of the roles of calcium, calcitriol and phosphorus in secondary hyperparathyroidism

Actions	
Calcium	<p>Activates the CaR on parathyroid cells to suppress PTH synthesis and secretion in the presence of elevated Ca^{2+} [5]. Negatively regulates transcription of PTH gene [5].</p> <p>Extracellular calcium is thought to have an inhibitory effect on parathyroid proliferation. Low calcium intake enhanced parathyroid cell proliferation in rats [50], but low Ca^{2+} concentration <i>in vitro</i> did not directly stimulate proliferation of cultured parathyroid cells from dialysis patients [51]. Elevated extracellular calcium may inhibit parathyroid cell proliferation under conditions of normal or high CaR expression, but stimulate it under low CaR expression [51].</p> <p>Plays a role in VDR regulation [52].</p>
Calcitriol	<p>Enhances intestinal absorption of calcium and phosphate [1].</p> <p>Reduces pre-pro-PTH gene transcription via the VDR [1].</p> <p>Inhibits parathyroid cell proliferation indirectly via increases in serum calcium and directly via CaR up-regulation [113]. Decreased c-myc expression [85], de novo TGF-α expression [114], and enhanced p21 expression [87] may also play a role.</p> <p>Up-regulates VDR in parathyroid cells via ligand-induced stabilization and increased VDR mRNA levels [1].</p> <p>Plays a role in CaR regulation [115].</p>
Phosphate	<p>Directly stimulates PTH synthesis and secretion [7,8], by a post-translational mechanism [116–118].</p> <p>Inhibits calcitriol production [119,120].</p> <p>Prevents the reduction in PTH induced by calcitriol administration [100].</p> <p>Sustained hyperphosphataemia leads to increased parathyroid cell proliferation [101,121–123]. Phosphorus exerts a calcium- and calcitriol-independent stimulatory effect on parathyroid cells [124]. This may be mediated by enhanced expression of TGF-α [125].</p> <p>Impairs the calcaemic action of PTH [126].</p>

respond to increased demand by increasing PTH secretion and synthesis, parathyroid cells subsequently begin to proliferate, leading to a diffuse hyperplasia [13]. Subsequently, transformation from a polyclonal to a more aggressive monoclonal or multiclonal growth

pattern occurs [14]. The glands become grossly enlarged and exhibit a nodular hyperplasia [13,14] (Figure 2). Such nodules are composed of more tightly packed cells featuring larger nuclei and a greater prevalence of cell cycle markers, oxyphil cells and acinar

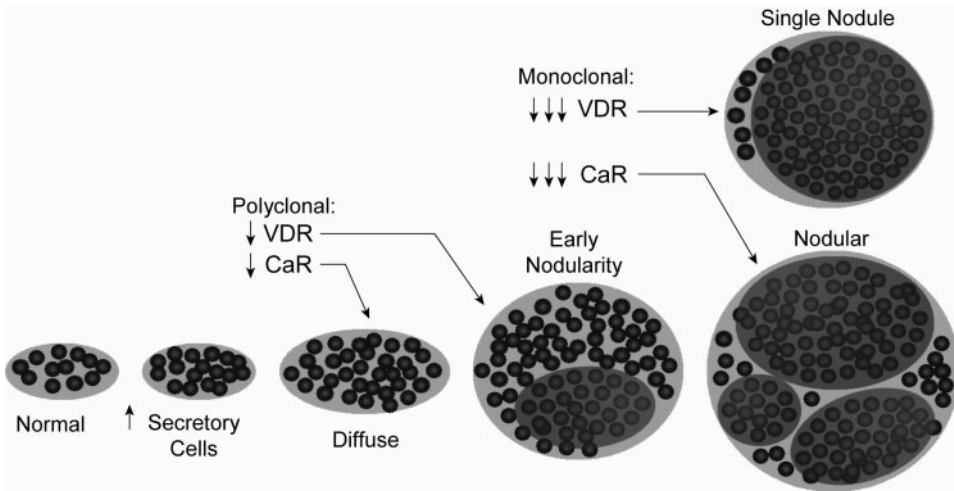


Fig. 2. Postulated evolution of parathyroid hyperplasia in renal hyperparathyroidism. Adapted from Tominaga *et al.* [131] with permission.

cell arrangements compared with those seen in diffuse hyperplasia [12,15]. Nodules may eventually coalesce to form a single large tumour, which may in rare cases ultimately undergo malignant transformation [16]. Increased cell volume (cell hypertrophy) appears to play only a minor role in parathyroid gland enlargement caused by uraemia, in contrast with that induced by hypocalcaemia or hyperphosphataemia in the presence of normal renal function, where parathyroid cell hypertrophy prevails over cell proliferation [12].

