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Original Paper

Pyridinium Cross-links in Multiple Myeloma: Correlation with Clinical Parameters and Use for Monitoring of Intravenous Clodronate Therapy—A Pilot Study of the German Myeloma Treatment Group (GMTG)

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The relevance of quantitative determinations of urinary deoxypyridinolines (DPY) and pyridinolines (PY), and of serum type I collagen carboxyterminal cross-linked telopeptides (ICTP), has been evaluated for patient monitoring in multiple myeloma (MM). In 178 untreated MM patients, a clear correlation was found between ICTP concentrations, bone destructions and serum calcium levels. Furthermore, serum ICTP, urinary DPY and PY concentrations were estimated before and during treatment in a further 33 MM patients randomly allocated to four groups receiving intravenous melphalan/prednisone (MivP) chemotherapy alone, or MivP in combination with three different doses of i.v. clodronate. 1800 mg of i.v. clodronate combined monthly with MivP induced a rapid and sustained reduction in bone resorption parameters to the normal range, a result not obtained with either MivP alone, or with a lower clodronate dose. While confirming the relevance of determining pyridinium cross-links for estimating bone resorption in MM, our data indicate that measurements of these parameters could be useful for dose finding and monitoring of bisphosphonate therapy.

Key words: multiple myeloma, clodronate, pyridinium cross-links, bone resorption

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INTRODUCTION

THE VALUE of bisphosphonates for the treatment of malignant hypercalcaemia and osteolytic bone metastases is well documented [1]. Studies in patients with multiple myeloma (MM) using prolonged oral [2, 3], intravenous, and intramuscular bisphosphonate applications [4, 5] have indicated that progress of bone destruction and its sequelae, as well as the formation of new lytic bone lesions, can be significantly reduced. However, the doses for long-term bisphosphonate treatment have been based on tolerability rather than efficacy. Moreover, it has been difficult to distinguish the effect of bisphosphonates from that of concomitant chemotherapy which also slows down bone destruction by reducing the tumour cell mass [6, 7].

With recently developed methods for the quantitative determination of pyridinium cross-links in serum and urine, accurate, specific and sensitive parameters for estimating ongoing bone resorption have become available [8, 9]. Elevated levels of urinary deoxypyridinolines (DPY) and pyridinolines (PY), which are largely derived from collagen type I in bone matrix, have been found in malignant hypercalcaemia and metastatic bone disease [10, 11]. Another related marker for measuring increased bone resorption is the serum concentration of type I collagen carboxyterminal cross-linked telopeptides (ICTP) which can be measured by a specific radioimmune assay (RIA) [12]. ICTP serum levels have been found to be related to prognosis in MM [13].

In view of the particular importance of preserving bone stability in MM, both the dependable detection of ongoing

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bone resorption and the early biochemical proof of a successful therapeutic intervention appear highly desirable. We have correlated serum ICTP concentrations in the serum of 178 untreated patients at diagnosis to clinical parameters indicating involvement. In addition, we have monitored serum ICTP, urinary DPY and PY in 33 IIA or IIIA MM patients during first-line therapy, randomised to standard intravenous melphalan/prednisolone (MivP) treatment [1] alone, or to MivP with three different additional monthly intravenous clodronate doses.

PATIENTS AND METHODS

Patients and treatment schedules

Serum samples from 178 newly diagnosed MM patients of stage I ($n = 24$), stage II ($n = 76$), or stage III ($n = 78$) (for classification see [6]) were used for measuring pretreatment ICTP concentrations. They were selected from sera collected by the multicentre trial MM02 [14]. The criterium for selection was creatinine <1.35 mg/dl, since ICTP is regularly increased in patients with renal impairment.

33 additional patients with MM of stages IIA or IIIA with X-ray proven lytic bone lesions fulfilling GMTG criteria for commencing chemotherapy [14, 15] wer randomly assigned to four groups receiving MivP alone (melphalan 15 mg/m^2—day 1 i.v. plus prednisolone 60 mg/m^2—days 1–4 orally (group A, $n = 11$), or additional infusions of clodronate (Ostac®)—600 mg, day 1 (group B, $n = 8$); 1200 mg given in daily doses of 600 mg, days 1, 2 (group C, $n = 7$); 1800 mg given in daily doses of 600 mg, days 1, 2, 3 (group D, $n = 7$). The same treatment schedule was repeated starting on day 29, i.e. the study was continued for two full cycles until day 58. 600 mg clodronate were diluted in 500 ml 0.15 M NaCl and infused slowly over a period of 2–4 h. All patients gave written consent to participate in the study. The trial protocol was approved by the ethics committee of the Medizinische Hochschule Hannover. Exclusion criteria included impaired kidney function (serum creatinine > 2.0 mg/dl); hypercalcaemia > 3.0 mmol/l; and treatment with any bisphosphonate during the last 3 months, or with other substances known to influence bone metabolism such as mithramycin, calcitonin or thiazides.

