SP580: RESPONSE OF HYPERPLASTIC PARATHYROID GLAND TO WITHDRAWAL OF A CALCIMIMETIC COMPOUND

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Abstract

Background: Calcimimetics have been shown to have suppressive effects on PTH secretion and parathyroid cell proliferation. However, little is known about the mechanistic change in parathyroid cells when calcimimetics are withdrawn. We studied the response of parathyroid glands to the withdrawal of cinacalcet in 5/6th nephrectomized uremic rats. **Methods:** Uremic rats (Nx) were fed a 1.2% high phosphate diet for 2 weeks to develop secondary hyperparathyroidism and then divided by 3 treatment groups: 1) with vehicle (UC), 2) with cinacalcet (15 mg/kg/day, gavage) (Cina), and 3) with cinacalcet and a VDRA 22-oxacalcitriol (OCT: 0.15µg/kg, 3 times a week, intraperitoneally) (Cina+Maxa). After 2 weeks treatment, vehicle and cinacalcet were withdrawn while OCT treatment was continued. Immediately (day 0) and 7 days (day 7) after the withdrawal, blood and parathyroid tissues were obtained. Normal rats with vehicle at day 0 and 7 were utilized as normal controls (NC). **Results:** Cina and Cina+OCT showed equally and significantly lower PTH than Veh at day 0 whereas PTH in the Cina and Cina+OCT was increased up to indistinguishable levels in the Veh at day 7. Serum P was comparable among Veh, Cina, and Cina+OCT in both day 0 and 7. Cinacalcet treatment significantly decreased ionized Ca compared with Veh at day 0, and this decrease was restored by day 7. At day 0, PCNAmRNA was significantly increased in Veh compared with that in NC and this increase was significantly suppressed in Cina and Cina+OCT. At day 7, however, Cina, but not Cina+OCT showed significant increase in PCNAmRNA compared with Veh. **Conclusions:** These results suggest that, although PTH rebound is not present, simultaneous use of VDRA is preferred regarding parathyroid cell proliferation when cinacalcet is withdrawn.

Experimental protocol

All studies were approved by the Showa University Animal Studies Committee in accordance with federal regulations. Renal insufficiency was induced by 5/6 nephrectomy (Nx) in a group of male Sprague-Dawley rats weighing 225-250 g. Nx involves ligation of several branches of the left renal artery and excision of the right kidney. All rats were fed a normal P (NP, P 0.5%, Ca 0.8%, and vitamin D1000IU/kg) diet. Experiments were started 2 weeks after the Nx or sham operation (Sham) to allow time for recovery. Nx rats were fed a high P diet (P 1.2%, Ca 0.8%, and vitamin D1000IU/kg) for 2 weeks to establish SHPT and then divided by 3 treatment groups: 1) with vehicle (UC), 2) with cinacalcet (15 mg/kg/day, gavage) (Cina), and 3) with cinacalcet and a VDRA 22-oxacalcitriol (OCT: 0.15µg/kg, 3 times a week, intraperitoneally) (Cina+OCT). After 2 weeks treatment, vehicle and cinacalcet were withdrawn while OCT treatment was continued. Immediately (day 0) and 7 days (day 7) after the withdrawal, blood and parathyroid tissues were obtained. Normal rats with vehicle at day 0 and 7 were utilized as normal controls (NC).

Introduction

Continuous stimulation of the parathyroid glands by a combination of elevated extracellular phosphate (P) concentration, decreased extracellular ionized calcium (Ca) concentration, and markedly reduced serum calcitriol leads to increased PTH synthesis and release, and parathyroid hyperplasia, both of which are main characteristics of secondary hyperparathyroidism (SHPT). In the development of parathyroid hyperplasia, the down-regulation of Ca-sensing receptor (CaSR) and vitamin D receptor (VDR) expressions renders the parathyroid cells unable to respond appropriately to the change in serum Ca and calcitriol levels. Experimental studies in normal and a rat model of renal failure has demonstrated that parathyroid cell proliferation is associated with a decrease in CaSR and VDR expression (1-3). Both VDR activators (VDRAs) and calcimimetics are useful tools for suppressing parathyroid hyperactivity by mediating these receptors. Animal studies have demonstrated that increasing parathyroid doses of calcitriol have been shown to up-regulate parathyroid VDR expression (4) and activation of the CaSR by calcimimetics has induced not only parathyroid CaSR (1)but also VDR expression (5) in uremic rats.

At the clinical level, resistance to VDRAs can also create challenges, specifically in patients who have severe SHPT that progresses to a nodular parathyroid hyperplasia from diffuse hyperplasia (6). An important indicator for the success or failure of treatment is the size of the parathyroid gland. VDRAs generally control SHPT well in patients with smaller parathyroid gland size than larger glands (7-9). Calcimimetics, such as cinacalcet, are positive allosteric modulators of the CaSR, have now been used mainly for patients with SHPT which is refractory to conventional therapy including VDRAs.