Both CaR [17–20] and VDR [15,21–24] are progressively down-regulated in the course of parathyroid hyperplasia. Nodular hyperplasia in patients with CKD is associated with a lower density of both CaR [17] and VDR [21,22] than diffuse hyperplasia, and VDR density was reported to be negatively correlated with both the weight and proliferative activity of the glands [21].

Enlargement of the parathyroid glands markedly increases the capacity for PTH production. Indeed, basal calcium-independent (non-suppressible) PTH secretion, that parallels the increased gland size [25], becomes an important factor in elevated PTH levels when the parathyroid glands are 50–100 times their normal size. Moreover, as CaR and VDR expression are reduced in the course of hyperplasia, the parathyroid glands become increasingly resistant to regulation by calcium and calcitriol [15,26,27]. Thus, PTH becomes sufficiently elevated to overcome skeletal resistance and mobilize calcium and phosphorus from bone. A vicious cycle ensues, whereby hypersecretion of PTH increases serum calcium and phosphorus levels, but resistance of the parathyroids to calcium regulation allows PTH secretion to continue unabated ('tertiary hyperparathyroidism') (Figure 1). Surgical parathyroidectomy may be required if PTH levels cannot be controlled by pharmacological means.

The key factors that mediate the transformation of diffuse parathyroid hyperplasia to aggressive tumour-like growth remain to be elucidated [28]. Changes in the expression of various growth factors/growth factor

Table 2. Summary of changes in expression of various receptors, genes, growth factors and other molecules observed in hyperplastic parathyroid tissue

↓CaR	Gogusev <i>et al.</i> ; Kifor <i>et al.</i> [17,18]
↓VDR	Fukuda <i>et al.</i> ; Tokumoto <i>et al.</i> 2002; Wang <i>et al.</i> 2001; Carling <i>et al.</i> [21–23, 127]
↑c-myc	Kremer <i>et al.</i> [85]
↓p21 and p27	Cozzolino <i>et al.</i> [29]
↑p53	Martin <i>et al.</i> [15]
↑TGF- α /EGFR	Gogusev <i>et al.</i> ; Dusso <i>et al.</i> [114,125]
↑Acidic fibroblast growth factor	Sakaguchi <i>et al.</i> [128]
↓Parathyroid-hormone-related peptide	Matsushita <i>et al.</i> [129]
↑Endothelin-1	Kanesaka <i>et al.</i> [130]

↑, enhanced or *de novo* expression; ↓, decreased expression; CaR, calcium-sensing receptor; EGFR, epidermal growth factor receptor; TGF, transforming growth factor; VDR, vitamin D receptor.

receptors and tumour enhancer/suppressor genes have been observed in hyperplastic parathyroid tissue, as summarized in Table 2. It is not clear whether these changes are a cause or a consequence of parathyroid hyperplasia, but such factors may act as autocrine or paracrine regulators of parathyroid cell proliferation in response to Ca^{2+} , phosphate and/or active vitamin D. For instance, the three main modulators of parathyroid cell proliferation, namely calcium, phosphate and vitamin D, all modulate signalling via the highly mitogenic transforming growth factor- α /epidermal growth factor receptor (TGF- α /EGFR) growth loop and also regulate p21 expression [29]. Reduced expression of VDR-dependent p21/p27 may play a key role in nodular parathyroid gland growth, as these genes regulate progression from the G_1 to the S phase of the cell cycle, via inhibition of cyclin-dependent kinase [30].

The role of the calcium-sensing receptor in SHPT

Parathyroid cells are extremely sensitive to minute alterations in extracellular Ca^{2+} , rapidly producing large changes in PTH production and release, and therefore, plasma PTH levels. The CaR is an evolutionarily conserved G protein-coupled cell surface receptor cloned by Hebert and Brown in 1993 [4] and identified as the sensor for extracellular calcium-mediated regulation of PTH secretion. The CaR has three major domains: a large (612-amino-acid) extracellular ligand-binding N-terminal; a smaller hydrophobic core with 7 membrane-spanning domains (250 amino acids) and an intracellular C-terminal (approximately 250 amino acids). Stimulation of the CaR by elevated extracellular Ca^{2+} levels in turn activates the mitogen-activating protein kinase C pathway, via both G-protein-linked phospholipase C and tyrosine phosphorylation of Shc (Src homolog and collagen) [31,32], resulting in activation of phospholipase A2 and production of arachidonic acid [31]. Arachidonic acid and its metabolites suppress PTH secretion [32–35]. CaRs are expressed in many tissues, with the highest density being found in the chief cells of the parathyroid gland. CaRs in the kidney also participate in calcium homeostasis [5].

