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Effects of Zoledronic Acid on Proteinase Plasma Levels in Patients with Bone Metastases

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Abstract. *Background: The effects of the bisphosphonate derivative zoledronic acid (ZA) on the circulating levels of matrix metalloproteinase-2 (MMP-2), matrix metallo-proteinases-9 (MMP-9), cathepsin B (Cath B) and urokinase-type plasminogen activator (uPA) in patients with bone metastasis (BMTS) and the possible correlation with the symptomatic response induced by this drug in these patients were evaluated. Patients and Methods: Proteinase levels were determined by enzyme-linked immunosorbent assay (ELISA) in the plasma of 30 patients with painful bone metastases from breast or prostate cancer undergoing multiple treatment with ZA (4 mg i.v., every 4 weeks). Healthy subjects (HS) of both genders (12 female and 30 male) served as the control group. The symptomatic response to ZA was assessed by the visual analog scale score (VAS). Results: The median MMP-2 and MMP-9 pretreatment levels were more elevated in BMTS as compared to HS ($p \leq 0.0001$). Conversely, uPA levels were lower in BMTS $p=0.0033$; no significant difference was observed for Cath B. ZA administration was associated with a symptomatic response (VAS score ≤ 4) in 25/30 patients (83.3%) ($p<0.0001$). This phenomenon paralleled a decrease of Cath B and MMP-2 plasma concentrations from baseline values on week 12 ($p=0.05$). A similar trend, although not statistically significant, was also noted for MMP-9 and uPA. However, no direct relationship was observed between the analgesic effect induced by ZA and changes in the circulating levels of these enzymes. Conclusion: These data show that ZA administration may provide relief from bone pain in patients with diffuse skeletal metastases and confirm a possible implication of cysteine proteinases and matrix metalloproteinases in bone metastasis formation, but not in the pathogenesis of metastatic bone pain.*

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Zoledronic acid (ZA) is new potent inhibitor of osteoclast-mediated bone resorption, currently used for the palliative treatment of tumor-associated osteolysis and hypercalcemia (1). Interestingly, recent studies have highlighted that, in addition to its anti-osteoclastic activity, ZA may affect tumor cell growth and metastasis (1, 2). The specific mechanism(s) underlying these effects are currently under investigation (1, 2). In this context, several studies have shown that this agent may decrease the invasive potential of human breast and prostate carcinoma cells *in vitro* and that this phenomenon appears to be, at least in part, due to the inhibiting effects of this drug on the activity of some proteinases involved in bone metastasis formation, namely, matrix metalloproteinases-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) (3-5). Moreover, recent clinical investigations have revealed that the administration of ZA to cancer patients may also relieve the pain associated with bone metastases (1, 6-9). These findings suggest a possible correlation between the analgesic response to ZA treatment and inhibition of MMP-2 and MMP-9 expression levels in tumor cells (1, 5-9). To test this hypothesis, a pilot study was carried out to assess whether the administration of ZA to patients with metastatic bone disease (BMTS) alters the plasma levels of MMP-2 and MMP-9 and those of two other main proteolytic enzymes implicated in the metastatic process, namely cathepsin B (Cath B) and urokinase type plasminogen activator (uPA) (10, 11), and whether these alterations correlate with the analgesic response to ZA .

Patients and Methods

The study were carried out on 30 consecutive patients with diffuse skeletal metastases from breast or prostate cancer (Table I), undergoing multiple treatment with zoledronic acid (Zometa® Novartis, Basel, Switzerland, 4 mg *i.v.* by a 20-min infusion every

Table I. *Patients' characteristics (n=30).*

Median age (range)	66.6 years (range 48-83)
Female/male	13/17
Median performance status ECOG score (range)	1 (0-2)
Tumor type:	
Breast carcinoma	13
Prostate carcinoma	17
Metastasis:	
bone only	18
bone + visceral	12

4 weeks). The patients were enrolled between January 2002 and September 2004. The elegibility criteria included scintigraphic and radiographic confirmation of bone metastasis, normal hepatic and renal function, no chemotherapy for at least one month before ZA treatment and pain not controlled by analgesic drugs (either FANS or opioids). Patients receiving endocrine therapy were included if under treatment for at least 3 months. Exclusion criteria were: i) chemotherapy or radiation therapy within the previous 3 months, ii) any previous bisphosphonate treatment, iii) the presence of severe cardiovascular disease, refractory hypertension or symptomatic coronary artery disease, iv) serum creatinine >3.0 mg/dL, v) serum calcium >8.0 mg/dL. All patients provided written informed consent to the study, which was approved by the local ethical committee and carried out in accordance with the Declaration of Helsinki (12). The baseline evaluation consisted of bone scan, skeletal X-ray, serum chemistry, complete blood cell count, evaluation of serum tumor markers Ca15.3 and prostate specific antigen (PSA) and that of some markers of bone metabolism, namely bone alkaline phosphatase, c-telopeptide and parathyroid hormone. The symptomatic response to ZA treatment was assessed according to Serlin *et al.* (13) by using a visual analog scale score (VAS), which ranged from 0 (no pain at all) to 10 (unbearable pain). A VAS score ≤4 was considered as an acceptable analgesic response.

