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Original Paper

Monitoring the Action of Clodronate with Type I Collagen Metabolites in Multiple Myeloma

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In our previous double-blind trial, we reported that clodronate reduced the incidence of bone lesions, fractures, pain and hypercalcaemia in multiple myeloma. Recently, it has been assumed that the antiresorptive effect of bisphosphonates on the osteoclasts is mediated through the osteoblasts. We therefore determined, in 244 patients of the same trial, serum assays of aminoterminal propeptide of type I procollagen (PINP) and type I collagen degradation product (ICTP). PINP is an early synthesis product of proliferating osteoblasts, in comparison to the alkaline phosphatase (AP) which is secreted by differentiated osteoblasts during the maturation phase of collagen. ICTP circulates in serum when old bone is resorbed. Our results indicate that after 25 months, the PINP levels decreased in the clodronate group (from 68.9 ± 4.4 μg/l to 37.2 ± 3.5 μg/l; $P < 0.001$) but not in the control group (from 61.5 ± 3.2 μg/l to 69.3 ± 7.5 μg/l; $P <$ NS). The fall in the ICTP levels was markedly steeper in the patients receiving clodronate (from 8.38 ± 0.80 μg/l to 4.58 ± 0.32 μg/l; $P < 0.01$) than placebo (from 7.84 ± 0.53 μg/l to 6.45 ± 0.95 μg/l; P = NS). A significant difference between the study groups was seen at 4 months in the PINP, at 7 months in the ICTP and at 13 months in the AP levels, suggesting that clodronate affected through the proliferating osteoblasts, the osteoclasts, and through the osteoclasts, the differentiated osteoblasts. High baseline ICTP, PINP and AP levels indicated a poor prognosis. The decrease of the markers by clodronate was more marked in survivors than in non-survivors. Copyright © 1996 Elsevier Science Ltd

Key words: clodronate, osteoclasts, osteoblasts, type I collagen metabolites, multiple myeloma, prognosis

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INTRODUCTION

BONE DISEASE is common in multiple myeloma. The unbalanced bone remodelling of this disease is characterised by enhanced bone resorption which is not followed by bone formation to the same extent. Increased resorption and diminished formation of bone can lead to pathological fractures, pain and hypercalcaemia. The rate of both bone formation and resorption can now be measured using radioimmunoassays for type I collagen synthesis and degradation products. Type I collagen is the main constituent of the organic matrix of mineralised bone [1].

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We recently reported the major findings of a double-blind controlled multicentre trial in 350 patients with newly diagnosed multiple myeloma [2]. All patients received a standard melphalan–prednisolone (MP) treatment, and after 1 month of MP treatment were randomised to receive either clodronate 2.4 g daily or placebo for 2 years. Clodronate use was associated with less osteolytic lesions and fractures, less hypercalcaemic episodes and bone pain. A 50% response was achieved in the clodronate patients and 67% of the placebo patients. There were 68 deaths in the placebo group and 54 in the clodronate group at the end of the trial [2].

In this study, we determined serum assays of aminoterminal propeptide of type I procollagen (PINP) and type I collagen degradation product (ICTP) from the same trial patients.

PINP is an synthesis product of lining cells and proliferating osteoblasts [3–4]. ICTP circulates in serum when old bone is resorbed by osteoclasts [5]. Recently, it has been assumed that the effect of clodronate on osteoclasts may be mediated through the osteoblast cell family [6–10]. Our aim was to determine the action of clodronate on PINP and ICTP levels.

PATIENTS AND METHODS

Serum samples for the PINP and ICTP analysis were collected from 244 of the 350 patients of the randomised, placebo-controlled double-blind trial. The only criterion for the selection of this subgroup was the availability of a serum sample taken before any treatment. The clinical and laboratory findings of these patients are given in Table 1. Detailed information about the inclusion criteria, the design of the trial, the assessment of treatment and participating doctors and hospitals have been previously described. The activity of alkaline phosphatase (AP) in serum was determined according to the recommended method of the Scandinavian Committee on Enzymes [11].

The blood samples for the PINP and ICTP assays were obtained before starting MP treatment (0 month), before starting clodronate therapy (1 month) and at 4, 7, 13, 18 and 25 months. An equilibrium type of radioimmunoassay for ICTP was utilised [5]. PINP was purified from the ascitic fluid of patients with malignant tumours. As expected, the protein contained two different polypeptide chains, in a ratio 2:1. Its identify was verified by aminoterminal sequencing. For the radioimmunoassay for PINP, polyclonal antibodies raised in rabbits and chloramine T-labelled PINP were used. Of the two antigen species related to PINP and present in human serum, this assay almost exclusively detects the major form, eluting in the position of standard PINP in gel filtration chromatography [3]. The reference interval for ICTP was 1.7–4.5 μg/l, that for PINP 10–79 μg/l and that for AP 60–275 U/l.

