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The Effect of Clodronate on Bone in Metastatic Prostate Cancer. Histomorphometric Report of a Double-blind Randomised Placebo-controlled Study

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57 patients with advanced prostate cancer and a failure of prior hormonal treatment were selected for a double-blind placebo-controlled trial, in which they were randomly allocated to receive either clodronate (C) or placebo concomitantly with the basic cancer treatment, estramustine phosphate (E) (560 mg daily). The treatment was started intravenously with 300 mg of C or placebo in 5 consecutive days, and thereafter maintained orally with 1600 mg of C or identical placebo daily for 3 months. Bone biopsies were taken at admission and at 3 months. Measurements of serum calcium, phosphate, alkaline phosphatase, prostate-specific antigen and creatinine were made at the time of both bone biopsies and at 1 month. Serum intact parathyroid hormone and vitamin D metabolites were measured at admission and at 3 months. Because of several discontinuations, the study groups at final analysis comprised 20 patients taking E+C and 19 patients taking E and placebo. Bone resorption, as judged by eroded surface and osteoclast number, was markedly increased especially in biopsies taken from tumour-involved bone. Treatments with E+C or E both induced a significant decrease in bone resorption, but were associated with the development of hypocalcaemia, secondary hypoparathyroidism, hypophosphataemia and severe impairment of mineralisation of newly formed bone, i.e. osteomalacia. Since the patients were not vitamin D deficient, we conclude that osteomalacia resulted from a relative deficiency of calcium and phosphate. The transiency of pain relief achieved with anti-resorptive agents in the treatment of bone metastases from prostate cancer may be due to the development of osteomalacia.

Key words: clodronate, estramustine phosphate, bone resorption, osteomalacia, histomorphometry, prostate cancer, bone metastases
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INTRODUCTION

MORE THAN 90% of the skeletal metastases from prostate cancer are osteosclerotic in character [1, 2]. However, a number of histomorphometric studies have indicated that the high rate of osteoblastic bone formation within metastases is accompanied with markedly increased osteoclastic bone resorption, which may also be accelerated at tumour-free sites of the skeleton [3–5]. Bone disease in patients with metastatic prostate cancer is an important cause of morbidity. One of the major features is bone pain, which has recently been associated with increased bone resorption [6, 7].

Basic cancer treatment is directed to reduce tumour growth,

which usually relieves pain. Most patients with prostate cancer and bone metastases respond to the first-line hormonal therapy, but the response rate to any treatment (hormonal or cytotoxic) after first relapse is poor [8–10]. Thus, it has been suggested that such patients might benefit from treatments directed at reducing the activity of the metabolic bone disease induced by the tumour, for instance by suppressing bone resorption. Indeed, it has recently been shown that bisphosphonates, which are specific inhibitors of osteoclast-mediated bone resorption, relieve bone pain in patients with skeletal metastases from prostate cancer [6, 7, 11–13]. In our recent study, pain relief occurred with simultaneous inhibition of bone resorption, as judged by measurements of serum calcium and serum ICTP (cross-linked carboxyterminal telopeptide region of type I collagen) during treatment with oral clodronate (C) and estramustine phosphate (E) [7]. The effect of treatment was, however, transient, lasting only for 1 month.

The aim of this study was to examine the effect of treatment with C on skeletal disease due to metastatic prostate cancer using histomorphometric techniques. The objectives were to determine whether C inhibited osteoclastic bone resorption, whether the given treatment had any adverse effects on bone remodelling and, in particular, to find out the reason why the earlier reported pain relief was transient.

PATIENTS AND METHODS

57 patients (mean age 74 years, range 52–87) with cytologically or histologically proven prostate cancer were selected for study. All patients had progressing skeletal metastases, as judged by bone scan or radiography, and had failed at least one hormonal treatment. The expected survival time was at least 3 months. Patients with severe liver insufficiency or renal failure, or radiotherapy in the 2 months proceeding the study were excluded from the trial. E (Estracyt, Kabi Pharmacia, Sweden, 280 mg twice daily by mouth) was selected for a basic cancer treatment during the trial in all patients. The patients were randomised to receive either C (Bonefos, Leiras, Finland) or placebo using a double-blind study design. Treatment was started with intravenous administration of 300 mg of C or placebo over 3 h in 5 consecutive days, and thereafter maintained orally with 1600 mg of C or identical placebo daily for at least 3 months.

