Purpose: The purpose of our study was to determine the influence of cinacalcet on bone markers such as tartrate resistant acid phosphatase TRAP5b and iPTH in management of secondary hyperparathyroidism.

Methods: We studied group of 22 patients with sHPT with PTH> 450 pg/ml (mean 757±264 pg/ml) (F=10, M=12; peritoneal dialysis 5 patients and hemodialysis 17 patients; mean age 59±17). There were 2 subgroups of patients with sHPT: group A treated with cinacalcet and group B treated with traditional methods (calcium carbonate and alphacalcidol). The following parameters were determined in serum: iPTH, Ca, P and tartrate resistant acid phosphatase TRACP5b as a marker of bone resorption. TRACP 5b activity was measured using assay Bone TRAP™ (SBA Finland). Intact PTH was measured using immunoradiometric assay (Diasorin USA). These parameters were measured before the start of cinacalcet therapy and after the 3rd and 6th months of therapy.

Results: Patients treated with cinacalcet (group A) had significant lower values of iPTH and TRAP 5b after the 3rd and 6th months of therapy in comparison to group B. We observed 61%/67% respectively reduction of iPTH level and 82%/76% reduction of TRAP 5b level. We did not find any significant difference between examined parameters in group B.

Conclusion: Treatment of secondary hyperparathyroidism with cinacalcet in dialysed patients was effective and allowed the significant reductions of iPTH and TRACP 5b levels. Monitoring of TRACP5b might be useful in assessment of bone resorption in the course of cinacalcet treatment.

	iPTH (pg/ml)	TRACP (U/L)	Ca (mg/dl)	P (mg/dl)
Group A	sHPT treated with cinacalcet			
Start 0	796±290*	5.6±2.8**	8.7±0.8	7.0±2.3
3 months	312±183	1.0±0.4	8.6±0.8	6.8±1.7
6 months	266±142*	1.3±0.5**	8.7±0.9	6.4±1.4
Group B	sHPT treated with calcium carbonate and alphacalcidol			
Start 0	718±327	5.1±2.2	9.0±1.4	7.3±2.7
3 months	640±258	4.2±2.5	9.8±1.8	7.5±2.8
6 months	683±292	4.0±1.9	9.4±1.5	7.1±2.1

*$p<0.001$.

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Calcium response in bone cell network to mechanical stimulations

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Objective: Calcium signaling in bone cell networks is essential for bone formation and resorption, but little is known about the quantitative molecular mechanism behind calcium signal propagation between bone cells. This study quantitatively examined the spatiotemporal characteristics of intracellular calcium ($[Ca^{2+}]_i$) responses in a well controlled bone cell chain under AFM indentation, and how these characteristics were influenced by gap junctions, ATP and intra/extracellular calcium sources.

Methods: In the present study, linear bone cell chains, in which six osteoblast-like MC3T3-E1 cells were interconnected in a row with functional gap junctions, were constructed using microcontact printing and self-assembled monolayer technologies. The cell at one end of a chain was mechanically stimulated by AFM indentation of 60 nN, and the intracellular calcium responses of the whole chain were recorded and analyzed. In addition to the untreated group, six groups treated with specific biochemical agents were also tested to identify the calcium sources and molecular pathways of calcium propagation in the bone cell chain.

Results: For the untreated and gap junction blocking groups, calcium transfer to one or two neighboring cells was observed in about 50% of the tests, and no significant difference was observed in these two groups. However, this percentage decreased to 10% for the ATP blocking group. When both ATP and gap junction pathways were inhibited, calcium transfer between cells was completely abolished. However, the indented cell still exhibited $[Ca^{2+}]_i$ response to mechanical stimulation. When the internal calcium in ER store was depleted, the indented cell still responded with calcium transients, but no signal transfer to the neighboring cells was observed. Moreover, no $[Ca^{2+}]_i$ response was detected in either the indented or its neighboring cells when external calcium was removed.

Conclusion: These results imply that ATP signaling may be the principal pathway for intercellular calcium signal propagation instead of gap junction, and confirm that internal calcium source is required for calcium propagation between cells as well as external calcium sources are responsible for the activation of calcium response under mechanical stimulations.

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In vivo studies of anabolic action of PTH and Wnt signaling pathway in bone

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Teriparatide [recombinant human PTH (1–34)] is an approved treatment of osteoporosis that stimulates new bone formation. Human and experimental animal studies have shown that PTH increases osteoblast activity and number via increased differentiation and survival, increases bone mass and bone strength, and decreases fracture rate. Given the important roles of the Wnt signaling pathway in regulating bone mass, we investigated the role of PTH on the canonical Wnt signaling pathway. In parathyroidectomized rats, continuous infusion of PTH (1–38) upregulated the mRNA expression of LRP6 and FZD-1 and decreased LRP5 and DKK-1. An in vitro study in the UMR 106 cells not only showed the similar gene responses as in vivo, but also showed an increased β-catenin protein level by PTH resulting in functional activation of a downstream Wnt responsive TBE6-luciferase (TCF/LEF-binding element) reporter gene activity. When PTH and a small molecule GSK-3 (glycogen synthase kinase-3) α and β dual-inhibitor were given to 7-month-old osteopenic, ovariectomized (Ovx) rats for 2 months, both treatments completely restored Ovx-induced bone loss on cancellous sites and significantly increased bone mineral content over both Sham and Ovx controls on cortical bone sites. Histomorphometric analyses revealed an elevated bone formation activity by significantly increasing trabecular mineral appositional rate and cortical periosteal bone formation rate with PTH and GSK-3 inhibitor treatments. Biomechanical analysis found that both treatments significantly improved vertebral strength, stiffness, and work-to-failure relative to Ovx. Furthermore, whole genome array profiling and pathway mapping analyses of bone marker genes, obtained from the distal femur after 2 months of treatment, found that both PTH and the GSK3 inhibitor reversed the Ovx-induced suppression of osteogenic markers with concomitant elevation of adipogenic markers. Of note, a subset of 158 genes altered by Ovx was restored by both PTH and GSK-3 inhibitor treatments. These data suggest that the Wnt signaling may be one of the pathways wherein PTH stimulates bone formation resulting in anabolic actions on bone.

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