There are several lines of evidence to support the involvement of the CaR in both excessive PTH secretion and synthesis and parathyroid hyperplasia:

- (i) The relationship between calcium sensing and abnormalities of the CaR gene. Loss-of-function mutations of the CaR gene are associated with an increased calcium set-point, as shown by an increase in the concentration of Ca^{2+} required to inhibit PTH release [36–38]. Moreover, the presence of two, rather than one, abnormal alleles for the CaR gene is associated with more marked elevation of the calcium set-point, higher serum PTH and calcium levels, and parathyroid hyperplasia. Thus, patients with familial hypocalciuric hypercalcaemia (FHH), who are heterozygotes, have a slight increase in the calcium set-point, with mild asymptomatic hypercalcaemia and normal or slightly elevated PTH levels [39]. Patients with neonatal severe hyperparathyroidism (NSHPT), who are homozygotes, exhibit a more pronounced increase in the set-point, with more severe hypercalcaemia and PTH elevation, resulting in parathyroid hyperplasia and bone disease [39]. Knockout mouse models have confirmed these observations, with animals heterozygous for inactivating mutations of the CaR gene exhibiting signs consistent with FHH and those homozygous for such mutations showing NSHPT-like symptomatology [40]. The development of parathyroid hyperplasia in these models, despite elevated serum calcitriol levels, supports the key role of calcium-dependent signalling, rather than vitamin D-mediated pathways, in parathyroid hyperplasia. Conversely, activating

mutations of the CaR are associated with autosomal dominant hypocalcaemia, characterized by hypocalcaemia with inappropriately normal or low PTH levels [41,42].

An increased set-point for Ca^{2+} may also be present in patients with SHPT [43,44], although this is not a consistent observation [45,46]. It appears to be more evident in patients with advanced SHPT, autonomous (tertiary) hyperparathyroidism or primary hyperparathyroidism [47,48].

- (ii) The association of parathyroid hyperplasia with down-regulation of the CaR in uraemic animals [19,20] and humans [17,18], as already discussed.
- (iii) The activity of calcimimetics in SHPT. In both animal models and patients with hyperparathyroidism, calcimimetics are able to reduce plasma PTH, calcium and/or phosphorus levels, as discussed in subsequent sections.

Calcium, the endogenous ligand for the CaR, negatively regulates transcription of the PTH gene [5]. Sustained hypercalcaemia results in reduced proliferation of parathyroid cells, as shown in uraemic rats (Figure 3). Calcium loading significantly decreased both the weight of parathyroid glands and the number of proliferating cells [49]. Although low calcium intake has been found to be associated with markedly enhanced parathyroid cell proliferation in rats [50], low Ca^{2+} concentration *in vitro* did not directly stimulate proliferation of cultured parathyroid cells isolated from glands from haemodialysis patients with severe SHPT [51]. Based on these *in vitro* observations, it has been suggested that increasing extracellular calcium may inhibit parathyroid cell proliferation under conditions of normal or high CaR expression, but stimulate it under low CaR expression [51].

Calcium also appears to regulate VDR expression by parathyroid cells independently of calcitriol [52]. VDR mRNA and protein levels were lower in hypocalcaemic, than in normocalcaemic, rats and this prevented the inhibitory effect of calcitriol on PTH mRNA. Thus, hypocalcaemia may increase PTH mRNA directly via a postranscriptional effect, or indirectly by reducing VDR expression.

Calcimimetics in SHPT

Calcimimetics are ligands that either mimic or potentiate the effects of extracellular Ca^{2+} at the CaR. Type I calcimimetics are agonists that directly stimulate the CaR by interacting with its extracellular domain and include inorganic and organic polycations [53]. Type II calcimimetics are allosteric modulators of the CaR. They appear to interact with the membrane-spanning domain of the receptor, inducing a conformational change that enhances signal transduction, thereby increasing sensitivity to extracellular Ca^{2+} . They include L-amino acids and phenylalkylamines [53]. The type II phenylalkylamine derivatives include the early compounds NPS R-568 and NPS R-467, the