Histological assessment

Bone biopsies were taken from the anterior ilium under local anesthesia using a Bordier trephine (6 mm internal diameter) at admission and at 3 months. The patients underwent *in vivo* tetracycline labelling before pre- and post-treatment biopsies, using 500 mg of tetracycline hydrochloride twice daily for 4 and 2 consecutive days on two occasions separated by an interval of 10 days. Histological measurements were made double-blinded on trabecular bone utilising a semi-automated technique with side-arm attachment to the microscope, and a digitising tablet from which surfaces and areas are automatically computed. The measurements are expressed according to the recommendations of the American Society of Bone and Mineral Research [14] and comprise the following: eroded surface (ES/BS); the proportion (%) of the bone surface occupied by erosion, assumed to be due to bone resorption. Osteoclast number (N.Oc/BS); the number of multinucleated and mononucleated osteoclasts present per mm of the trabecular bone surface. Osteoid surface (OS/BS); the proportion (%) of the trabecular bone surface (BS) occupied by unmineralised bone. Osteoblast number (N.Ob/BS); the number of active looking osteoblasts per mm of the trabecular bone surface. Osteoid thickness (OTh); the width of osteoid seams measured directly expressed in microns and corrected for obliquity. Osteoid volume (OV/BV); the unmineralised bone matrix expressed as a percentage of bone volume (BV). Mineral apposition rate (MAR); the interval between tetracycline labels expresssed as microns per day. Values were corrected for obliquity. Mineralisation lag time (Mlt); this was calculated by dividing the mean osteoid seam width (OTh) by adjusted apposition rate. The values are given in days.

Reference values for static histomorphometric measurements were calculated from an autopsy material of 20 men (mean age 71 years, range 52–82), who had died suddenly as a result of accident or acute illness with no history of bone disease or medical treatment affecting the skeleton. Reference values for dynamic measurements of bone formation in elderly men have been given previously [15].

Biochemistry

Serum calcium (Ca), phosphate (Pi), alkaline phosphatase (ALP), prostate specific antigen (PSA) and creatinine (Crea) were measured in serum after an overnight fast at admission, and at 1 and 3 months. Serum was collected for measurements of intact parathyroid hormone (iPTH) and vitamin D metabolites at admission and at 3 months. The serum levels of 1,25-dihydroxyvitamin D_3 and 25-hydroxyvitamin D_3 were determinated essentially as described previously [16]. The between-assay variations of the method were 18 and 15%, respectively. Serum intact parathyroid hormone was measured with an immunoradiometric assay kit from Nichols institute (San Juan Capistrano, California, U.S.A.).

Statistical analysis

The significance of differences between the treatment groups at each time point, and the significance of changes between the groups were calculated using the Mann–Whitney test. The significance of changes within the treatment groups were calculated using Wilcoxon's test for paired data. P values less than 0.05 were regarded as statistically significant.

The study was approved by the local ethics committee.

RESULTS

Of the 57 patients recruited, 42 completed the study. Reasons for discontinuations and withdrawals are presented in Table 1. In 3 patients, one of the biopsies was taken from tumour-free bone and the other one from tumour-involved bone. Since these paired bone biopsies did not contain comparable areas of trabecular bone, they were excluded from analysis. Thus, paired bone biopsies for the analysis of response to treatment were available from 39 patients. The results reported in this study are based on the data of these 39 patients.

Histological response

For the statistical analysis of response to treatment, biopsies from tumour-free and tumour-involved bone within both treat-

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Table 1. Clinical characteristics, previous treatments, pretreatment laboratory values and causes of discontinuation of the study patients in the estramustine + clodronate (E+C) and in the estramustine (E) groups