The mechanism and time course of VDRAs' effect on parathyroid hormone (PTH) differ from those of calcimimetics. While VDRAs reduce PTH gene transcription and hormone synthesis over a period of several hours or even days (10), a calcimimetic cinacalcet inhibits PTH secretion within minutes, with a maximal decrease occurring within 2 hours of dosing in patients with SHPT. Other data indicate that activation of the CaSR by calcimimetics decreases PTH mRNA stability via the post-translational modification of the PTH-mRNA binding protein AUF1 (11). Little is known about the change in hyperplastic parathyroid cells caused by withdrawal of these different types of compounds. In the present study we studied the effect of withdrawal of cinacalcet treatment on parathyroid glands with or without a VDRA, oxacalcitriol (OCT) in 5/6 nephrectomized uremic rats.

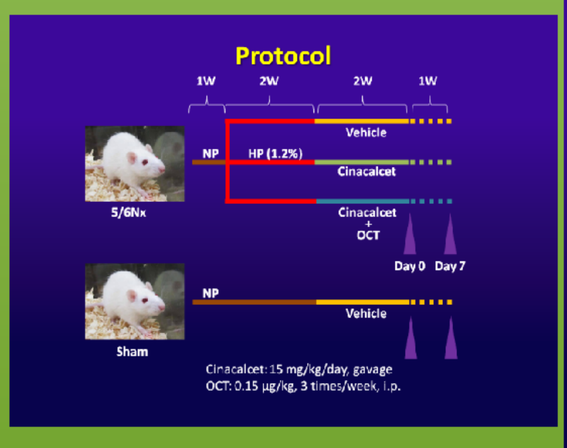
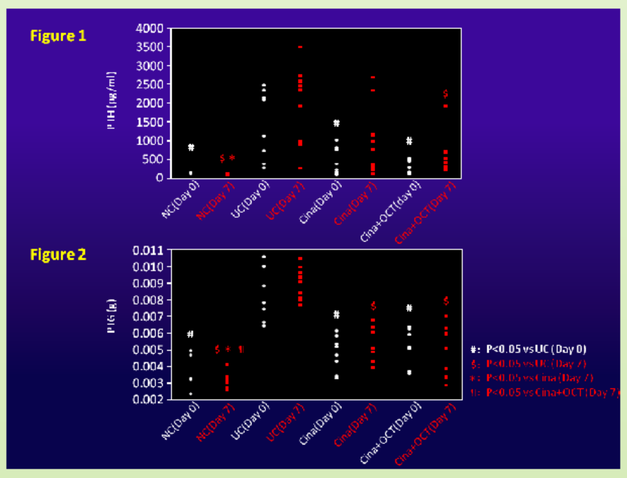


Table 1. Serum chemistries

		Alb g/dl	Cre mg/dl	iCa mmol/l	P mg/dl
NC	Day 0 (6)	3.8±0.1	0.25±0.02	1.35±0.03	7.1±0.3
	Day 7 (6)	3.6±0.1	0.18±0.02	1.34±0.03	7.9±0.1
UC	Day 0 (8)	3.3±0.1a	0.70±0.15a	1.23±0.06	11.6±2.7a
	Day 7 (10)	3.2±0.1	1.13±0.19c	0.97±0.05c	15.3±2.6
Cina	Day 0 (8)	3.3±0.1a	0.79±0.09a	0.85±0.03a,b	13.2±1.9a
	Day 7 (9)	3.4±0.1	0.74±0.36c	1.18±0.08	10.9±2.2
Cina+OCT	Day 0 (7)	3.3±0.2a	0.87±0.21a	0.99±0.03a,b	14.4±2.5a
	Day 7 (9)	3.4±0.2	0.70±0.16c	1.24±0.04	11.6±2.1

a: P<0.05 vs NC(Day 0), b: P<0.05 vs UC(Day 0), c: P<0.05 vs NC(Day 7)



