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Effects of a Short Course of Oral Phosphate Treatment on Serum Parathyroid Hormone(1-84) and Biochemical Markers of Bone Turnover: A Dose-Response Study

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Summary. To investigate the possible use of oral phosphate as an activator of bone remodeling in coherence treatment of osteoporosis, 82 postmenopausal females, aged 50–75 years, were randomized to treatment with oral phosphate (750, 1500, or 2550 mg/day) or placebo for 7 days and followed for 4 months thereafter. All patients had sustained at least one previous fracture of the distal forearm and had a bone mineral content of the contralateral forearm or bone mineral density of the lumbar spine lower than normal mean for age. Urinary phosphate/creatinine ratio increased in a dose-dependent fashion during treatment ($P < 0.001$), whereas no significant changes were seen in serum phosphate or serum calcium. Serum parathyroid hormone (PTH) rose significantly ($P < 0.05$) during treatment to a maximum of 36 and 33% in the groups receiving 1500 and 2250 mg/day, respectively, whereas serum 1,25-dihydroxycholecalciferol remained unchanged. In the group receiving 1500 mg/day, mean serum osteocalcin was increased in the period from day 1 to day 28 ($P < 0.05$), but no significant changes were observed in urinary hydroxyproline/creatinine ratio, or serum bone alkaline phosphatase. We conclude that a short course of oral phosphate treatment increases serum PTH considerably. Furthermore, 1500 mg/day but not 2250 mg/day increases serum osteocalcin. No clear biochemical evidence, however, of increased activation of bone remodeling could be demonstrated in either group.

Key words: Osteoporosis – Coherence treatment – Phosphate – Parathyroid hormone – Bone markers.

The ideal treatment or secondary prophylaxis of osteoporosis should increase bone mass and restore bone structure (i.e., create a positive bone balance per remodeling cycle), and at the same time increase the birth rate of bone remodeling units (BRU). One suggested approach to achieve this is coherence therapy, also called activate-depress-free-repeat (ADFR) treatment [1, 2]. In theory, a cohort of new BRUs is initiated by a short course (pulse dose) of a drug or hormone resulting in partial synchronization of the remodeling processes (activation). This allows selective pharmacological inhibition of the resorptive phase of the new BRUs by antiresorptive agents (depression) leading to more shallow resorption lacunae, and enables the cavities to be filled in completely or even overfilled during the formation period which is left free from intervention (free). After completion of the formative period, the sequence is repeated (repeat). Thyroid hormones [3, 4], growth hormone [5], and parathyroid hormone (PTH) [3, 6] are known activators and calcitonin and bisphosphonates have been used as depressors. PTH is not yet available for clinical use, however, oral phosphate supplementation might activate bone remodeling indirectly by lowering serum calcium and in turn increase endogenous production of PTH [2, 7]. Although phosphate has been used in several protocols [8–10], there is no conclusive evidence that it does activate bone remodeling. Moreover, basic knowledge on dose-response relationships are lacking. In this study, we examined the effect of short-term treatment with three different dosages of oral phosphate on serum levels of PTH and biochemical markers of bone turnover.

Patients and Methods

Subjects and Experimental Design

Eighty-two postmenopausal (cessation of menstruation for more than 12 months) females, aged 50–75 years, were included in the study. All patients had previously sustained at least one low-energy distal forearm fracture. Furthermore, bone mineral content in the distal forearm or bone mineral density of the lumbar spine was lower than normal mean for age. None of the patients had been treated with glucocorticoids, calcitonin, estrogens, or PTH within 6 months before study, or had ever received bisphosphonates or fluoride. Two patients were on hydroflumetiazide (50 mg/day) and two were on furosemide (40 and 80 mg/day, respectively) and continued this throughout the study. Patients with malignant diseases, diabetes mellitus, liver disease, drug or alcohol abuse, or other diseases likely to affect calcium metabolism were not allowed to participate. All patients were fully ambulatory during the study and had normal renal function, as judged from serum creatinine (<110 μmol/liter). No dietary restrictions were made. Two patients in whom a diagnosis of primary and secondary hyperparathyroidism, respectively, were made as a result of the screening procedure were excluded from the study and replaced. Furthermore, one patient who developed upper abdominal discomfort after a single dose of the study drug was excluded and not replaced. Data from this patient are only included in the report on side effects. Thus, the results are based on 79 women.