	E+C		E	
Total no. of patients recruited	28		29	
Discontinuations and withdrawals				
Refusal of the medication	1		2	
Nausea	1		1	
Progression/ineffective medication	5		4	
Cause unknown	0		1	
Biopsies containing unpaired data	1		2	
No. of patients analysed	20		19	
Mean age, years (range)	73	(54–81)	77	(60–87)
Duration of skeletal disease (months)				
Mean (range)	9	(1–45)	14	(1–44)
Median	4		5	
Treatment prior to the study				
Orchidectomy	15		15	
Oestrogen/estramustine	3		6	
LHRH-agonists	1		3	
Anti-androgens	3		1	
Radiotherapy (to the prostate)	2		0	
Biochemistry at admission				
S-Ca < 2.20 mmol/l	5	(25%)	6	(32%)
S-P_i < 0.80 mmol/l	0	(—)	0	(—)
S-ALP > 275 U/l	11	(55%)	14	(74%)
S-Crea > 115 μmol/l	3	(15%)	4	(21%)
Bone biopsies from				
Tumour-free bone	15		14	
Tumour-laden bone	5		5	

LHRH, luteinising hormone releasing hormone; S-Ca, serum calcium; S-P_i, serum phosphate; S-ALP, serum alkaline phosphatase; S-Crea, serum creatinine.

ment groups were pooled (Table 2), but the most important parameters are also illustrated separately according to tumour-free and tumour-laden biopsies in both groups before and after treatment (Figure 1).

Bone resorption. At admission, there were no significant differences in the measurements of bone resorption between the treatment groups. The number of osteoclasts was significantly higher than in controls both in tumour-laden as well as in tumour-free bone, which showed increased cell numbers in 20% of the biopsies. Eroded surface was increased only in tumour-involved bone (Figure 1a). The number of osteoclasts decreased significantly during treatment in both groups (Table 2). The decrease in the eroded surface did not reach statistical significance. The differences of changes between the treatment groups were not significant.

Bone formation. The increase in the amount of osteoid and osteoblast number at admission was clearly localised in tumour-involved bone (Figure 1c and d), in which the presence of woven bone and osteoid was a prominent feature. During treatment, there was a significant increase in osteoid surface, osteoid thickness and volume in both treatment groups (Table 2). The increase was noted in tumour-laden as well as in tumour-free biopsies (Figure 1c and d, Figure 2). Osteoblast numbers did not significantly decrease in either treatment group. The differences of changes between the treatment groups were not significant.

Mineralisation of bone. At admission, mineral apposition rate in both treatment groups was locally increased in tumour-involved bone, but mineralisation lag time did not differ from that in tumour-free bone (Figure 1e and f). During treatment, mineral apposition rate decreased significantly in both groups (Table 2). Mineralisation lag time, which was at admission significantly higher in patients on E ($P = 0.014$; Table 2), extended significantly during treatment in both treatment groups. The differences of changes between the groups were non-significant. Post-treatment biopsies from 4 and 5 patients on E+C and E, respectively, showed wide and irregular tetracycline labels instead of double labelling, as a sign of osteomalacia (Figure 3). In these biopsies, mineral apposition rate or mineralisation lag time was not measurable.

Biochemical response

The mean serum calcium was normal in both treatment groups at admission, although slightly decreased concentrations were measured in 25 and 32% of the patients taking E+C and E, respectively (Table 1). Serum calcium decreased significantly even to below the lower limit of normal range in both groups during the course of the trial. In patients taking E+C, the decrease was significant at 1 month ($P = 0.003$), whereas in patients taking E, it reached statistical significance at 3 months ($P = 0.044$). The difference of change between the groups was not statistically significant (Table 3).

None of the patients were hypophosphataemic at admission. During treatment, the mean serum phosphate concentration

Table 2. Histomorphometric data of 20 patients on estramustine+clodronate (E+C) and 19 patients on estramustine (E)