The homogeneity of the four groups was determined on the basis of pretreatment clinical and laboratory data using Kruskal and Wallis's H-test which revealed no significant differences with respect to age, Karnofsky index, weight, anaemia, platelet counts, serum creatinine concentrations, and tumour cell mass (TCM) according to Salmon and Wampler [16], bone index, urinary DPY, PY and ICTP.

Response to chemotherapy was evaluated after two cycles of MivP measuring TCM changes. A TCM reduction of $\geqslant 25\%$ of the pretreatment value was defined as remission, an increase by $\geqslant 25\%$ was progression, and values of $\pm 25\%$ indicated a no-change result.

Laboratory methods

ICTP serum concentrations (normal range 1.8–5 µg/l) were investigated using a commercially available RIA (Telopeptide ICTP (^{125}J) Radioimmunoassay Kit, Orion Diagnostica, Pharmacia GmbH, Freiburg, Germany).

Urinary DPY (normal range 20–52 µg/g creatinine) and PY (normal range 123–268 µg/g creatinine) levels were determined by reversed phase HPLC after extraction, hydrolysis, and chromatographic purification of urine samples, the results being expressed as µg/g creatinine [17, 18]. Urine samples were regularly collected as second-morning specimens.

For all patients investigated, a staging according to GMTG criteria [1] (X-ray evaluation of the skeleton, bone marrow biopsy, etc.) was done before treatment. For the 33 patients randomised in the clodronate study an additional standard evaluation programme consisting of blood counts, serum electrolytes, phosphate, creatinine, urea, alkaline phosphatase, protein; serum electrophoresis, and urine calcium was carried out during treatment. Laboratory results are not reported in detail if no significant changes were observed during the study.

Statistical evaluation

All data were evaluated descriptively. For continuous variables, arithmetic mean and median values were calculated. Other variables were combined in frequency tables and evaluated as per cent of the respective treatment groups. Groups were compared using the Mann–Whitney *U*-test. Inference statistical investigations were performed non-parametrically using Kruskal–Wallis H-tests and multiple contrasts according to Dunn [19]. Because of the explorative character of the investigation, α-adjustment was not carried out.

RESULTS

ICTP was measured in 178 untreated MM patients (Table 1). Significantly higher ICTP values were found in stage II and in stage III patients compared with stage I. There was also a clear correlation between ICTP concentrations and bone damage as determined by conventional X-ray evaluations. Furthermore, patients with a serum calcium > 2.55 mmol/l had a significantly higher serum ICTP than those with a lower serum calcium, obviously indicating enhanced bone resorption in the first group of patients.

In 33 MM patients randomly allocated for no or three different doses of clodronate treatment, serum ICTP and urinary DPY and PY were measured before and during

Table 1. ICTP in 178 MM patients with serum creatinine < 1.35 mg/dl

Stage (D/S)	Median ICTP [µg/l] (range)	
Stage I	2.9	
$n = 24$	(0.8–6.5)	$P = 0.02$
Stage II	3.6	
$n = 76$	(0.6–29.1)	$P = 0.001$
Stage III	4.9	
$n = 78$	(0.2–34.1)	
No bone lesions	2.9	
$n = 32$	(0.6–10.0)	$P = 0.07$
Osteoporosis	3.6	
$n = 24$	(1.3–12.1)	$P = 0.03$
1–3 lesions	3.7	
$n = 31$	(1.3–26.4)	$P = 0.0001$
< 3 lesions	4.7	
$n = 91$	(0.2–34.1)	
Serum calcium $\geqslant$ 2.55 mmol/l	3.6	
$n = 142$	(0.2–34.1)	
		$P = 0.001$
Serum calcium > 2.55 mmol/l	5.5	
$n = 36$	1.2–29.4)	