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Table 1. Baseline characteristics of the study population

	Group I	Group II	Group III	Group IV
Dose of phosphate	750 mg	1500 mg	2250 mg	Placebo
n	19	19	20	21
Age (years) (range)	64 (54–73)	64 (50–75)	62 (46–73)	62 (51–75)
Height (cm)	162 ± 6	163 ± 6	162 ± 8	160 ± 5
Weight (kg)	62.3 ± 8.7	66.4 ± 13.9	65.6 ± 11.8	61.8 ± 9.1
Serum				
Phosphate (mmol/liter)	1.04 ± .08	1.07 ± .11	1.08 ± .14	1.12 ± .11
Calcium (mmol/liter)	2.47 ± .06	2.44 ± .07	2.45 ± .07	2.46 ± .05
PTH (pg/ml)	32 ± 13	30 ± 11	32 ± 11	26 ± 9
Osteocalcin (ng/ml)	45 ± 23	41 ± 18	53 ± 22	49 ± 21
AP (U/liter)	180 ± 44	181 ± 35	202 ± 58	187 ± 47
Bone-AP (U/liter)	84 ± 29	79 ± 26	89 ± 27	82 ± 35
$1,25(OH)_2D_3$ (pmol/liter)[a]	154 ± 44	138 ± 50	152 ± 72	156 ± 59
25 OHD_3 (nmol/l)[b]	56 ± 33	64 ± 35	76 ± 38	50 ± 27
Fasting urinary				
Phosphate creatinine (mmol/mmol)	2.02 ± 0.43	2.20 ± 0.56	2.20 ± 0.54	1.96 ± 0.49
Calcium/creatinine (mmol/mmol)	0.44 ± 0.20	0.36 ± 0.17	0.41 ± 0.22	0.45 ± 0.23
Hydroxyproline/creatinine (μmol/mmol)	24 ± 8	25 ± 6	26 ± 5	25 ± 7

[a] Measured in 61 patients; [b]measured in 74 patients

Spot urine samples were collected after an overnight fast, and serum samples were drawn between 7.30 a.m. and 11.00 a.m. (before administration of study drugs) at day −7, 1, 2, 3, 4, 5, 6, and 7, and at week, 2, 3, 4, 6, 10, and 16. As a rule, the individual patient attended the clinic at the same hours at every visit.

The study was approved by the regional Ethical Committee and Danish National Board of Health. Each individual gave informed consent prior to the study, which was conducted according to the Declaration of Helsinki II.

Treatment

The study was performed in a double-blind manner. Patients were randomized in blocks of four of the following treatments: group I received 750 mg phosphorus in the morning and placebo at noon and in the evening; group II received 750 mg phosphorus in the morning and evening and placebo at noon; group III received 750 mg phosphorus three times daily; and group IV received placebo three times daily for 7 days. Phosphorus was given as effervescent tables (Phosphore Sandoz) containing ammonium phosphate (1.518 g), potassium phosphate (0.650 g), and glycerol phosphate (1.540 g). Coded drugs were delivered by Sandoz, Basel, Switzerland. No calcium supplementation was given.

Biochemistry

Samples were divided into aliquots and stored immediately at −80°C (serum) or −20°C (urine) until analysis. Serum intact PTH was measured using a commercial kit (Allegro Intact PTH IRMA) from Nichols Institute, San Juan Capistrano, CA. Intraassay coefficient of variation (CV) was 5%.

Serum osteocalcin was measured by radioimmunoassay (RIA) [11] modified from Price and Nishimoto [12] using rabbit antisera against bovine osteocalcin. Intact, purified bovine osteocalcin [13], verified by amino acid analysis and antisera to bovine osteocalcin, was generously provided by Dr. J. Poser (Procter and Gamble, Cincinnati, OH). The antisera showed full cross-reactivity between human and bovine osteocalcin. The intra- and interassay CV were 5 and 10%, respectively (mean = 10 ng/ml, n = 10).

