

## SHORT AND LONG TERM EFFECTS OF $1,25(\text{OH})_2\text{D}_3$ ON SERUM CONCENTRATIONS OF BONE-GLA PROTEIN IN PRE-DIALYSIS CHRONIC RENAL FAILURE

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### Summary

Serum GLA protein, produced by the osteoblasts, is elevated in chronic renal failure and increases with advancing renal damage, in spite of the known  $1,25(\text{OH})_2\text{D}_3$  deficit. The effects of  $1,25(\text{OH})_2\text{D}_3$  treatment on serum bone-GLA protein (BGP) were studied in 24 pre-dialysis patients. Twelve patients received  $1\mu\text{g}$ /daily for two weeks and 12 were treated with  $0.25\mu\text{g}$  daily for one year. Blood for calcium, phosphate, alkaline phosphatase,  $1,25(\text{OH})_2\text{D}_3$ , BGP and iPTH was drawn basally and at the end of treatment. Administration of  $1,25(\text{OH})_2\text{D}_3$  for two weeks induced a significant fall in iPTH, and a significant increase in BGP and alkaline phosphatase. Long-term treatment with  $1,25(\text{OH})_2\text{D}_3$  induced significant falls in iPTH, BGP and alkaline phosphatase. Short-term administration of  $1,25(\text{OH})_2\text{D}_3$  in pre-dialysis chronic renal failure induces an increase in serum BGP levels, probably due to direct stimulation of the osteoblasts. Long-term treatment causes a parallel decline in BGP and iPTH presumably due to prolonged suppression of parathyroid gland and reduction in number of the osteoblasts.

### Introduction

The bone GLA protein (BGP), or osteocalcin, is a vitamin k-dependent protein produced by the osteoblasts [1]. The protein is the most abundant (25%) non-collagenous protein of bone. In vitro [1] and experimental in vivo studies [2] have shown that production of BGP is modulated by  $1,25(\text{OH})_2\text{D}_3$ . Addition of  $1,25(\text{OH})_2\text{D}_3$  to cultures of human osteoblasts induces a rapid dose dependent increment in BGP concentration in the medium [1]. Administration of  $1,25(\text{OH})_2\text{D}_3$  to vitamin D depleted rats increases the serum concentrations of the protein. However, a basal production of BGP can be observed even in vitamin D depleted animals [2]. In pre-dialysis chronic renal failure serum BGP is higher than normal [3], in spite of the known  $1,25(\text{OH})_2\text{D}_3$  deficit [4].

The aim of this study was to explore the effects on serum BGP of short and long term  $1,25(\text{OH})_2\text{D}_3$  administration in pre-dialysis chronic renal failure. The results suggest that osteoblasts respond to  $1,25(\text{OH})_2\text{D}_3$  by increasing production of BGP. In the long-term observation serum BGP decreases, probably as a consequence of reduction in osteoblasts.

## Materials and methods

The study was performed on 24 patients with slowly developing chronic renal failure. Twelve patients (age  $48.58 \pm 15.33$  years, serum creatinine  $4.44 \pm 1.07$  mg/dl; mean  $\pm$  SD) were treated with oral  $1,25(\text{OH})_2\text{D}_3$   $0.5 \mu\text{g}$  twice daily at 8 a.m. and 8 p.m. for two weeks. Blood for calcium, phosphate, alkaline phosphatase, BGP,  $1,25(\text{OH})_2\text{D}_3$  and iPTH was obtained basally and at the end of treatment. In a second group of 12 patients comparable for age and serum creatinine (age  $55.33 \pm 14.24$  years, serum creatinine  $4.31 \pm 1.45$  mg/dl),  $1,25(\text{OH})_2\text{D}_3$   $0.25 \mu\text{g}$ /day, was administered for about one year. The same blood parameters as for the short-term experiment, were explored basally and every four months for one year. Serum samples for BGP and  $1,25(\text{OH})_2\text{D}_3$  were stored at  $-30^\circ\text{C}$  until the assay. Measurements of these parameters were carried out each in the same assay in order to avoid interassay variation. All patients were on a diet moderately restricted in protein ( $0.8 \text{g/kg/body weight}$ ) and phosphate ( $12 \text{mg/kg/body weight}$ ) with calcium supplements ( $\text{Ca}^{++}$   $500\text{--}1000 \text{mg/day}$ ). Some patients were treated with anti-hypertensive medication (frusemide,  $\alpha$ -methyldopa, clonidine). A lower dose of  $1,25(\text{OH})_2\text{D}_3$  in the long-term observation was chosen in order to avoid hypercalcaemia and renal damage [5].