	Controls		E+C					E					
			Admission		3 months			Admission		3 months			
	Mean	(S.E.)	Mean	(S.E.)	Mean	(S.E.)	P^1	Mean	(S.E.)	Mean	(S.E.)	P^2	P^3
Bone resorption													
ES/BS (%)	7.5	(1.3)	9.3	(1.7)	6.8	(1.1)	0.167	10.3	(1.7)	7.8	(1.4)	0.073	0.736
N.Oc (/mmBS)	0.06	(0.02)	0.22	(0.06)	0.09	(0.04)	0.012	0.14	(0.05)	0.04	(0.02)	0.028	0.456
Bone formation													
OS/BS (%)	5.4	(1.2)	13.9	(4.0)	25.7	(7.2)	0.022	11.6	(3.1)	20.0	(4.1)	0.051	0.736
N.Ob (/mmBS)	0.36	(0.10)	1.71	(0.67)	1.38	(0.55)	0.526	1.20	(0.55)	1.09	(0.48)	0.366	0.833
OTh (μm)	12.6	(0.7)	13.7	(0.7)	25.2	(4.3)	0.008	14.7	(1.4)	20.0	(2.4)	0.007	0.457
OV/BV (%)	0.9	(0.1)	3.6	(2.1)	8.9	(3.9)	0.020	1.7	(0.3)	4.2	(1.0)	0.003	0.725
Mineralisation													
MAR (μm/day)	0.492*	(0.038)	0.467	(0.031)	0.236	(0.028)	<0.001	0.453	(0.038)	0.295	(0.042)	0.006	0.289
Mlt (days)	29.0*	(6.0)	31.4	(2.3)	107.9	(19.8)	0.001	40.8†	(2.3)	82.0	(12.6)	0.004	0.249

P^1 and P^2 values denote the significance of change within the treatment groups during treatment (Wilcoxon's test for paired data). P^3 values denote the significance of change between the treatment groups (the Mann–Whitney test). *For reference, see [15]. The abbreviations for histomorphometric parameters are explained in the text. †$P = 0.014$ between E+C and E group at admission.