Cath B, MMP2, MMP9 and uPA plasma levels. Venous blood was drawn into ethylenediammine tetra-acetic acid (EDTA)-coated tubes just prior to ZA administration on day 0, week 4 and week 12. The blood samples were centrifuged at 3,500 rpm for 15 min (Hereus Omnifuge 2.0 RS, Hereus Sepatech). The plasma aliquots were stored at –80˚C until assays. Cath B, MMP-2, MMP-9 and uPA concentrations were determined by commercially available enzyme-linked immunosorbent assay (ELISAC) kits according to the manufacturers' instructions (Cath B ELISA kit, KrKa, Novo Mesto, Slovenia; Biotrak MMP-2 and MMP-9 ELISA kits, Amersham Bioscience, UK; IMUBIND uPA ELISA kit, product no. 894, American Diagnostic, USA). The reported detection limits were 0.9 ng/ml for CB, 0.37 ng/ml for MMP-2, 0.6 ng/ml for MMP-9 and 10 pg/ml for uPA, respectively. Forty-two registered healthy blood donors (HS) (30 male and 12 female, aged 19-62 years) were used as the control group.

Statistical analysis. The statistical analysis was performed with the Medcalc statistical software package version 7.4 (14). The data were tested for normal distribution by the Kolmogorov-Smirnov test. Because of their uneven distribution, statistical analysis was performed, where required, by the non-parametric Mann-Withney *U*-test, the Wilcoxon signed rank test or the Spearman rank correlation test. The relationship between symptomatic response to ZA treatments (VAS score trend) and the time-course variation of proteinase plasma concentrations was evaluated by linear regression analysis. The 0.05 level of probability was taken as significant.

Results

The plasma distribution of MMP-2, MMP-9, Cath B and uPA in healthy subjects (HS) and in patients with metastatic bone disease (BMTS) is shown in Table II. No gender-related differences were observed in the levels of any of these proteolytic enzymes in HS (data not shown). The median MMP-2 and MMP-9 concentrations prior to ZA treatment were 2- to 4-fold higher in BMTS as compared to HS ($p<0.0001$ and $p=0.0001$, respectively) (Table II). Conversely, the Cath B and uPA concentrations were 1.5- to 2.0-fold lower in BMTS (Table II). However, this phenomenon was statistically significant only in the case of uPA ($p=0.0033$) (Table II). No substantial difference was noted in the plasma level of these enzymes between patients with bone metastases from breast cancer and those with bone metastases from prostate cancer or between patients with metastases confined only to the bones and those with additional visceral metastases (data not shown). Among the parameters considered, a positive correlation was highlighted only between Cath B and MMP-9 basal values ($r=0.556$, $p=0.019$). The administration of ZA was associated with a significant symptomatic response in 25/30 patients (83.3%), while no analgesic effect was seen in the other 5 patients (16.7%). Furthermore, ZA administration induced a significant decrease of Cath B and MMP-2 concentrations from baseline values on week 12 ($p=0.05$) (Table III). A similar phenomenon, although not statistically significant, was also noted for MMP-9 and uPA (Table III). However, significant fluctuations in uPA plasma levels were observed between week 4 and week 12 ($p=0.049$) (Table III). Finally, linear regression analysis did not show any significant correlation between the time-course alteration of proteinase levels and symptomatic response to ZA treatment (Cath B:$r=0.22$, $p=0.31$; uPA:$r= 0.23$, $p= 0.85$; MMP-2: $r= 0.06$, $p=0.95$; MMP-9: $r = 0.97$, $p= 0.13$)

Discussion

The results of this pilot study indicated that patients with diffuse skeletal metastases present a different pattern of expression levels of some proteinases involved in tumor progression. These findings further support the concept of a

Table II. *Proteinase circulating levels in healthy subjects (HS) and in patients with bone metastases (BMTS).*