The Mann–Whitney *U*-test and Wilcoxon test were used for statistical analysis. The effect of the pretreatment serum PINP and ICTP concentrations on 2-year mortality was calculated using univariate logistic regression analysis.

RESULTS

The two patients groups were similar for gender and body mass index, but patients to be treated with clodronate were somewhat younger than those in the placebo group. The baseline values of the laboratory tests routinely used were similar between the groups. The distributions of myeloma protein in serum and in the urine were also similar. However, there were more patients in Stages I and III in the clodronate group than in the placebo group (Table 1).

The basic myeloma therapy was started with a combination of melphalan and prednisolone. In both groups, the PINP levels increased after the first month of MP therapy. Clodronate therapy was randomised to begin at month 1. Both the PINP and ICTP levels decreased significantly after clodronate therapy (Table 2; Figure 1a,b). In contrast, in the control group, the PINP levels increased initially but the ICTP levels did not change markedly (Figure 1a,b). The significance of the differences between the control and clodronate groups was seen earlier in the PINP levels (at 4 months) than in the ICTP levels (at 7 months) (Figure 1a,b). The activity of serum alkaline phosphatase was similar between the two groups (Table 2; Figure 1c).

Patients who died during the trial had initially higher ICTP, PINP and AP concentrations than those who were alive at the end of the trial (Table 3). In univariate logistic regression analysis, high ICTP ($P = 0.0007$), PINP ($P = 0.0273$) and AP levels ($P = 0.016$) predicted mortality. Throughout the trial,

Table 1. Clinical and laboratory findings at baseline

	Placebo ($n = 119$)	Clodronate ($n = 126$)	P*
M/F	63/56	58/68	NS
Age (year + S.E.M.)	67 ± 1	63 ± 1	0.009
Weight (kg)	71 ± 1	71 ± 1	NS
BMI (kg/m^2)	26.1 ± 0.4	26.4 ± 0.4	NS
Hb (g/l)	110 ± 2	111 ± 2	NS
S-creatinine (μmol/l)	124 ± 8	121 ± 6	NS
S-urate (μmol/l)	353 ± 11	368 ± 12	NS
S-calcium (alb.corr. mmol/l)	2.61 ± 0.03	2.65 ± 0.03	NS
S-Pi (mmol/l)	1.26 ± 0.03	1.26 ± 0.03	NS
S-ALAT (U/l)	19 ± 1	23 ± 2	NS
S-albumin (g/l)	35 ± 1	36 ± 1	NS
M-protein (g/l)	30 ± 2	34 ± 2	NS
DU-protein (mg/24 h)	1131 ± 203	825 ± 159	NS
U-calcium (mmol/mmol creat)	0.79 ± 0.13	0.85 ± 0.10	NS
Stage†			
I (%)	21.8	28.6	
II (%)	58.8	42.1	0.03
III (%)	19.2	29.4	
A† S-creatinine <170 μmol/l (%)	89.9	86.5	NS
B† S-creatinine >170 μmol/l (%)	10.1	13.5	

*Mann–Whitney U-test. †Clinical staging after Durie and Salmon [17].
S, serum; M, myeloma; DU, urine; M/F, male/female; BMI, body mass index; Hb, haemoglobin; Pi, precipitable iodine; ALAT, alanine aminotransferase.