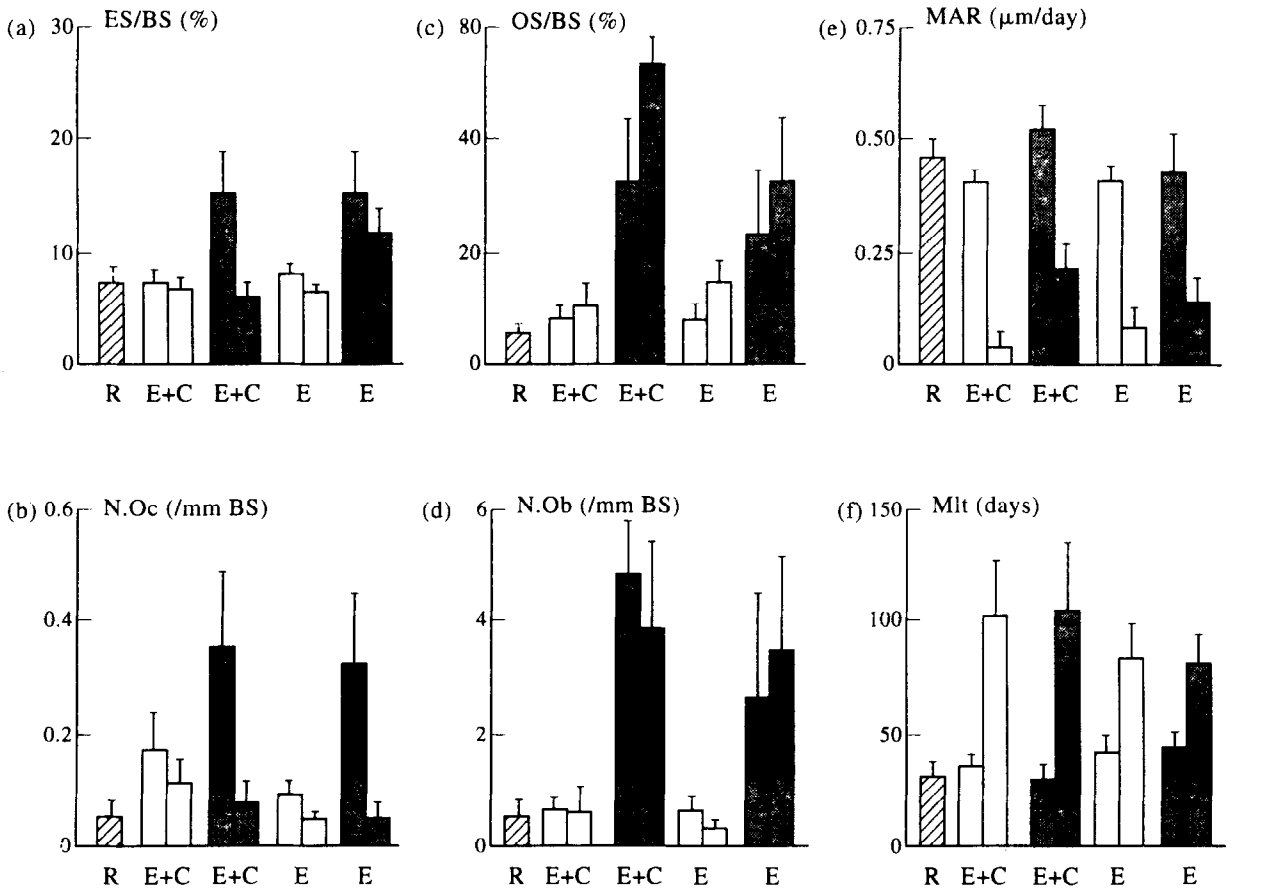

Figure 1. Measurements of bone resorption and bone formation in tumour-free (open bars) and tumour-laden (solid bars) bone before and after treatment in patients with prostate cancer. R, controls (striped bars). (a) Eroded surface; (b) osteoclast number; (c) osteoid surface; (d) osteoblast number; (e) mineral apposition rate; (f) mineralisation lag time.

decreased significantly at 1 month in both E + C and E groups ($P < 0.001$ and $P = 0.001$, respectively). The values decreased below the lower limit of the normal range in as many as 55% of the patients on E+C and in 16% of the patients on E. The difference of change between the groups was not statistically significant (Table 3).

Mean serum iPTH at admission was normal in both treatment groups despite the value of 298 ng/l measured in 1 patient in E group—this patient had a serum creatinine value of 245 umol/l because of urinary obstruction, low serum calcium but normal serum phosphate levels, low values of both serum 25-hydroxyvitamin D_3 and 1,25-dihydroxyvitamin D_3, but no signs of osteomalacia in bone biopsy. During treatment, levels of serum iPTH increased significantly in both groups, and the difference of change between the groups was non-significant (Table 4).

The means of serum 25-hydroxyvitamin D_3 and 1,25-dihydroxyvitamin D_3 were normal at admission in both treatment groups despite low values in 15 and 21% patients in E+C and E groups, respectively. The values of 1,25-dihydroxyvitamin D_3 increased significantly during treatment in both groups (Table 4). The difference of change between the groups was non-significant. Only 1 patient had abnormally low serum 1,25-hydroxyvitamin D_3 at 3 months. He was the same patient who had high iPTH at admission. Despite low 1,25-hydroxyvitamin D_3, the increase in mineralisation lag time was only moderate (being 78 days at 3 months) in this patient.

Serum alkaline phosphatase (Table 3) and serum prostate-specific antigen were highly increased in both treatment groups at admission, and increased further towards the end of the trial time. Despite moderately increased values in some patients (Table 1), the mean serum creatinine was normal at admission, and decreased significantly at 1 month in both E+C and E groups ($P = 0.008$ and $P = 0.014$, respectively), and remained well within the normal range in both groups during the study (data not shown). There were no significant differences between the groups at any time point of the trial.

DISCUSSION

C has been shown to relieve bone pain, especially in patients with lytic bone disease due to breast cancer or multiple myeloma [17–19]. The mechanism of pain relief is very likely associated with the inhibition of osteoclastic bone resorption. C is also effective in the treatment of Paget's disease of bone, in which bone turnover is highly augmented [20], and which has many features similar to metastatic bone disease due to prostate cancer, such as increased osteoclast-mediated bone resorption, osteosclerosis and the formation of woven bone. The aim of this study was to examine the effects of treatment with C on bone turnover in patients with metastatic prostate cancer. The entry criteria for the study demanded progressive bone metastases and a failure of prior hormonal therapy, because we wanted to examine the features of bone metabolism particularly in advanced skeletal disease. Even though the expected response to any cancer treatment was poor, basic cancer treatment was given to all patients for ethical reasons, since C is not an anti-tumour agent. We chose E for the basic cancer treatment for our patients, since in Finland it is the most commonly used second-line treatment in patients with advanced prostate cancer.