	HS (n=42) Median (Range)	BMTS (n=30) Median (Range)	% HS	*p* value[a]
Cath B (ng/ml)	4.2 (0-26.8)	2.5 (0-19.2)	–40.5%	0.28 (n.s.)
uPA (ng/ml)	1.09 (0.2-4.8)	0.43 (0.026-2.28)	–60.6%	0.0033
MMP-2 (μg/ml)	0.75 (0.36-1.2)	1.46 (0.77-2.72)	+194.6%	<0.0001
MMP-9 (ng/ml)	29.4 (10.2-189.1)	101.7 (17.3-796.0)	+345.9%	0.0001

[a]Mann-Whitney *U*-test; n.s.= not significant

Table III. *Time-course variation of VAS score and proteinase circulating levels in patients with metastatic bone disease treated with zoledronic acid (4 mg i.v. by a 20-min infusion every 4 weeks).*

	VAS score	Cath B (ng/ml)	MMP-2 (μg/ml)	MMP-9 (ng/ml)	uPA (ng/ml)
Baseline	7.0 (9.0-3.0) n=30[a]	2.5 (0-19.2) n=30	1.46 (0.77-2.72) n=30	101.7 (17.3-796.0) n=30	0.43 (0.026-2.28) n=22
Week 4	4.0 (8.0-2.0)* n=30	1.6 (0-21.3) n=22	1.50 (1.03-2.78)§ n=30	59.05 (4.7-535) n=30	0.60 (0-4.1) n=22
Week 12	3.0 (7.0-2.0)*$^{\#}$ n=21[a]	0 (0-5.6)$^{\bullet\dagger}$ n=13	1.29 (1.12-2.06)$^{\circ}$ n=10	43.5 (1.9-232.7) n=10	0.31 (0-0.96)$^{+}$ n=11

Data are expressed as median value and (range); Statistical analysis was computed by the Wilcoxon signed rank test.
[a]Due to some missing samples and/or patient follow-up, the number of evaluable subjects at each time-point may vary.
VAS $^{*}p<0.0001$ *vs.* baseline and $^{\#}p=0.012$ *vs.* week 4; Cath B $^{\dagger}p=0.048$ *vs.* baseline and $^{\bullet}p=0.016$ *vs.* week 4; MMP-2 $^{\S}p=0.037$ and $^{\circ}p=0.05$ *vs.* baseline; uPA $^{+}p=0.049$ *vs.* week 4.

possible involvement of these enzymes in different steps of bone metastasis formation (15). Thus, the lower levels of uPA and Cath B in the plasma of patients with advanced metastatic bone disease prior to ZA administration suggest that these enzymes may be actively secreted in the early phases of metastasis formation thereby facilitating the initial steps of this phenomenon, such as the activation of growth factors and that of the proteolytic cascade which triggers off the metastatic process (15). Instead, the elevated levels of MMP-2 and MMP-9 suggest that these proteinases may be preferentially more secreted in subsequent steps and promote later key events of bone metastasis formation, including tumor cell-induced osteolysis (5, 8, 15-20). This may also, partially, explain the positive correlation highlighted between the baseline levels of Cath B and MMP-9.

The administration of ZA to patients with bone metastases was associated with a significant symptomatic response which lasted throughout the observation period. These findings further confirm our previous observations and those of other studies (1, 6, 7-9). Interestingly, the analgesic response to ZA treatment paralleled significant changes in the plasma concentrations of Cath B and MMP-2 from the baseline values. A similar trend, although not statistically significant, was also observed for MMP-9 and uPA. However, these alterations do not seem to be due to a direct growth inhibitory activity of this agent on metastatic cells. This hypothesis is corroborated by the lack of clinical evidence of antitumor response to ZA treatment in these patients during the time-course of the study: evident. On the other hand, proof of the direct antiproliferative effects of ZA *in vivo* on bone metastatic cells in humans has still not been provided. Because matrix metalloproteinases and cysteine proteinases are actively secreted by osteoclasts during normal and malignant remodeling processes of bone tissue (1, 5, 8, 10, 16-24), it is probable that their altered levels may be the result of the inhibiting effects of ZA on osteoclastic activity. Therefore, the evaluation of the circulating levels of these proteinases may be useful as additional biochemical markers to monitor the clinical response of patients with bone metastases treated with bisphosphonates. Nevertheless,

the lack of a significant relationship between the analgesic response and alteration of the proteinase plasma concentrations following ZA administration seems to rule out a direct involvement of these enzymes in the pathogenesis of pain associated with skeletal metastasis.

In summary, our data further demonstrate that multiple ZA administrations may induce a significant symptomatic response in patients with metastatic bone disease and confirm a possible implication of cysteine proteinases and matrix-metalloproteinases in bone metastasis formation, but not in the pathogenesis of metastatic bone pain.

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