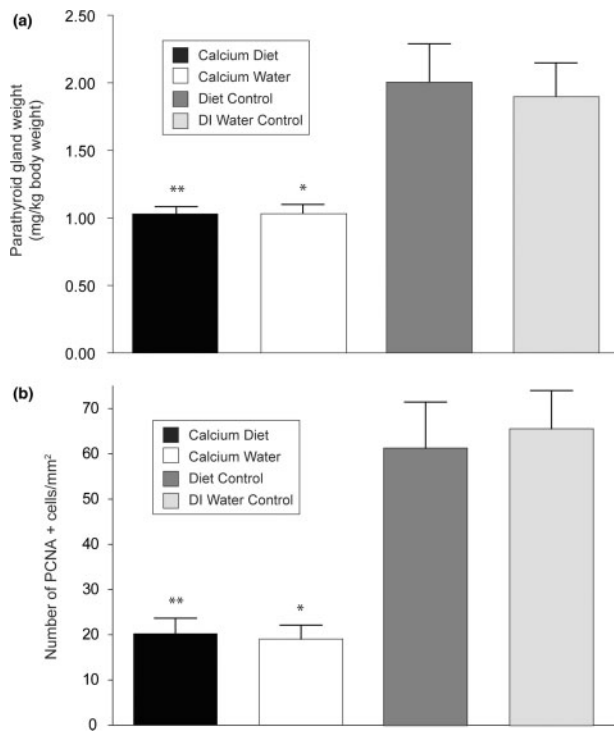


Fig. 3. Effect of dietary calcium on parathyroid weight (a) and cell proliferation, as assessed by PCNA (b) in 5/6 nephrectomized rats [49]. Male Sprague–Dawley rats (300–350 g) were anaesthetized (3% isoflurane in O₂) and the upper bifurcation of the left renal artery ligated. Animals were allowed to recover for 1 week, after which they were anaesthetized and the right kidney removed. After recovery, animals received for 40 days: calcium gluconate 3% in chow, calcium gluconate 3% in water, control diet (standard chow), or deionized (DI) water and standard chow. Animals were sacrificed on day 40 and the parathyroids removed, weighed and processed for PCNA staining, as described previously by Colloton *et al.* [56]. The number of PCNA-positive cells was counted by a treatment-blinded observer. Data shown as mean \pm SEM. * $P=0.0003$ vs DI water control; ** $P=0.0002$ vs diet control.

second generation compound cinacalcet HCl (AMG 073; KRN 1493; Mimpara[®]/Sensipar[®]), and AMG-641.

Type II calcimimetics increase the sensitivity of parathyroid cells to extracellular calcium, inhibiting PTH release and shifting the calcium–PTH response curve to the left *in vitro* [53,54]. In uraemic rats, calcimimetics induce rapid, dose-dependent decreases in serum PTH and dose-dependent increases in serum calcitonin levels [54–56]. In general, calcimimetics are at least 10 times more potent in reducing serum PTH levels than in increasing calcitonin levels [54], although the reasons for this are not clearly understood. The mechanism and time-course of action of calcimimetics differ from those of vitamin D sterols, which reduce PTH gene transcription and hormone synthesis over a period of several hours or even days [57]. Cinacalcet inhibits PTH secretion within minutes, with a maximal decrease occurring within 2 h in patients with SHPT [58]. Metabolism by a number of CYP450 enzymes results in a cyclic pattern of serum PTH during continued administration [54] that may have anabolic

Table 3. Comparison of the effects of calcimimetics and vitamin D on serum Ca and P and parathyroid hormone (PTH)

Parameter	Calcimimetics	Vitamin D
Serum Ca and P		
Serum calcium	↓ ^a	↑
Serum phosphorus	↓ ^a ↑ ^b	↑
Serum PTH reduction		
Onset of action	Fast (minutes)	Slow (days)
Pattern of reduction	Cyclic	Continuous

^a In patients with ESRD.

^b In preclinical models (with remnant kidney).

↑, increased; ↓, decreased.

NOTE: For references, see text.

effects on bone [59]. In addition, recent data indicate that activation of the CaR by calcimimetics decreases PTH mRNA stability, by post-translational modification of the PTH-mRNA binding protein AUF1 [60].

The sustained long-term efficacy of calcimimetics in controlling PTH levels in dialysis patients [61,62] may reflect their mechanism of action as allosteric activators, rather than receptor agonists, at the CaR. The substantial PTH reductions achieved in patients with primary parathyroid adenomas [63], as well as in PTH–cyclin D1 transgenic mice (a model of primary hyperparathyroidism) [64], suggest that CaR signalling is largely preserved in advanced parathyroid hyperplasia and parathyroid adenomas, despite the marked reduction in CaR expression that accompanies these conditions.

Table 3 compares the effects of calcimimetics and vitamin D sterols on serum calcium and phosphorus and PTH. It should be noted that calcimimetics are usually administered in conjunction with existing vitamin D therapy and/or phosphate binders in dialysis patients and may have synergistic or additive effects on PTH, as well as counteracting the calcaemic and phosphataemic actions of vitamin D sterols.