Serum BGP was measured by radio-immunoassay employing bovine standard and anti-serum against the bovine BGP, according to Price et al [6]. Normal values are  $3.89 \pm 1.45 \text{ng/ml}$ .

Serum iPTH was measured by a radio-immunoassay based on an antiserum against the C-terminal portion of the molecule. Normal values are  $0.34 \pm 0.19 \text{ng/ml}$ .

Serum  $1,25(\text{OH})_2\text{D}_3$  was measured by receptorial method, with cytosol receptors obtained from chicken intestinal mucosa as described by Eisman et al [7]. Purification of the metabolite following extraction from the serum sample, is performed on Sep-pak C18 and Sep-pak Silica cartridges, and finally with HPLC on direct phase columns (uPorasil, Waters Ass). Normal values are  $34.8 \pm 10.2 \text{pg/ml}$ .

Calcium was determined with atomic absorption spectrophotometry. Serum creatinine, phosphate and alkaline phosphatase were measured with standard laboratory techniques.

## Results

Basal laboratory data and following the short and long term  $1,25(\text{OH})_2\text{D}_3$  administration are reported in Table I and Figures 1 and 2. Short and long term treatments induced comparable changes in serum  $1,25(\text{OH})_2\text{D}_3$ , calcium

TABLE I. Calcium, phosphate and 1,25(OH)<sub>2</sub>D<sub>3</sub> serum levels in the short and long term observation

	Basal	2 weeks	Basal	6-12 months
Ca (mg/dl)	9.05±0.45	9.49±1.05*	9.03±0.58	9.56±0.75**
P (mg/dl)	3.46±0.62	4.19±0.81*	3.84±0.49	4.42±0.81*
1,25(OH) <sub>2</sub> D <sub>3</sub> (pg/ml)	16.36±11.06	30.54±21.37***	16.58±7.81	32.32±8.94***

\* p<0.05; \*\* p<0.01; \*\*\* p<0.005. Values are the mean ± SD

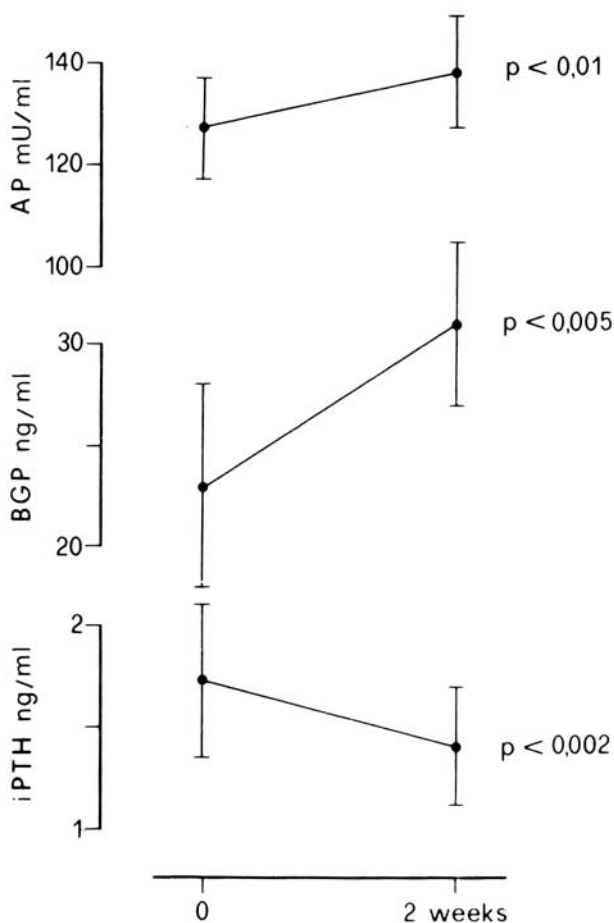


Figure 1. Changes in serum alkaline phosphatase, BGP and iPTH levels following two weeks 1,25(OH)<sub>2</sub>D<sub>3</sub> treatment