Total alkaline phosphatase activity (AP) in serum was measured spectrophotometrically using p-nitrophenylphosphate as substrate according to the method recommended by the Scandinavian Committee on Enzymes [14]. Intraassay CV was 2.5% (mean 246 U/liter, n = 10) and interassay CV was 5% (mean = 246, U/liter, n = 25).

Serum bone isoenzyme alkaline phosphatase activity (bone-AP) was determined by lectin-precipitation as described [15, 16]. Samples (300 μl) were pretreated with 30 μl Triton X-100 (20 g/liter) for 30 minutes at 37°C. An aqueous solution of wheat germ lectin (Sigma L-9640, 5 g/liter in distilled water) was then added and the samples were mixed and incubated for 30 minutes at 37°C. After centrifugation at 2000 × *g* for 10 minutes, the AP activity was determined as above and serum bone-AP was calculated as the difference between total and supernatant activity. The intraassay CV was 8% (mean 131 U/liter, n = 10) and the interassay CV was 25% (mean 69 U/liter, n = 10).

Serum 1,25-dihydroxycholecalciferol ($1,25(OH)_2D_3$) was determined by RIA following a two-step purification procedure [17]. Intra- and interassay CVs were 7 and 14%, respectively. Serum 25-hydroxycholecalciferol ($25OHD_3$) was measured by RIA using the extraction and purification procedure described by Lund et al. [18]. Intra- and interassay CVs were 6.4 and 10%. Both analyses were performed at Medicinsk Laboratorium, Copenhagen, Denmark.

Fasting urinary hydroxyproline (OHP) was measured using a commercial kit (Organon Teknika, B.V. Boxtel, The Netherlands) [19]. Serum calcium, phosphate, albumin, creatinine, and fasting urinary calcium, phosphate, and creatinine were analyzed according to standard laboratory methods. All serum calcium concentrations were corrected for individual variations in serum albumin using the formula:

$$\text{corrected serum calcium (mmol/liter)} = 0.0011\,[650 - \text{serum albumin}(\mu\text{mol/liter})] + \text{measured serum calcium (mmol/liter)}.$$

Concerning serum PTH, osteocalcin, AP, bone-AP, OHP, and $1,25(OH)_2D_3$, all samples from a single patient were analyzed in the same run.

Statistical Analysis

Baseline values (mean of day −7 and day 1) among the study groups were compared using one-way analysis of variance (ANOVA). Differences between groups in response to treatment were identified by