Table 2. ICTP, PINP and AP levels during the trial

	ICTP					PINP			AP		
Month	Placebo Mean + S.E.M.	*n*	Clodronate Mean + S.E.M.	*n*	*P**	Placebo Mean + S.E.M.	Clodronate Mean + S.E.M.	*P**	Placebo Mean + S.E.M.	Clodronate Mean + S.E.M.	*P**
0	7.84 ± 0.53	119	8.38 ± 0.80	125	NS	61.5 ± 3.2	68.9 ± 4.4	NS	193 ± 7	188 ± 9	NS
1§	7.90 ± 1.51	47	7.13 ± 0.80	66	NS	74.6 ± 7.2¶	95.9 ± 14.1**	NS			
4	7.61 ± 0.88	66	6.56 ± 0.52	71	NS	76.2 ± 6.7**	73.4 ± 10.1	†	210 ± 8**	213 ± 10**	NS
7	6.75 ± 0.70	72	5.61 ± 0.37¶	72	NS	75.9 ± 5.0**	53.4 ± 5.1¶	‡	202 ± 8‖	189 ± 7	NS
13	7.45 ± 0.71	67	5.46 ± 0.36‖	65	†	69.6 ± 4.8¶	44.7 ± 3.4¶	‡	196 ±9	164 ± 6‖	†
18	6.33 ± 0.46	50	5.48 ± 0.65¶	57	†	61.1 ± 4.0	44.9 ± 4.4¶	‡			
25	6.45 ± 0.94	43	4.58 ± 0.32¶	47	†	69.3 ± 7.5	37.2 ± 3.5**	‡	204 ± 13‖	212 ± 43	NS

ICTP, type I collagen degradation product; PINP, aminoterminal propeptide of type I procollagen; AP, alkaline phosphatase.
*Mann–Whitney *U*-test between the groups (†$P < 0.01$; ‡$P < 0.001$). §Time of randomisation to placebo and clodronate groups. Wilcoxon test within the groups (‖$P < 0.05$; ¶$P < 0.01$; **$P < 0.001$).

Table 3. Baseline ICTP, PINP and AP indicate prognosis

	ICTP	PINP	AP
2-year mortality ($n = 244$)			
Beta coefficients*	0.0732	0.0074	0.0037
*P**	0.0007	0.0273	0.016
Alive ($n = 157$)			
Mean ± S.E.M.	6.81 ± 0.53	60.6 ± 3.1	180 ± 9
Dead ($n = 87$)			
Mean ± S.E.M.	10.62 ± 0.91	73.9 ± 5.2	212 ± 12
P†	<0.001	0.013	0.008

For abbreviations see legend to Table 2.
*Univariate logistic regression analysis. †Mann–Whitney *U*-test.

there was a tendency for the non-survivors to maintain higher levels and for the survivors to maintain lower levels (Figures 2a–c).

At the time of meyloma diagnosis, the PINP correlated significantly to ICTP ($r = 0.444$; $P < 0.01$), PINP to AP ($r = 0.47$; $P < 0.01$) and AP to ICTP ($r = 0.224$; $P < 0.01$).

DISCUSSION

The mode of action of clodronate is still not completely known and there may be many mechanisms operating at the same time [6–10]. Bisphosphonates may have different biochemical effects on various cell types involved in bone remodelling. They may act by inhibiting recruitment and survival of osteoclasts. The most common hypothesis suggests that clodronate inhibits the resorptive capacity of current osteoclasts [6]. Clodronate may also have action on lining cells, which are the members of the osteoblast cell family that carry out *in vivo* the various actions of osteoblast-like cells on bone resorption demonstrated *in vitro* [8], either to promote release of inhibitory factor [9] or inhibit release of stimulatory factor (IL-6) [10].

Type I collagen accounts for most of the organic matrix of mineralised bone [1]. Its synthesis, deposition and mineralisation involves the ordered expression of a number of genes by the osteoblasts. The developmental sequence of an osteoblastic cell phenotype has been divided into three consecutive phases: proliferation, extracellular matrix maturation, and mineralisation [5]. Each phase involves the expression of a characteristic set of genes and is a necessary prerequisite for the next. Production of type I collagen is an early event, taking place during the proliferation of osteoblast precursor cells. If deposition of a collagenous matrix by proliferating osteoblastic cells *in vitro* is interfered with, the cells do not enter the next developmental phases of the osteoblast phenotype [4]. The expression of alkaline phosphatase characteristically starts immediately after cessation of cell proliferation, reaches a maximum during the phase of matrix maturation and declines as matrix mineralisation commences. Among the genes expressed during matrix mineralisation are those for the calcium binding proteins osteocalcin and osteopontin [4].

The fall in PINP levels suggests that clodronate retards the proliferation of osteoblast precursor cells, probably by its action on lining cells. The subsequent decrease in type I collagen production might be a signal for the current osteoclasts to inhibit collagen degradation or to interrupt the release of stimulatory factors such as IL-6. As a result, bone resorption decreased with a concomitant fall in the ICTP levels and collagen maturation, measured as activity of alkaline phosphatase, is significantly retarded. The delayed inhibition of AP activity may also reflect coupling phenomenon: osteoclast inhibition acts to reduce osteoblast function. When the study groups were compared with each other, a significant and stepwise fall of the markers was seen: PINP at 4 months, ICTP at 7 months and AP at 13 months.