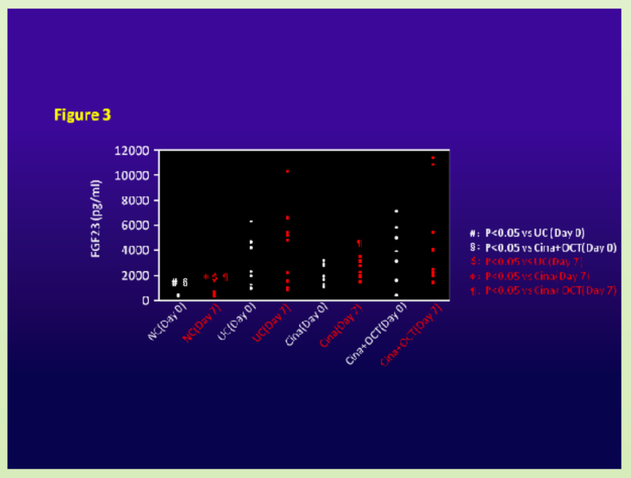
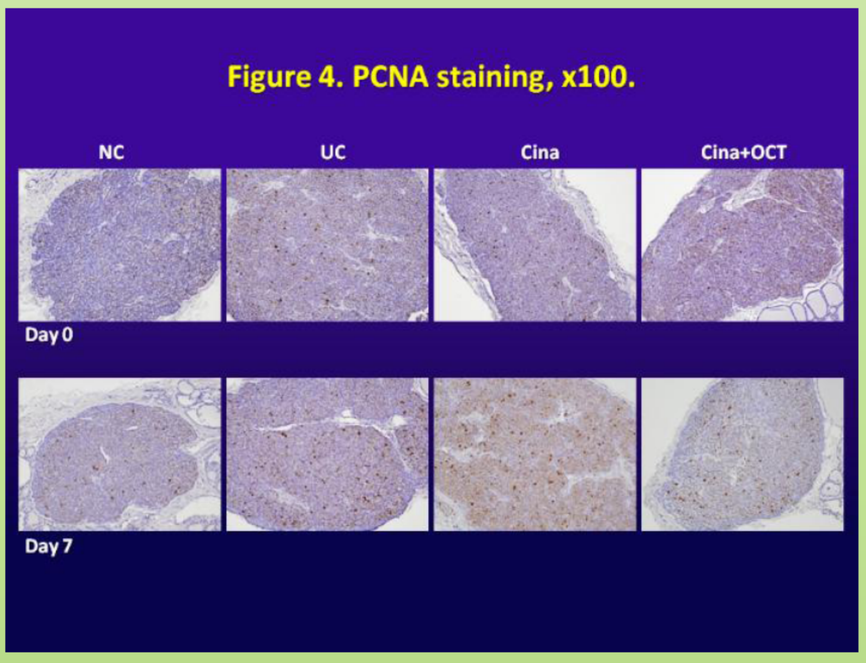


Table 2. mRNA levels (fold to NC in each day)

		PCNA	PTH	VDR	CaR
NC	Pre(Day 0)	1.0±0.4	1.0±0.1	1.0±0.2	1.0±0.1
	Post(Day 7)	1.0±0.2	1.0±0.2	1.0±0.2	1.0±0.2
UC	Pre(Day 0)	2.5±0.4a	2.0±0.4a	0.3±0.1a	0.2±0.1a
	Post(Day 7)	2.4±0.3	2.2±0.2c	0.3±0.2c	0.2±0.0c
Cina	Pre(Day 0)	1.6±0.4b	1.0±0.2b	0.7±0.1	0.6±0.0
	Post(Day 7)	12.1±2.5c,d	1.8±0.1c	0.4±0.1	0.4±0.1
Cina+OCT	Pre(Day 0)	0.6±0.1b	0.8±0.2b	0.9±0.1b	0.6±0.1
	Post(Day 7)	6.8±1.2c	1.5±0.1	0.8±0.2	0.5±0.2

a: P<0.05 vs NC(Day 0), b: P<0.05 vs UC(Day 0), c: P<0.05 vs NC(Day 7), d: P<0.05 vs UC(Day 7)



Results

1. Serum Cre, P, and ionized Ca levels were comparable between Cina and Cina+OCT groups both in day 0 and 7 (Table 1).
2. PTH levels were equally and significantly suppressed both in Cina and Cina+OCT groups compared with UC group in day 0, while the levels in Cina group increased up to the same levels as UC group in day 7 (Figure 1).
3. Parathyroid weight was equally and significantly suppressed in Cina and Cina+OCT groups in day 0 and 7 (Figure 2).
4. FGF23 levels were comparable between Cina and Cina+OCT groups in day 0, while the levels in Cina+OCT were significantly higher than those in Cina group in day 7 (Figure 3).
5. Comparing with UC group, PTHmRNA levels were equally and significantly suppressed in Cina and Cina+OCT groups in day 0, while the levels in Cina group were significantly higher than those in NC in day 0 (Table 2).
6. The decrease in VDRmRNA and CaSRmRNA levels were marginally rescued in Cina and Cina+OCT groups in day 0, while CaSRmRNA levels showed a trend to decrease in Cina and Cina+OCT groups in day 7 and this trend was apparent in Cina group (Table 2).
7. Comparing with UC group, PCNAmRNA levels were significantly suppressed in Cina and Cina+OCT groups in day 0. The levels were significantly increased in Cina and Cina+OCT groups in day 7, and the increase was distinguished in Cina group (Table 2).
8. The similar trend was observed in PCNA staining (Figure 4).

Conclusion

These results suggest that, although PTH rebound is not present, simultaneous use of VDRA is preferred regarding parathyroid cell proliferation when cinacalcet is withdrawn.

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