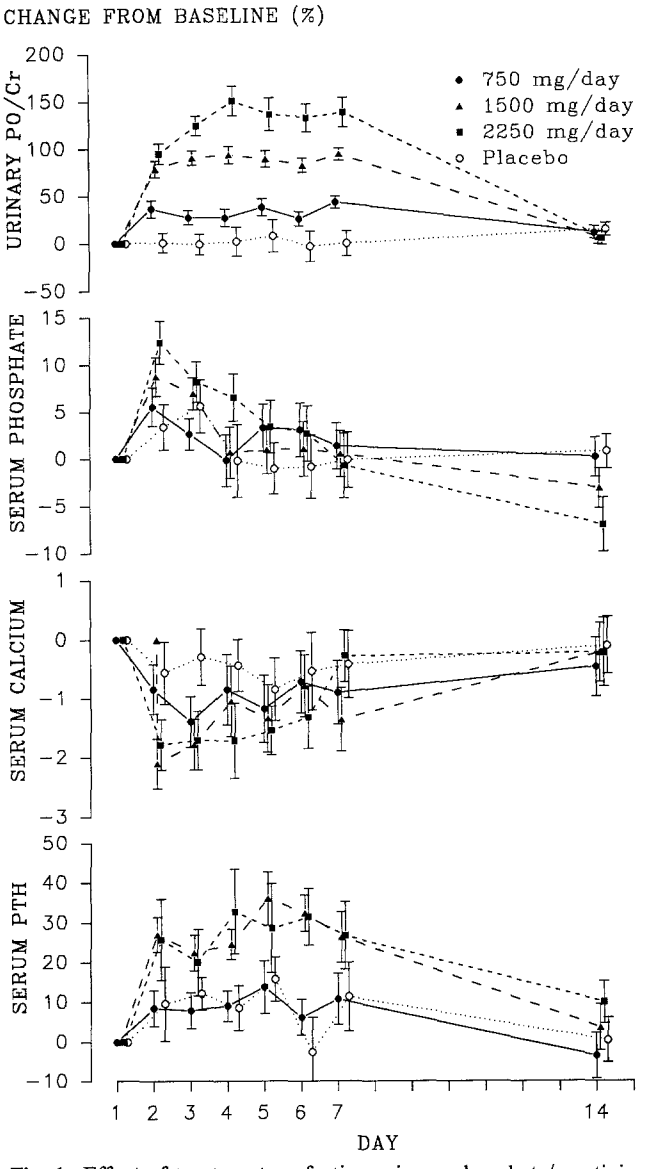

Fig. 1. Effect of treatment on fasting urinary phosphate/creatinine (Po/Cr), serum phosphate, calcium, and parathyroid hormone (PTH) in 79 women. Phosphate or placebo were administered orally from day 1 to 7. Data are shown as percentage of change from baseline (mean ± SE). Only the changes in urinary Po/Cr ($P < 0.001$, ANOVA) and serum PTH ($P < 0.05$) were statistically significant.

repeated measures ANOVA using the SPSS (Statistical Package for Social Sciences). *A posteriori* analysis within each group was done using *t*-tests. Furthermore, data were transformed to indicate percentage of deviation from baseline values and plotted against time. Finally, mean deviation from baseline for the periods from day −7 to day 28 and from day 42 to day 112 were calculated, and differences between groups were analyzed using one-way ANOVA. *P*-values less than 0.05 were considered significant. All results are given as means ± SE unless otherwise stated.

Results

Clinical characteristics and baseline biochemistry of the 79 patients in the study are given in Table 1. No significant differences in baseline parameters were seen between the groups.

Urinary phosphate/creatinine ratio increased significantly in a dose-dependent manner during treatment ($P < 0.001$, ANOVA) and returned to baseline by day 14 (Fig. 1). The increase was evident in all patients in groups II and III, indicating good compliance with the treatment. According to the drug counting, a total of only eight doses were missed and no patient missed more than a single dose of the study drug.

An insignificant, dose-dependent increase was seen in serum phosphate ($P = 0.10$, ANOVA) (Fig. 1). This increase was most evident on day 2, and on day 7, serum phosphate had returned to baseline, At day 14, the pattern was reversed and serum phosphate was slightly decreased in a dose-dependent fashion. No significant changes were seen in serum calcium (Fig. 1) or urinary calcium/creatinine ratio (not shown).

During treatment, serum PTH increased significantly ($P < 0.05$, ANOVA) (Fig. 1). In group I the increase was slight, but the maximal increase in groups II and III were 36 and 33%, respectively, reaching maximum at days 4 and 5 and returning to baseline at day 14. There were no significant differences between the response in groups II and III.

Serum was available for measurement of vitamin D metabolites in only some of the patients. Sera from days −7 + 1, days 3 + 4, and days 6 + 7 were pooled. When all data were included in the analyses, a marginally significant variation with time was observed ($P = 0.07$, ANOVA, n = 46) due to a 20% decrease in $1,25(OH)_2D_3$ at days 3 + 4 in group III. However, the difference between groups III and IV did not attain statistical significance when compared alone (Table 2).

Serum osteocalcin changed significantly during the study period ($P < 0.05$, ANOVA) (Fig. 2), however, paired examinations showed that this was due to differences between groups I and III. None of the treatment groups were significantly different from the placebo group. The mean change in serum osteocalcin during days 1–28 was significantly higher in group II compared with group IV ($P < 0.05$) (Table 3) and the same trend was seen in the period from day 42 to day 112, but this did not reach significance ($P = 0.09$). No significant changes were seen in serum total or bone-AP, or in fasting urinary hydroxyproline/creatinine ratio (OHP/Cr) when analyzing raw data by repeated measures ANOVA. Mean bone-AP in the period from day 42 to day 112, however, differed significantly between the groups ($P < 0.02$), but none of the treatment groups were different from the placebo group (Table 3).