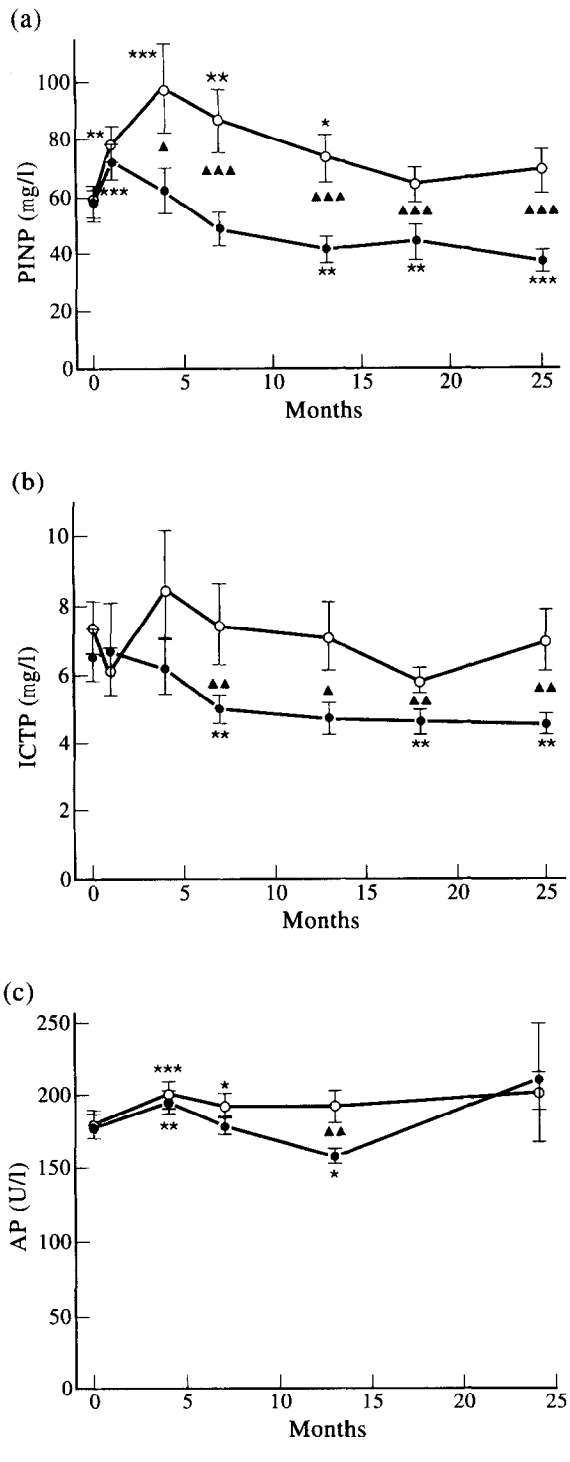

Figure 1. Effect of clodronate on PINP (a), ICTP (b) and AP (c) levels in patients who survived 2 years from the start of the trial. (● clodronate group; ○ control group) (Wilcoxon within the groups: * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$) (Mann–Whitney test between the groups: ▲ = $P < 0.05$; ▲▲ = $P < 0.01$; ▲▲▲ $P < 0.001$).