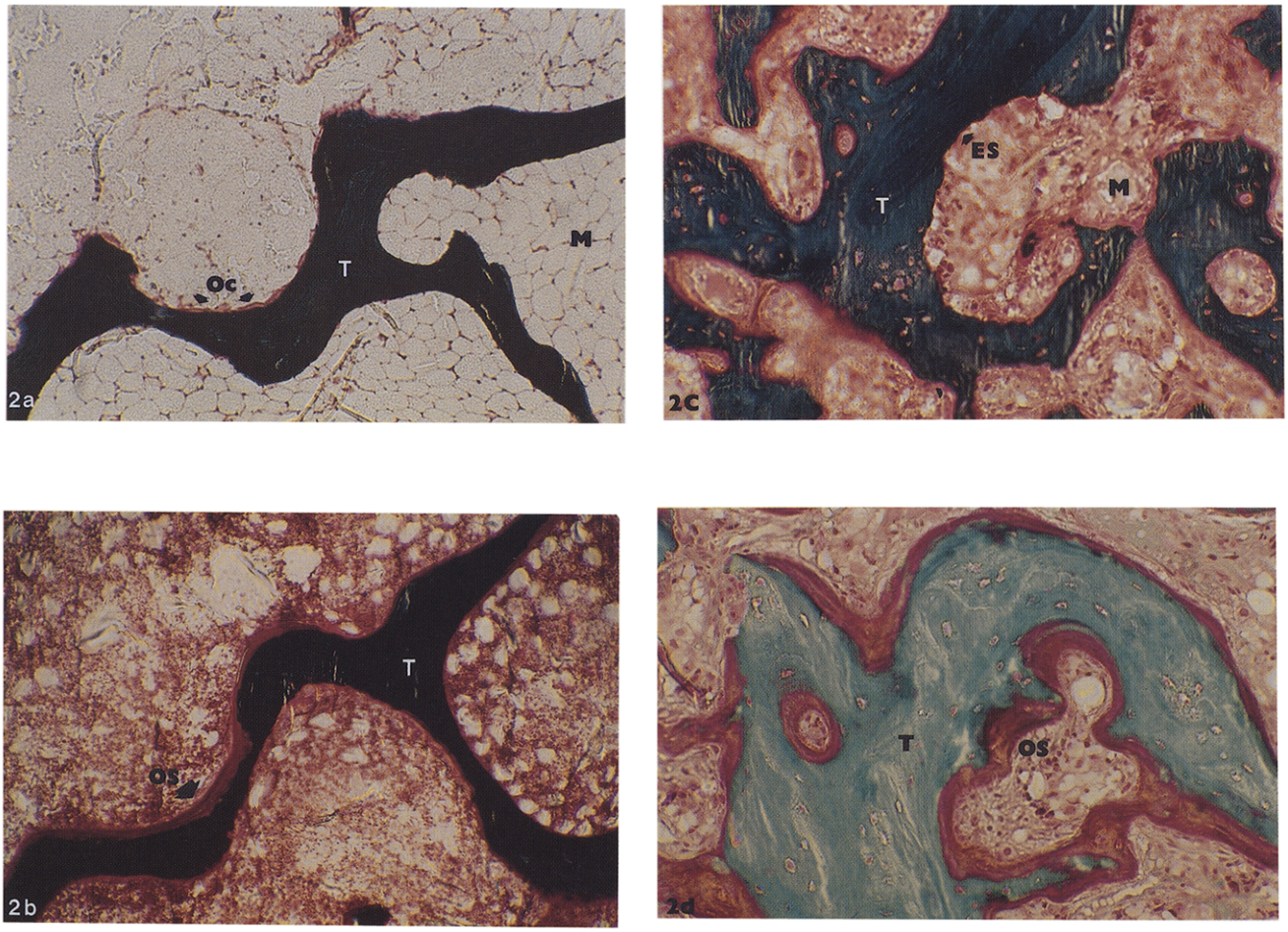

Figure 2. Photomicrographs from tumour-free and tumour-laden bone in prostate cancer. (a) Tumour-free trabecular bone (T) before treatment. Osteoclasts (OC) are gathering on the trabecular surface. Bone marrow (M) is occupied by fat cells. (b) Tumour-free trabecular bone (T) after 3 months treatment with E. Bone surfaces are mainly covered with unmineralised osteoid (OS) (Goldner's tricrome, × 100). (c) Tumour-involved trabecular bone (T) before treatment. Trabecular bars are irregular, bone surfaces are eroded (ES). Bone marrow (M) is occupied by tumour-tissue. (d) Tumour-involved trabecular bone (T) after 3 months treatment with E. Bone surfaces are covered with thick, unmineralised osteoid (OS) (Goldner's tricrome, × 200).