Side effects were clearly dose dependent. Two patients in group I, three in groups II, and seven in group III complained of gastrointestinal side effects (nausea, loose stools or diarrhea, or vomiting) and one patient in group III complained of dizziness at a single occasion. One of the patients in group III, who complained of nausea after the first dose of the study drug, refused to continue treatment, but apart from this patient medication did not have to be stopped prematurely.

Discussion

Our study demonstrates that oral phosphate supplementation increases serum PTH and thus confirms earlier reports

Table 2. Serum $1,25(OH)_2D_3$ (pmol/liter) in response to treatment

	Group I	Group II	Group III	Group IV
Dose of phosphate	750 mg	1500 mg	2250 mg	Placebo
n	9	11	13	13
Day −7 + 1	156 ± 14	144 ± 13	166 ± 21	153 ± 17
Day 3 + 4	141 ± 16	130 ± 10	131 ± 12	151 ± 14
Day 6 + 7	147 ± 12	144 ± 10	145 ± 14	151 ± 18

None of the changes were significant. Data are shown as mean ± SE.

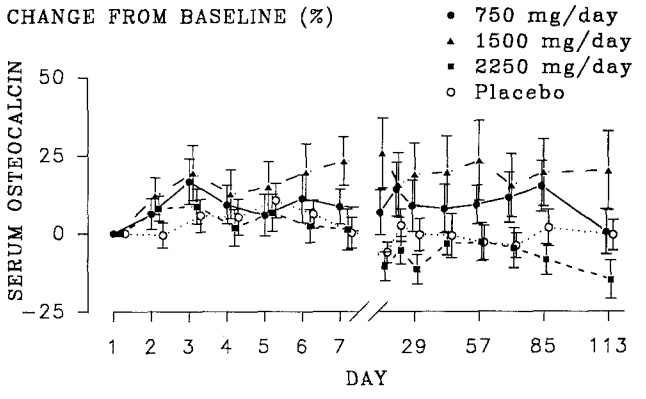

Fig. 2. Effect of treatment on serum osteocalcin. Data are shown as mean ± SE. See text for further legend.

in patients [7, 20] and normal volunteers [21, 22]. The increase in serum PTH showed no linear dose-response relationship, and the effect of 1500 mg/day and 2250 mg/day of phosphate on serum PTH and serum calcium were similar. No effect of the lowest dose of phosphate (750 mg/day) could be demonstrated; however, we may have underestimated this as blood samples in this group were drawn 24 hours after the last phosphate administration.

Activation of bone remodeling would be expected to be mirrored by an increase in urinary OHP/Cr followed by a later increase in serum osteocalcin and bone-AP. We found, however, no significant effects of phosphate administration on urinary OHP/Cr, and previous studies have been contradictory. Silverberg et al. [7] found no change in OHP excretion in response to oral phosphate (2000 mg/day) for 5 days in an uncontrolled study in younger subjects, whereas Calvo et al. [21] found a significant increase of 9% in young females and 16% in young males during high phosphate (1600 mg/day) low calcium (400 mg/day) diet for 8 days, but not in a later study following 4 weeks on a similar diet [22]. The changes in serum PTH in these studies were of the same magnitude as in the present, and skeletal responsiveness to bovine PTH(1-34) does not seem to be altered by the menopause or in osteoporosis [23]; however, the discrepancies may reflect differences in sensitivity to phosphate administration depending on sex, age, menopausal status, or calcium intake.