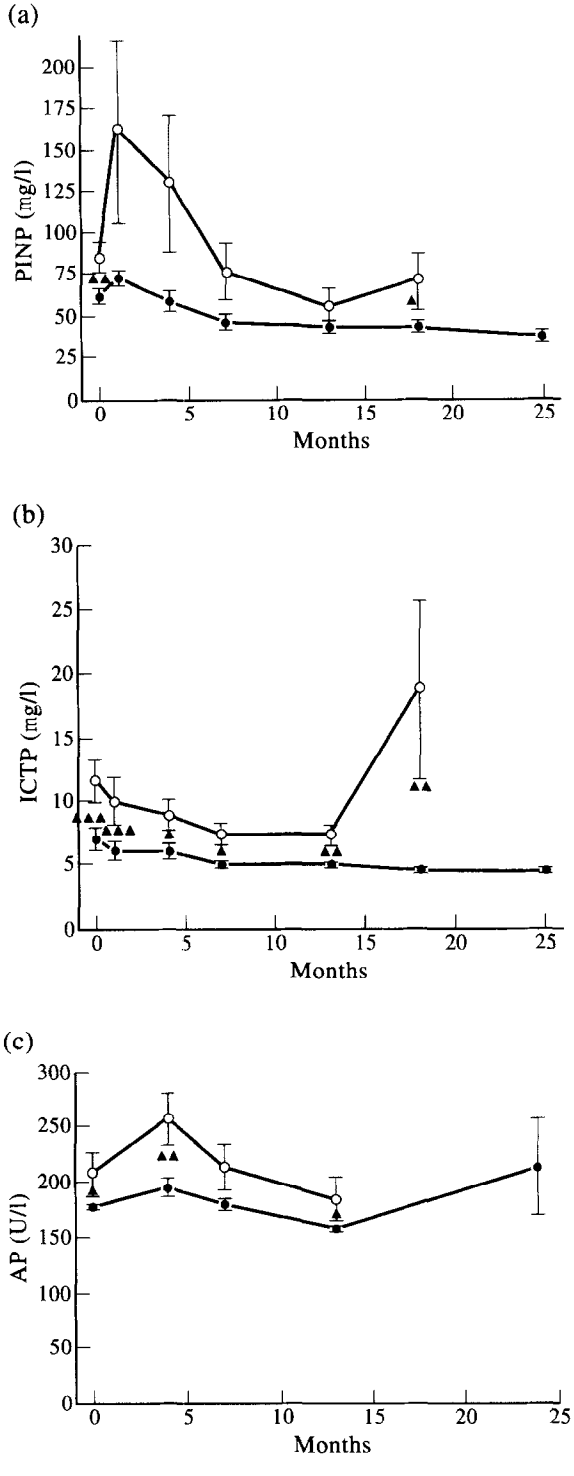

Figure 2. PINP (a), ICTP (b) and AP (c) levels in survivors and non-survivors of the clodronate group. (● survivors; ○ non-survivors) (Mann–Whitney test between the groups; ▲ = $P < 0.05$; ▲▲ = $P < 0.01$; ▲▲▲ $P < 0.001$).

In our previous pilot study of myelomatosis, the ICTP levels correlated strongly with the extent of bone lesions [12]. Baseline IL-6 levels in the patients of this trial (presented elsewhere) have been similarly shown to be correlated [13]. Furthermore, the number of osteolytic lesions at baseline were significantly associated with the risk of death during the 24 months follow-up [14]. Equally, initially high ICTP levels indicated poor prognosis in the present study. In addition, patients who had elevated PINP and AP levels at baseline showed a higher 2-year mortality.

Clodronate decreased significantly all the bone markers, thus influencing both osteoblasts and osteoclasts. This seemed to be a beneficial effect, since bone turnover declined and

clinical symptoms improved [2]. However, clodronate was not sufficient in its action in all patients: it decreased the markers in the survivors more effectively than in the non-survivors whose levels remained elevated throughout the study. The behaviour of the markers is supported by our subgroup analysis of the trial: if the patients responded to chemotherapy, clodronate diminished the complications of myelomatous bone disease more effectively than placebo. In contrast, patients who did not respond to cytotoxic drugs had no benefit from clodronate [15]. Consequently, a decrease of PINP, ICTP and AP levels during clodronate therapy indicates a good response to chemotherapy.

The function of osteoblasts has been assumed to be impaired or disturbed in myelomatosis, since chemotherapy does not induce osteoblasts to fill the lytic lesions in bone in myeloma. In this study, the MP therapy increased the PINP concentrations, suggesting that, before the treatment, the osteoblasts had been inhibited by myeloma cells. Chemotherapy, by reducing neoplastic cell mass, probably liberates many osteoblasts from inhibition factors secreted by myeloma cells. However, this effect of chemotherapy in this study was insufficient since, despite of high ICTP levels, the PINP concentrations were not elevated to the same extent. The same inbalance in the coupling phenomenon was observed in our previous myeloma study, where we measured type I collagen synthesis with PICP assay (carboxyterminal propeptide of type I procollagen) [12]. Similarly, Bataille and colleagues have shown that the lower the osteocalcin levels the myeloma patients have, the more extensive their lytic lesions [16].

We conclude that clodronate treatment decreases PINP, ICTP and AP levels, suggesting that the antiresorptive action of clodronate on osteoclasts is mediated via osteoblasts. The fall of these markers provides information, not only about the antiresorptive activity of clodronate, but also about the response to chemotherapy. The higher the ICTP, PINP and AP levels at baseline, the poorer the prognosis.

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