It has been suggested that in patients with advanced prostate cancer, bone resorption may be accelerated throughout the skeleton, since osteoclast-mediated bone resorption has been shown to be increased in biopsies taken from tumour-free bone [3–5]. We found normal bone resorption in 80% of the pretreatment biopsies from tumour-free bone. In the remainder of these biopsies, osteoclast numbers were increased. The likelihood exists, however, that these biopsies, although categorised as being tumour-free, were in reality very close to tumour tissue. Moreover, serum iPTH values were increased in less than 10% of the patients. It is, therefore, not possible to conclude that bone resorption in all these patients was increased throughout the skeleton. In contrast, the increase in osteoclastic bone resorption in biopsies from tumour-involved bone was significant, supporting the idea that the activation of osteoclasts was focal and mediated by the tumour cells [21, 22].

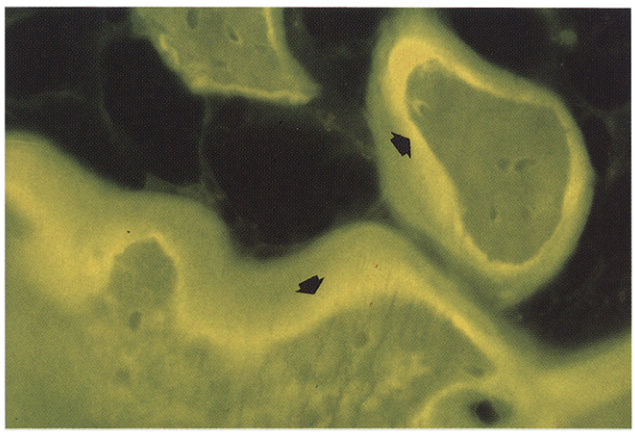

Figure 3. Tetracycline-labelled tumour-free bone after 3 months treatment with E. Double labelling is not seen. Instead, the labels (arrows) are wide and irregular as a sign of impaired mineralisation of bone, i.e. osteomalacia (ultraviolet light, × 200).

An interesting finding was that the number of osteoclasts decreased significantly in both treatment groups, indicating that the basic cancer treatment E inhibited osteoclast-mediated bone resorption. It is very likely that this effect was caused by oestradiol, since 17β-oestradiol has recently been shown to inhibit the formation of osteoclasts as well as to suppress osteoclast-mediated bone resorption in tissue cultures and in animal models, respectively, by inhibiting the production of interleukin-6 [23, 24].

The suppression of bone resorption was also indicated with a significant decrease in serum calcium levels in association with an increase in serum iPTH levels, suggesting the development of secondary hyperparathyroidism in both treatment groups.

Table 3. Biochemical data of 20 patients on estramustine + clodronate (E+C) and 19 patients on estramustine (E) during the study

Determination (reference range)	E+C Mean	(S.E.)	E Mean	(S.E.)	T	P
S-CaA (2.20–2.65 mmol/l)						
Admission	2.27	(0.03)	2.22	(0.04)	0–1	0.129
1 month	2.20*	(0.02)	2.20	(0.03)	0–3	0.194
3 months	2.15*	(0.04)	2.15†	(0.03)	1–3	0.436
S-Pi (0.80–1.40 mmol/l)						
Admission	1.15	(0.04)	1.17	(0.05)	0–1	0.456
1 month	0.82‡	(0.04)	0.87‡	(0.03)	0–3	0.760
3 months	0.91‡	(0.06)	0.88‡	(0.04)	1–3	0.631
S-ALP (60–275 U/l)						
Admission	535	(134)	746	(226)	0–1	0.496
1 month	687§	(256)	735	(225)	0–3	0.937
3 months	868	(261)	991	(370)	1–3	0.405

P values denote the significance of changes between the treatment groups at given time intervals (T; the Mann–Whitney test). Significance of differences within the groups between admission and given time points are given as follows: $*P \leq 0.003$, $†P = 0.044$, $‡P \leq 0.001$, $§P = 0.033$ (Wilcoxon's test for paired data). S-Ca, serum calcium; S-Pi, serum phosphate; S-ALP, serum alkaline phosphatase.