Effects on parathyroid hyperplasia

The effects of calcimimetics on parathyroid hyperplasia have been extensively investigated *in vitro*, in human parathyroid cells derived from uraemic patients and in uraemic rats (Table 4), particularly in the classic 5/6 nephrectomized rat model, which involves ligation of two of the three branches of the left renal artery and removal of the right kidney. This model probably represents an early stage in parathyroid hyperplasia: it has not been possible to replicate advanced, nodular, hyperplasia in uraemic rodents.

Calcimimetics have also been studied in the adenine model of SHPT. Dietary adenine feeding (0.75%) in rats induces irreversible chronic renal failure within a period of only 4 weeks, as precipitation of its metabolite 2,8-dihydroxyadenine and deposition in kidney tubules results in rapid tubular degeneration and

Table 4. Calcimimetic studies in uraemic rat models

Reference	Model	Calcimimetic dosing schedule
<i>Prevention of parathyroid hyperplasia</i>		
Wada <i>et al.</i> [66]	5/6 Nx	NPS-568 1.5 or 15 mg (free base)/kg/day po × 4d from d1 of Nx
Wada <i>et al.</i> [67]	5/6 Nx + dietary phosphate loading (1.2% P; 0.5% Ca)	NPS-568 30 or 100 µmol/kg/day po; or 20 µmol/kg/day sc × 8 wks from d6 post Nx
Miller <i>et al.</i> [68]	5/6 Nx	Cinacalcet 10 mg/kg po × 6 or 9 wks from 1 wk post Nx
Miller <i>et al.</i> [72]	Dietary adenine (0.75%) feeding	Calcimimetic (investigational) 3 mg/kg po × 1–4 wks from d1 of adenine feeding
<i>Attenuation of established parathyroid hyperplasia</i>		
Chin <i>et al.</i> [70]	(a) 5/6 Nx (b) 5/6 Nx + diet with high P: Ca ratio (0.8: 0.6%)	(a) NPS-568 20 µmol/kg/day sc × 8 wks from 4 wks post Nx (b) NPS-568 10 or 30 µmol/kg/d po or 20 µmol/kg/d sc × 8 wks from 11 weeks post Nx
Colloton <i>et al.</i> [56]	5/6 Nx	Cinacalcet 1, 5 or 10 mg/kg po × 4 wks from 6 wks post Nx

Sc, subcutaneous; d, day(s) Ca, calcium; Nx, nephrectomy; P, phosphate; po, oral (gavage); wk(s), week(s).

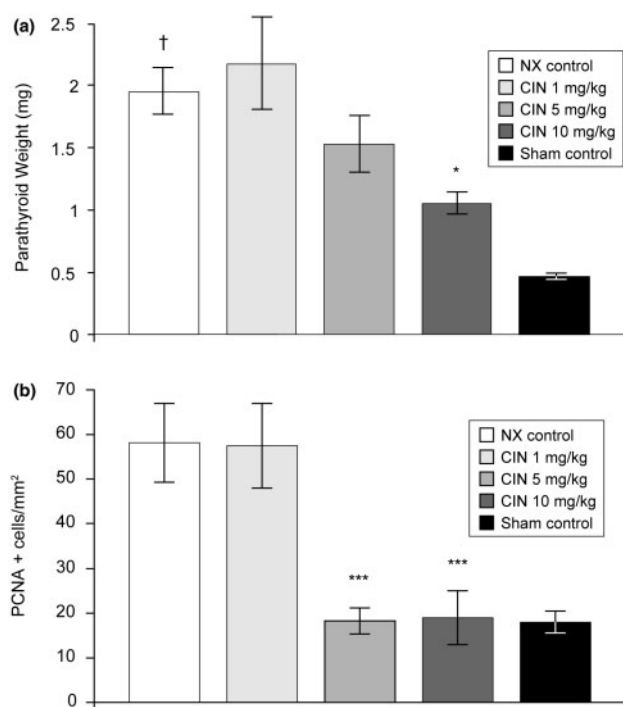


Fig. 4. Effects of 4 weeks' administration of cinacalcet HCl (CIN) on parathyroid weight (a) and proliferation (b) in 5/6 nephrectomized (Nx) rats. Colloton *et al.* [56]. Animals were administered cinacalcet HCl (p.o.) or vehicle (p.o.) for 4 weeks beginning 6 weeks post-surgery. At 10 weeks animals were sacrificed and parathyroid glands removed for determination of parathyroid weight and parathyroid proliferation by proliferating cell nuclear antigen (PCNA). Data shown as mean ± SEM † $P < 0.01$ 5/6 vs sham control; * $P < 0.05$ vs Nx control; *** $P < 0.001$ vs Nx control.

uraemia. This is accompanied by extremely elevated PTH and severe parathyroid hyperplasia, as well as severe bone lesions and metastatic calcification of soft tissues [65].