In contrast to previous studies, the follow-up period in the present study covered the expected formative phase of new bone remodeling units that might have been initiated by the phosphate treatment [3]; however, no consistent increase in markers of bone formation could be demonstrated. As the duration of the remodeling periods vary widely between subjects, and changes in biochemical bone markers therefore might develop asynchronously, we calculated the mean change from baseline in the periods roughly matching the resorptive and formative periods. This showed that serum osteocalcin increased in the period from day 1 to day 28 in group II and remained increased, however insignificantly, during days 42–112. Silverberg et al. [7] reported an increase in serum osteocalcin during phosphate treatment for 5 days, but others [21] found no effect. In our study, no effect on serum osteocalcin could be demonstrated in the group receiving the highest dose of phosphate. Serum $1,25(OH)_2D_3$ is a potent stimulator of osteocalcin synthesis [24], and previous studies have demonstrated that increased phosphate intake decreases serum $1,25(OH)_2D$ [25], but no such effect could be demonstrated in the present study. Furthermore, PTH directly impairs osteocalcin synthesis in osteoblasts *in vitro* [24, 26] and decreases serum osteocalcin upon infusion in normal humans [27] and patients with X-linked hypophosphatemic rickets [28].

Bone turnover measured histomorphometrically is increased in hyperparathyroidism [3] and accompanied by increased serum levels of osteocalcin and AP and urinary excretion of OHP [29]. These changes, however, are slight and often within normal limits despite higher levels of PTH than observed in our series. Furthermore, biochemical evidence of activation of bone remodeling has been reported following treatment with synthetic human PTH(1-38) [6]. This suggests that the increase in serum PTH induced by phosphate administration was too small or the treatment of too short duration to induce clearly measurable changes in bone markers. Moreover, it may be important that serum calcium and $1,25(OH)_2D$ are increased and serum phosphate decreased in both primary hyperparathyroidism [17] and during treatment with PTH(1-34) [6], though this is not the case during phosphate treatment.

Finally, *in vitro* studies have indicated that phosphate may directly inhibit bone resorption [30], and there is increasing evidence for PTH as an important anabolic hormone [31]. These effects however, may be too small to be picked up by the biochemical markers of bone turnover and our study does not rule out a beneficial effect of phosphate.

We observed only the well-known side effects of diarrhea and dyspepsia during treatment. Nevertheless, the side effects were clearly dose-dependent and would prevent the use of phosphate doses of 2250 mg/day or more in many patients, and larger increases in serum PTH, therefore, can hardly be achieved by phosphate administration.

In conclusion, our study has demonstrated that oral phosphate increases endogenous PTH secretion considerably and that there is no linear dose-response relationship, the effect of 1500 and 2250 mg/day being similar. Treatment with 1500 mg/day, but not 2250 mg/day, increased serum osteocalcin; however, no changes indicating increased activation of bone remodeling could be demonstrated in the biochemical markers of bone turnover.

Table 3. Mean values (expressed as percentage of baseline) of bone markers in periods corresponding to the expected resorptive (days 1–28) and formative (days 42–112) periods

	Group I	Group II	Group III	Group IV	ANOVA
Dose of phosphate	750 mg	1500 mg	2250 mg	Placebo	
n	19	19	20	21	
Mean day 1–28					
Osteocalcin	9.06 ± 30.66	21.69 ± 40.76[a]	−5.57 ± 25.99	.47 ± 24.78	$P < 0.05$
OHP/Creatinine	6.94 ± 28.26	−0.77 ± 19.47	2.26 ± 25.92	10.90 ± 39.89	NS
Bone-AP	9.91 ± 30.56	9.12 ± 27.35	−8.11 ± 21.60	1.07 ± 24.10	NS
AP	4.57 ± 10.54	4.08 ± 15.39	0.99 ± 11.26	3.17 ± 17.45	NS
Mean day 42–112					
Osteocalcin	8.36 ± 27.06	21.46 ± 46.16[b]	−3.42 ± 26.15	.53 ± 26.26	$P = 0.09$
OHP/Creatinine	16.20 ± 31.31	−2.17 ± 15.39	−1.24 ± 19.90	8.37 ± 46.63	NS
Bone AP	10.30 ± 29.76	19.14 ± 30.42	−7.57 ± 20.24	2.29 ± 26.59	$P < 0.02$
AP	3.93 ± 12.73	5.20 ± 15.69	0.07 ± 8.34	1.62 ± 13.35	NS

Data are shown as mean ± SE
[a] $P < 0.05$; [b] $P < 0.10$: differences between placebo and treatment groups (unpaired t test)

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