Treatment of Paget's disease or metastatic breast cancer with C is associated with a similar increase in serum iPTH levels as a sign of physiological adjustment for low serum calcium [25, 26]. In patients with Paget's disease, the suppression of osteoclastic activity is also followed by a subsequent decrease in the activity of osteoblasts as judged by serum alkaline phosphatase [25, 26]. In our patients, serum alkaline phosphatase increased during treatment suggesting that neither E nor C was capable of inhibiting the activity of osteoblasts through coupled bone turnover. This was not surprising, since in prostate cancer osteoblasts are directly stimulated by local factors released by the tumour cells [27]. Moreover, the basic cancer treatment with E did not seem to have an effect on tumour growth, since values of serum prostate-specific antigen remained highly increased during treatment.

Of particular interest is the histological finding that the mineralisation of newly formed collagen matrix was significantly impaired in both groups during treatment. A number of studies have reported osteomalacia associated with vitamin D deficiency or low concentrations of serum phosphate in patients with advanced prostate cancer [28, 29]. We saw a significant increase in serum 1,25-dihydroxyvitamin D_3 during treatment, which excluded the possibility that our patients would have had vitamin D deficiency associated with the syndrome of oncogenic osteomalacia. There was, however, a significant decrease in initially normal serum phosphate concentrations in both treatment groups of this study, but we do not believe that this was induced by the tumour. More likely, increased parathyroidal activity aggravated renal wasting of phosphate. Indeed, hypophosphataemia and impaired mineralisation of bone in patients with advanced prostate cancer have often been associated with oestrogen treatment, but the way in which oestrogens induce the decrease in serum phosphate has not been completely explored [30]. We believe that the mechanism is based on the suppression of osteoclast-mediated bone resorption.

We conclude that osteoclast-mediated bone resorption was increased in patients with metastatic prostate cancer. Treatments with E + C or E both decreased bone resorption. However, the

Table 4. Levels of serum intact parathyroid hormone (iPTH) and vitamin D metabolites in patients taking estramustine + clodronate (E+C) or estramustine (E) during the study

Determination (reference range)	E+C No.	Mean	(S.E.)	P^1	E No.	Mean	(S.E.)	P^2	P^3
S-iPTH (10–65 ng/l)									
Admission	17	34.7	(2.9)		15	60.4	(17.8)		
3 months	14	75.5	(8.7)	0.001	12	78.4	(20.8)	0.045	0.076
S-25-OH-D_3 (25–124 nmol/l)									
Admission	17	51.5	(6.6)		15	42.3	(3.9)		
3 months	13	69.5	(13.6)	0.003	11	53.7	(7.4)	0.563	0.325
S-1,25$(OH)_2$-D3 (50–215 pmol/l)									
Admission	16	86.8	(10.0)		15	89.5	(17.6)		
3 months	12	184.0	(20.6)	0.005	10	130.5	(20.9)	0.019	0.245

P^1 and P^2 values denote the significance of change within the treatment groups and P^3 values denote the significance of changes between the groups.

suppression of bone resorption led to the development of secondary hyperparathyroidism, which in turn aggravated renal wasting of phosphate. As a result, the patients became hypophosphaemic. Treatments with E+C and E were both associated with osteomalacia. Since the patients were not vitamin D deficient, we conclude that osteomalacia resulted from a relative deficiency of calcium and phosphate. The development of osteomalacia may be the reason why the relief of pain reported in our recent study [7] was transient, and why serum ICTP increased after the first month [7], in spite of the sustained decrease in osteoclastic activity indicated in the present study. It is likely that the increase in ICTP after the first month did not only reflect osteoclastic bone resorption, but also the breakdown of unmineralised collagen.

It is very likely that the development of osteomalacia during the treatment with E leads to insufficient binding of C to bone surfaces, which in turn, decreases the efficacy of C in these patients. It may, therefore, be prudent to avoid the use of C concomitantly with E, and the efficacy of C in the treatment of bone metastases from prostate cancer should be further investigated without simultaneous influence of oestrogens on bone turnover.

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