NPS R-568 [66,67] and cinacalcet [56,68] inhibit parathyroid cell proliferation in the 5/6 nephrectomy model, as indicated by marked reduction in the

numbers of S-phase (5-bromodeoxyuridine positive) cells [66], PCNA-positive cells [56,68,69] and overall parathyroid cell numbers [67,70]. Indeed, parathyroid cell proliferation was reduced to control levels by cinacalcet treatment (Figure 4) [56]. NPS R-467 also showed direct antiproliferative effects on human parathyroid cells in long-term culture [51].

The decrease in parathyroid cell proliferation induced by calcimimetics appears to be partly attributable to an increase in cells expressing the CDK inhibitor p21 [68]. In the 5/6 nephrectomy model, administration of cinacalcet more than doubled the number of p21-positive cells compared to vehicle control ($P < 0.01$) (Figure 5) [68]. In contrast to vitamin D, which inhibits proliferation of many different cell types, including intestinal mucosa [1,71], calcimimetics do not have any antiproliferative effects on intestinal epithelial cells [56,66] or thyroid C cells [66].

When initiated shortly after subtotal nephrectomy (i.e. from the onset of the uraemic state), calcimimetics consistently prevented the increase in parathyroid gland weight and/or volume that occurred in vehicle-treated animals [66,68]. Inhibition of hyperplasia was reversible within 2–3 weeks after discontinuation of the calcimimetic [68].

Calcimimetics were also active in models of severe SHPT (subtotal nephrectomy with a high-phosphate diet or the adenine model). For instance, an investigational calcimimetic prevented development of parathyroid hyperplasia, as evidenced by both parathyroid weight and the number of proliferating cells, in adenine-fed rats [72]. Parathyroid weight had increased to 2.5 mg/kg bodyweight after only 4 weeks in the vehicle group, compared to a weight of 0.9 mg/kg in the calcimimetic group, similar to that seen in rats fed a normal diet (Figure 6). Calcitriol (10 ng) was not active in this model, although it did reduce serum PTH.

When administered to animals with established parathyroid hyperplasia, starting 4–11 weeks after nephrectomy, calcimimetics halted progression of hyperplasia [56,70], even under conditions of

phosphate loading [70]. Indeed, parathyroid weight in animals treated with cinacalcet 10 mg/kg was approximately half that in vehicle-treated nephrectomized controls (Figure 4). Based on pharmacokinetic data from rats and humans, it was calculated that this dose level would approximate to a dose of 60 mg/day in the clinical setting [56]. While this study did not allow determination of whether actual regression of parathyroid hyperplasia had occurred, Chin *et al.* [70] demonstrated this effect by sacrificing groups of untreated animals at 4 or 11 weeks after nephrectomy.

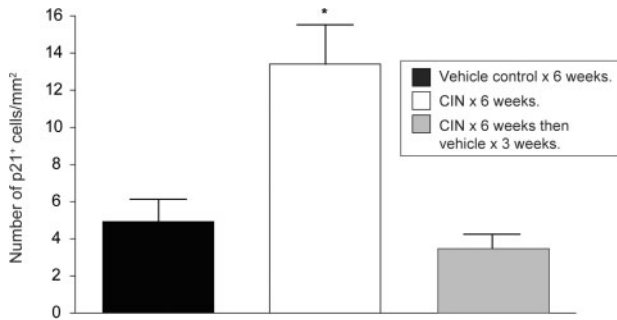


Fig. 5. Effects of cinacalcet administration on p21 expression in rat parathyroid cells. Male Sprague–Dawley rats underwent 5/6 nephrectomy, as described previously. Colloton *et al.* [56]. One week after surgery, rats were administered: vehicle (10% captisol in water), cinacalcet (10 mg/kg) by gavage for 6 weeks (sacrifice at 6 weeks), or cinacalcet (10 mg/kg) by gavage for 6 weeks, followed by vehicle for 3 weeks (sacrifice at 9 weeks). After sacrifice parathyroids were removed for p21 positive cell expression, determined in paraffin-embedded sections using a mouse anti-rat p21 monoclonal antibody (Santa Cruz Biotech, Santa Cruz, CA). Data shown as mean ± SEM ($n = 13 - 17$). Miller *et al.* [68]. * $P < 0.01$ vs vehicle control.