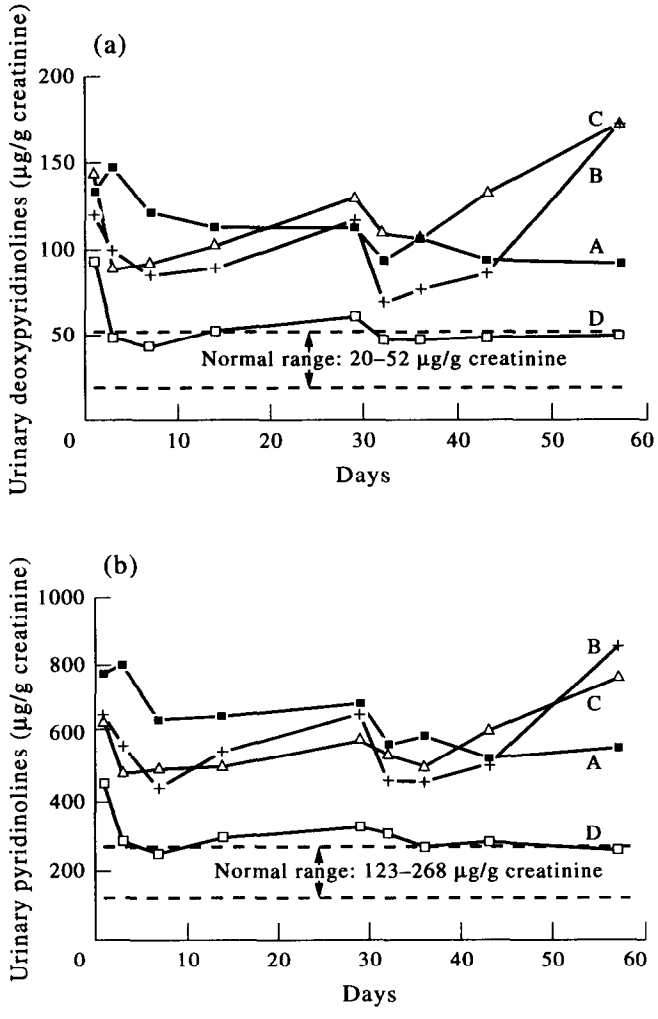

Figure 1. Urinary concentrations (µg/g creatinine) of (a) deoxypyridinolines (DPY) and (b) pyridinolines before (day 1) and during treatment cycles. Group A: MivP control group; group B: MivP plus 600 mg clodronate i.v. every 4 weeks; group C: MivP plus 1200 mg clodronate i.v. every 4 weeks; group D: MivP plus 1800 mg clodronate i.v. every 4 weeks. Only DPY concentrations of group D fell into the normal range shortly after commencing treatment.

treatment (Figures 1 and 2). Before commencing treatment, all four patient groups displayed elevated DPY, PY and serum ICTP levels indicating active bone resorption. Shortly after treatment was started, differences between the groups became apparent. DPY (Figure 1a) and PY (Figure 1b) values of group A showed a decreasing tendency, but remained above the normal range throughout the study period. Mean values for DPY and PY of both groups B and C showed a similar course, returning to the original levels towards the end of the first treatment period (day 28). After the second treatment application, a short reduction of DPY and PY concentrations was followed by a distinct though statistically non-significant increase during the last 15 days in both groups. In contrast, urinary DPY and PY concentrations of group D fell into the normal range within 3 days after commencing MivP plus clodronate treatment, and stayed there for the remaining study period, probably indicating successful interference with pathologically increased bone resorption. In particular, the differences for DPY