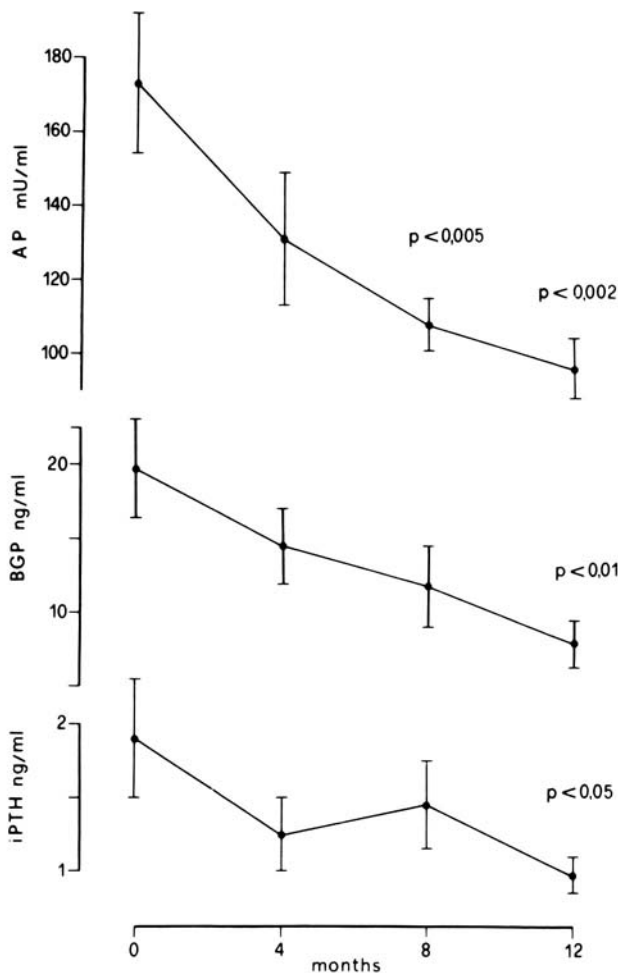


Figure 2. Time course of serum alkaline phosphatase, BGP and iPTH during treatment with low dose  $1,25(\text{OH})_2\text{D}_3$

and phosphate. Administration of  $1,25(\text{OH})_2\text{D}_3$  for two weeks induced a significant decrease in serum iPTH ( $p < 0.0025$ ) and an increase in serum BGP ( $p < 0.005$ ). A small but significant ( $p < 0.01$ ) increase of serum alkaline phosphatase was also observed. Long term treatment induced a significant fall in serum iPTH, alkaline phosphatase and BGP ( $p < 0.05$ ,  $p < 0.002$ ,  $p < 0.01$  respectively), noticeable four months from the start of  $1,25(\text{OH})_2\text{D}_3$  administration.

### Conclusion

The data indicate that short term  $1,25(\text{OH})_2\text{D}_3$  administration is able to provoke a fall in serum iPTH along with a sizeable significant increase in BGP and

a slight increment in serum alkaline phosphatase (Figure 1). On the contrary long term administration of the vitamin D metabolite induces a consensual fall in serum iPTH, alkaline phosphatase and BGP (Figure 2). The results suggest that in man, at least in this experimental situation,  $1,25(\text{OH})_2\text{D}_3$  can stimulate the osteoblasts to synthesize bone GLA protein, as observed in vitro and in animal experiments [1,2]. The increment in serum BGP after the short term  $1,25(\text{OH})_2\text{D}_3$  administration in spite of the fall in serum iPTH, indicates that parathyroid hormone has no direct stimulatory effect on BGP production by the osteoblasts. Accordingly parathyroid hormone was found to inhibit rather than stimulate BGP production by the osteoblasts in vitro [1]. Therefore the increment in serum BGP observed in secondary hyperparathyroidism of chronic renal failure is mainly due to increased active osteoblasts, under the chronic stimulus of parathyroid hypersecretion. We have already reported a strict correlation between serum BGP and the histomorphometric parameter Active Osteoblastic Surface in pre-dialysis chronic renal failure [8]. The progressive fall in serum BGP during long term treatment with  $1,25(\text{OH})_2\text{D}_3$  can probably be ascribed to parathyroid gland suppression and consequent gradual decrease in the number of the active osteoblasts.

As for the increment in serum  $1,25(\text{OH})_2\text{D}_3$  observed following administration of the metabolite, no difference was found between the values reached after two weeks' treatment with the higher dose and those after six to 12 months of the lower dose. This finding suggests that the divergent effect on serum BGP induced by the short and long term treatment cannot be ascribed to differences in the doses of the metabolite. However, the finding of similar levels of serum  $1,25(\text{OH})_2\text{D}_3$  following high and low dose treatment deserves further attention. The degradation metabolite of  $1,25(\text{OH})_2\text{D}_3$  in peripheral tissues is not entirely known [9]. It cannot be excluded that reduction of bone turnover and suppression of secondary hyperparathyroidism may slow vitamin D turnover and also lengthen the biological half-life of  $1,25(\text{OH})_2\text{D}_3$  during the long term treatment, thus allowing relatively higher levels of the metabolite to be reached. Alternatively long term treatment may induce a saturation of the metabolite tissue stores comparable to that obtained with short term higher dose treatment.

The fall in serum BGP during long term  $1,25(\text{OH})_2\text{D}_3$  treatment of secondary hyperparathyroidism of chronic renal failure suggests that serum BGP measurement is a useful tool in the assessment of bone turnover and of the efficacy of treatment.

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