Calcimimetic treatment reduced parathyroid gland volume (normalized for bodyweight) to below the size seen in the untreated group. When a standard diet was given, continuous subcutaneous infusion of NPS-568 for 8 weeks completely reversed parathyroid gland enlargement, such that mean gland volume was similar to that in sham-operated rats. This reduction was found to be attributable solely to a decrease in volume of the parathyroid cells. Conceptually, regression of gland mass could also occur via apoptosis of parathyroid cells. This would be technically very difficult to demonstrate, given the extremely slow turnover rate of these cells [12]. Indeed, Wada *et al.* [66] were not able to detect apoptosis, as evaluated by DNA fragmentation (deoxynucleotidyl transferase-mediated dUTP nick end-labelling; TUNEL), in calcimimetic-treated or untreated uraemic rats, or in sham-operated rats.

Inhibition of parathyroid hyperplasia by calcimimetics was independent of serum creatinine levels, indicating that this effect was not mediated by improved renal function [56,66].

Effects on CaR and VDR expression

Calcimimetics have also been shown to up-regulate decreased parathyroid CaR in uraemic rats [73], with both mRNA and protein being restored to control levels [69], and to up-regulate VDR both *in vitro* [74] and *in vivo* [73,75]. These agents also potentiated the effects of calcitriol on VDR mRNA and VDR protein in normal rats [74]. Suppression of parathyroid

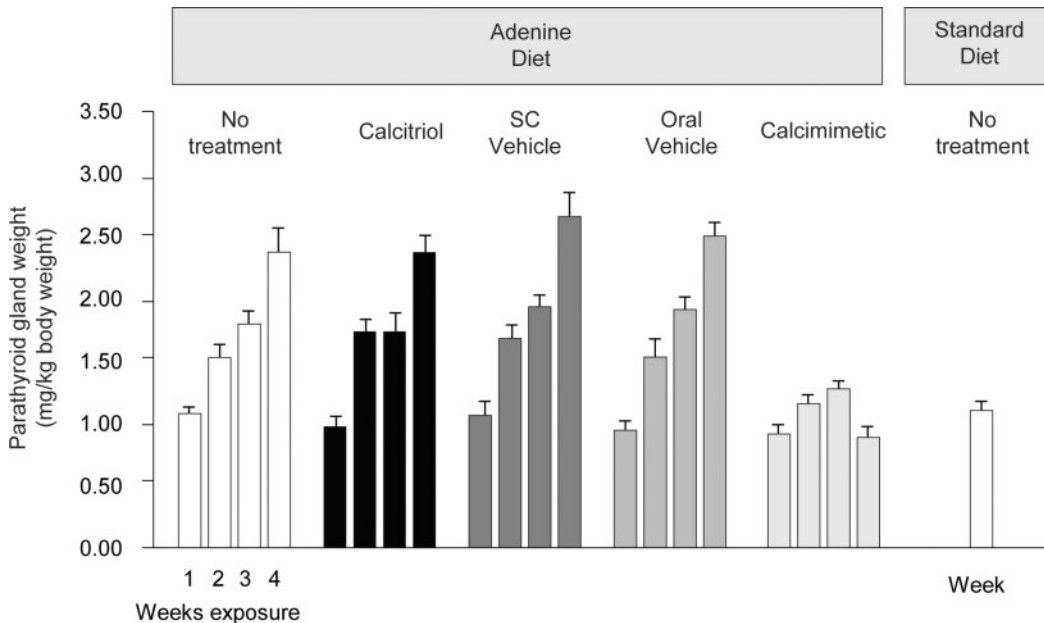


Fig. 6. Comparative effects of a calcimimetic and calcitriol in adenine-induced chronic renal failure with parathyroid hyperplasia. Male Sprague–Dawley rats (300–350 g) were fed a standard diet and received no treatment, or received a diet containing adenine 0.75% for 1–4 weeks and received daily: no treatment, calcitriol 10 ng SC; calcitriol vehicle SC; oral calcimimetic vehicle (by gavage); or calcimimetic (investigational compound) 3 mg/kg (by gavage). At the end of the treatment period, animals were sacrificed and the parathyroids removed and weighed ($n = 10$ animals/group/week at weeks 1–4). Data shown as mean ± SEM. Miller *et al.* [72].

proliferation preceded the recovery of both CaR and VDR expression [73].

Effects of other SHPT treatments on parathyroid hyperplasia

The key drivers of excessive PTH secretion and synthesis and parathyroid cell proliferation in SHPT are hypocalcaemia, hyperphosphataemia and low serum calcitriol levels. Traditional therapies such as vitamin D sterols and phosphate binders do not fully address these metabolic abnormalities. Indeed, the calcaemic and phosphataemic actions of vitamin D analogues lead to frequent episodes of hypercalcaemia and hyperphosphataemia in clinical practice. Thus, interruptions in therapy are common and can potentially lead to disease progression [76]. Moreover, response to vitamin D sterols is poor once parathyroid hyperplasia has progressed to the advanced nodular form [77]. The limited ability of conventional treatments to reduce parathyroid proliferation is reflected in the ongoing high rate of surgical parathyroidectomies among the dialysis population [78,79].