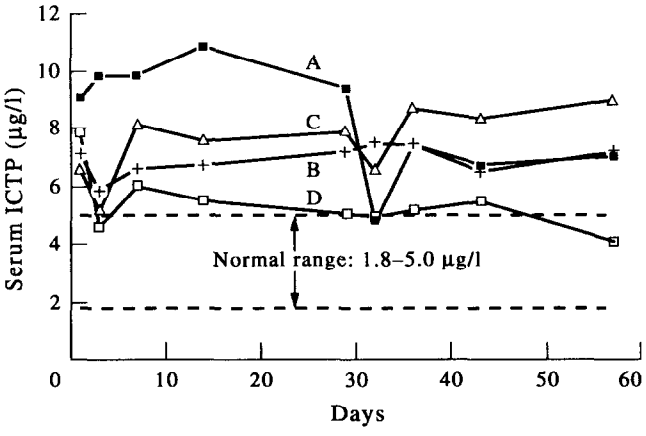

Figure 2. Serum type I collagen carboxyterminal cross-linked telopeptide (ICTP) concentrations (µg/l) before (day 1) and during treatment cycles. Group A: MivP control group; group B: MivP plus 600 mg clodronate i.v. every 4 weeks; group C: MivP plus 1200 mg clodronate every 4 weeks; group D: MivP plus 1800 mg clodronate i.v. every 4 weeks.

between groups A/B and group D were significant from day 7 on (A/D: $P = 0.03$; B/D: $P = 0.015$), whereas the differences between group C and D reached significance after day 36 (C/D: $P = 0.004$). Regarding PY concentrations, groups A, B, and C all differed significantly from group D after day 36, i.e. after the second treatment cycle (A/D: $P = 0.04$; B/D: $P = 0.02$; C/D: $P = 0.007$).

The course of ICTP serum concentrations showed a somewhat less impressive pattern (Figure 2), but again group D was the only one reaching the normal reference range towards the end of the trial. The differences between groups A, B, C and D did not reach statistical significance.

An analysis of the individual patients' data revealed different patterns of response not apparent if mean values of urinary pyridinium cross-links from patient groups were considered. Individual urinary PY data from 3 patients treated with monthly 1800 mg clodronate are illustrated in Figure 3. Patient 9 had elevated urinary PY before treatment which fell promptly and stayed in the normal range during therapy. One conclusion is that this patient received

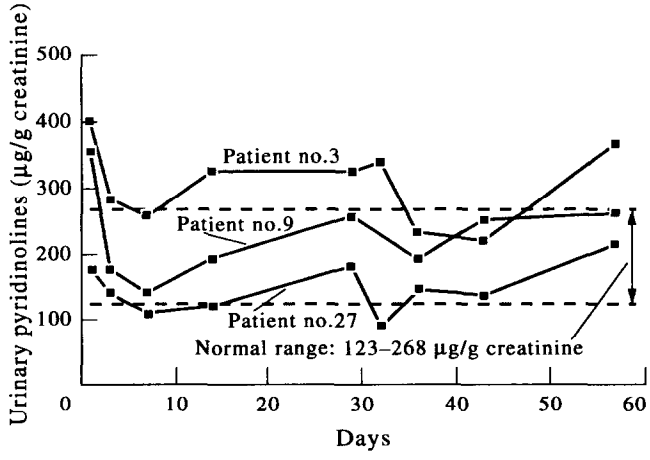

Figure 3. Concentrations (µg/g creatinine) of urinary pyridinolines (PY) before (day 1) and during two treatment cycles with MivP and 1800 mg clodronate measured in MM patients 3, 9 and 27.

Table 2. Baseline characteristics of 33 MM patients randomized for four different treatment groups (Group A: MivP control group, group B: MivP plus 600 mg clodronate i.v. every 4 w; group C: MivP plus 1200 mg clodronate i.v. every 4 w; group D: MivP plus 1800 mg clodronate i.v. every 4 w). Median values and range (in parentheses)

Group	A	B	C	D
n	11	8	7	7
Age in years (range)	65 (52–76)	65 (44–71)	64 (52–81)	62 (50–78)
DPY (μg/g creatinine)	105 (37–363)	108 (56–228)	122 (63–220)	95 (44–180)
PY (μg/g creatinine)	461 (239–2284)	580 (345–1350)	625 (274–944)	400 (175–942)
ICTP (μg/l)	5.8 (2.6–30.6)	7.0 (2.9–12.0)	6.1 (3.1–9.8)	6.0 (2.8–21.4)
No bone lesions (*n*)	0	0	0	0
Osteoporosis (*n*)	0	0	0	1
1–3 lesions (*n*)	1	2	0	1
>3 lesions (*n*)	10	6	7	5
TCM ($\times 10^{12}$ cells/m^2)	1.29 (0.83–1.62)	1.36 (1.18–1.64)	1.31 (1.20–1.56)	1.19 (0.82–1.79)
Response of TCM after two cycles of MivP (% of initial TCM)	− 16 (− 71–± 0)	− 14 (− 73–+ 13)	+ 4 (− 67–+ 26)	− 17 (− 85–+ 3)

TCM, tumour cell mass; w, weeks.

an optimal dose of clodronate. Alternatively, for patient 3, the dose of 1800 mg was obviously insufficient for maintaining normal PY concentrations. Patient 27 displayed normal urinary PY at the beginning of the treatment, so that clodronate treatment might not have been necessary at all.