In addition to the increased risk of vascular calcification posed by elevated $\text{Ca} \times \text{P}$, [80,81] animal and *in vitro* data suggest that calcitriol may itself induce vascular calcification, possibly by decreasing PTH-related peptide in vascular smooth muscle [82,83].

Although calcitriol inhibits parathyroid cell proliferation *in vitro* and *in vivo* [71,84–86], cultured parathyroid cells [51] and parathyroid tissue [86] from patients with SHPT responded only at very high calcitriol concentrations and tissue from patients with primary hyperparathyroidism did not respond [86]. The effects of active vitamin D sterols on parathyroid hyperplasia in uraemic animal models appear to be somewhat inconsistent, depending on the timing and doses administered, as well as the experimental model [71,87,88]. Calcitriol did not prevent adenine-induced parathyroid hyperplasia at a dosage comparable to that shown to induce vascular calcification (80 ng/kg) [72,83]. It is unclear whether vitamin D therapy can induce regression of existing parathyroid hyperplasia associated with SHPT, with negative animal data [71] and conflicting clinical observations [89,90] having been reported.

Repeated percutaneous injection of vitamin D sterols directly into the parathyroid gland over prolonged periods can reduce gland size [91–94] and induce parathyroid cell apoptosis [92,93,95], but this may reflect a toxic effect (of high local concentrations) rather than a true pharmacological response. Systemic calcitriol did not induce apoptosis in parathyroid cells in rodent models of SHPT [50,96].

The limited activity of vitamin D sterols in parathyroid hyperplasia may reflect several factors, including decreased VDR expression [15,21,97], tachyphylaxis and/or a change in signal transduction processes [98,99]. Failure to control serum phosphate

also contributes to resistance to calcitriol therapy [100,101], as clearly demonstrated in the clinical setting [100]. Additionally, recent data suggest that the CaR is a more important determinant of parathyroid hyperplasia than the VDR. In VDR-ablated mice, a high-calcium diet supplemented with lactose to enhance passive intestinal calcium absorption was able to prevent parathyroid gland hyperplasia [102]. In contrast, in CaR-ablated mice, the ensuing severe SHPT could be prevented only by concomitant ablation of the PTH [103] or *Gcm-2* [104] gene.

Accumulation of phosphate induces SHPT directly and indirectly via several mechanisms [105,106] (Table 1). Administration of the calcium-free phosphate binder sevelamer was reported to reduce parathyroid cell proliferation [107] and reduce parathyroid gland hypertrophy [108] in uraemic rats. Whether this represents a direct effect via improved control of hyperphosphataemia, or an indirect effect, mediated by increased serum calcium levels, or both, remains to be determined.

Conclusions

While development of parathyroid hyperplasia undoubtedly involves an extremely complex cascade of events, signalling through the CaR appears to be the most important driver of the disease process. Activation of the CaR by calcimimetics (given in conjunction with conventional treatments) allows long-term control of PTH in dialysis patients without increasing plasma levels of Ca^{2+} , phosphorus, and/or vitamin D. Indeed, serum calcium, phosphorus and calcium-phosphorus product are reduced [61,62]. Data from uraemic rodent studies indicate that calcimimetics can inhibit the development and progression of parathyroid hyperplasia, having shown activity even in models of severe and/or established hyperplasia. Regression of existing parathyroid hyperplasia has also been demonstrated. Consistent with these observations, preliminary data from a *post hoc* analysis indicate a >10-fold reduction in the risk for parathyroidectomy, as well as a significantly reduced risk of fractures and cardiovascular hospitalisation, in dialysis patients treated with cinacalcet [109]. Given also the role of parathyroid hyperplasia in increasing the capacity for PTH production, these findings imply that calcimimetics may be able to slow disease progression in SHPT and might potentially be beneficial if initiated in the earlier stages of CKD (stage 3–4). This approach is currently being evaluated, with positive preliminary findings [110] and long-term efficacy and safety data are awaited with interest. Additional animal data suggest that calcimimetics can also restore the decreased expression of CaR and VDR that accompanies parathyroid hyperplasia, thereby, potentially improving the response to calcitriol. These agents may also inhibit development of renal and cardiovascular changes associated with SHPT and attenuate calcitriol-induced vascular calcification [111,112].

Further research is warranted to determine whether calcimimetics are effective in attenuating parathyroid hyperplasia in humans.

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