Tumour response to MivP chemotherapy was not significantly different in all four patient groups (Table 2). The number of patients with remission was 3 in group A, 3 in group B, 2 in group C, and 1 in group D. A no-change result was found in 8 patients of group A, 5 patients of group B, 4 patients of group C and 6 patients of group D. In group C, 1 patient had progression at the end of the trial period. Altogether, the treatment period was too short for evaluating tumour response to chemotherapy, which indeed was not a result of the study.

DISCUSSION

Intractable bone pains and pathological fractures due to diffuse malignant osteopenia or osteolytic bone lesions, often complicated by nerve or spinal cord compression syndromes, incapacitate the majority of MM patients at the time of diagnosis. Bone destruction in MM is caused by bone marrow-infiltrating myeloma cells, producing several cytokines collectively called osteoclast activating factors [20, 21]. Increased numbers of stimulated osteoclasts are conspicuous in MM bone marrow biopsies [22, 23], and skeletal mobilisation of calcium in progressive MM has been documented [24]. In contrast to other markers, e.g. urinary hydroxyproline or γ-carboxyglutamic acid, urinary DPY and PY and serum ICTP concentrations are specific indicators for the extent of bone resorption [8, 9, 25]. The data presented in this paper show that MM patients with bone involvement display elevated DPY and PY concentrations along with serum ICTP levels well above the normal range (Table 1, Figures 1–3) before commencement of therapy. Accordingly, a significant correlation between serum ICTP on one hand and bone lesions and serum calcium on the other hand was found (Table 1). These data confirm and extend previous results [13], and indicate that pyridinium cross-links are useful markers for active bone resorption in MM.

There is evidence that currently practiced palliative chemotherapy falls short of terminating bone destruction completely and does not induce healing of lytic bone lesions. Even after successful reduction of the tumour cell mass by 1 or even 2 logs, myeloma cells in the order of 10^{10}–10^{11} remain, thus providing for continued osteoclast activation. Several studies [2–5] have pointed out that prolonged application of bisphosphonates, given along with standard chemotherapy in MM, significantly reduces bone pain, lowers the incidence of pathological fractures, and halts progression of osteolytic bone lesions. Risteli and associates [26] have shown that oral treatment of MM with a combination of melphalan/prednisone and clodronate led to a very slowly emerging fall of ICTP serum levels becoming significant after 7 months, in contrast to almost unchanged ICTP levels in the control group receiving chemotherapy alone. We demonstrate for the first time, by measuring DPY and PY excretion, that an intravenous bisphosphonate dose can be determined which may stop further bone destruction in MM within days: combined treatment with MivP and 1800 mg clodronate intravenously (group D) was followed by a prompt fall of both urinary DPY and PY concentrations into the normal range. A similarly sustained effect was neither observed with MivP treatment alone nor by MivP combined with lower clodronate doses (groups B and C). The intravenous application may play an important role since oral bisphosphonates are poorly and variably absorbed from the intestine [1].

The results of our pilot study also raise some evidence that bisphosphonate doses needed to achieve fast and sustained suppression of progressive bone destruction, may be determined individually within a short period of time by measuring the appropriate parameters of bone matrix metabolism. These parameters, estimated during bisphosphonate treatment, may thus be helpful for monitoring therapy effects and provide a rational basis for adjustment of bisphosphonate doses.

However, certain reservations have still to be made. We have studied a comparatively small number of patients, and data have been collected over a period of only 2 months, preventing the evaluation of established clinical criteria such as progress of lytic bone lesions or the incidence of pathological fractures. It may also be of interest to measure the effect of i.v. clodronate on osteoblast activity, in view of recent results indicating that inhibition of osteoclasts by bisphosphonates in osteolytic malignant bone disease should therefore, include suitable parameters for measuring both

osteoclast and osteoblast activities and also measurements of bone mineral density [28], in order to estimate more completely the effect of these drugs on bone metabolism.

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