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Changes in bone resorption and vascular endothelial growth factor after a single zoledronic acid infusion in cancer patients with bone metastases from solid tumours

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Purpose

Zoledronic acid (Zometa) is increasingly used to treat tumour-induced bone disease, and is also reported to have anti-angiogenic properties *in vivo*.[1,2] In this study we have investigated the correlations between changes in the pro-angiogenic cytokine VEGF and markers of bone resorption in a cohort of patients with metastatic bone disease, following a single infusion of zoledronic acid.

Experimental design

Twenty four consecutive selected cancer patients with scintigraphic and radiographic evidence of bone metastases were treated for the first time with a single infusion of 4 mg zoledronic acid. Patients were considered ineligible if they had received any steroid therapy, radiotherapy, chemotherapy, immunotherapy or haemopoietic growth factors during the study period and during the last 4 weeks before entry into the study. Circulating levels of vascular endothelial growth factor (VEGF) and β crosslinked Type I collagen C-telopeptide (βCTX) were measured at baseline and at 1, 2, 7 and 21 days following zoledronic acid infusion.

Results: The majority of our patients (23/24) developed a reduction in circulating levels of βCTX decreased significantly just one day after the single zoledronic acid infusion, median percentage change 67.05% (95% CI 52.39%; 76.27%). This reduction persisted at all following time points in almost all our patient population (day two: 95.8%; day seven: 100%; day twenty one: 91.7%). Median change at day 2 was 85.67% (95% CI 78.23%; 90.16%), at day 7: 67.38% (95% CI 67.38%; 86.98), and at day 21: 76.89% [95% CI: 35.00%; 83.16%]). All these reductions were reflected by a reduction of median circulating VEGF and βCTX circulating levels as reported by Figure1. Moreover, a linear regression model with variance analysis demonstrated a statically significant correlation between median VEGF and βCTX circulating levels at each of the time-points (1, 2, 7 and 21 days after zoledronic acid infusion), as demonstrated by Figure 2.

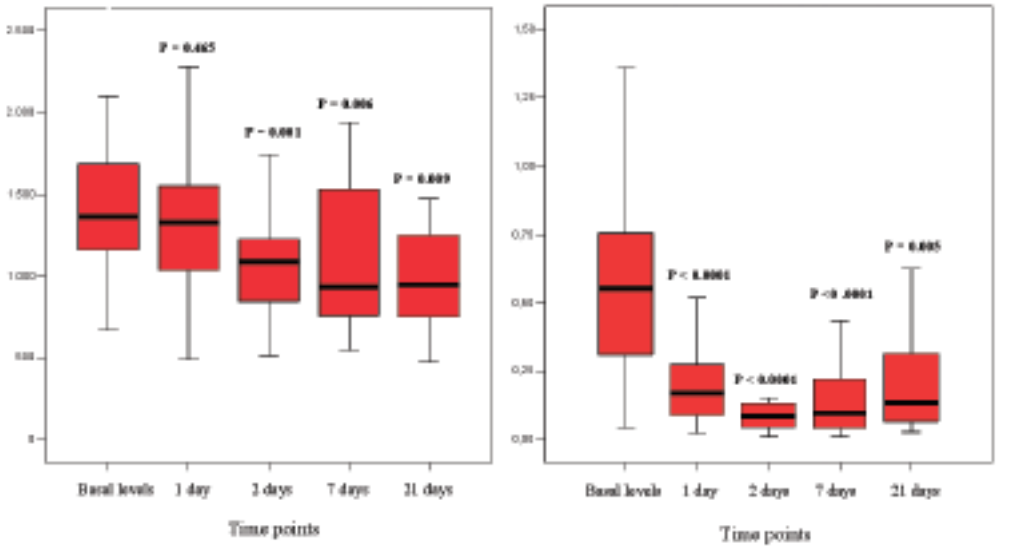

Figure 1. bCTX and VEGF levels at days 1, 2, 7 and 21 after Zoledronic Acid administration. Red boxes represent 95 percentiles. Horizontal black bar in the red boxes represent median value. Bottom and top horizontal bars indicate minimum and maximum values. P values are calculated according to Wilcoxon test for non parametric dependent continuous variable.

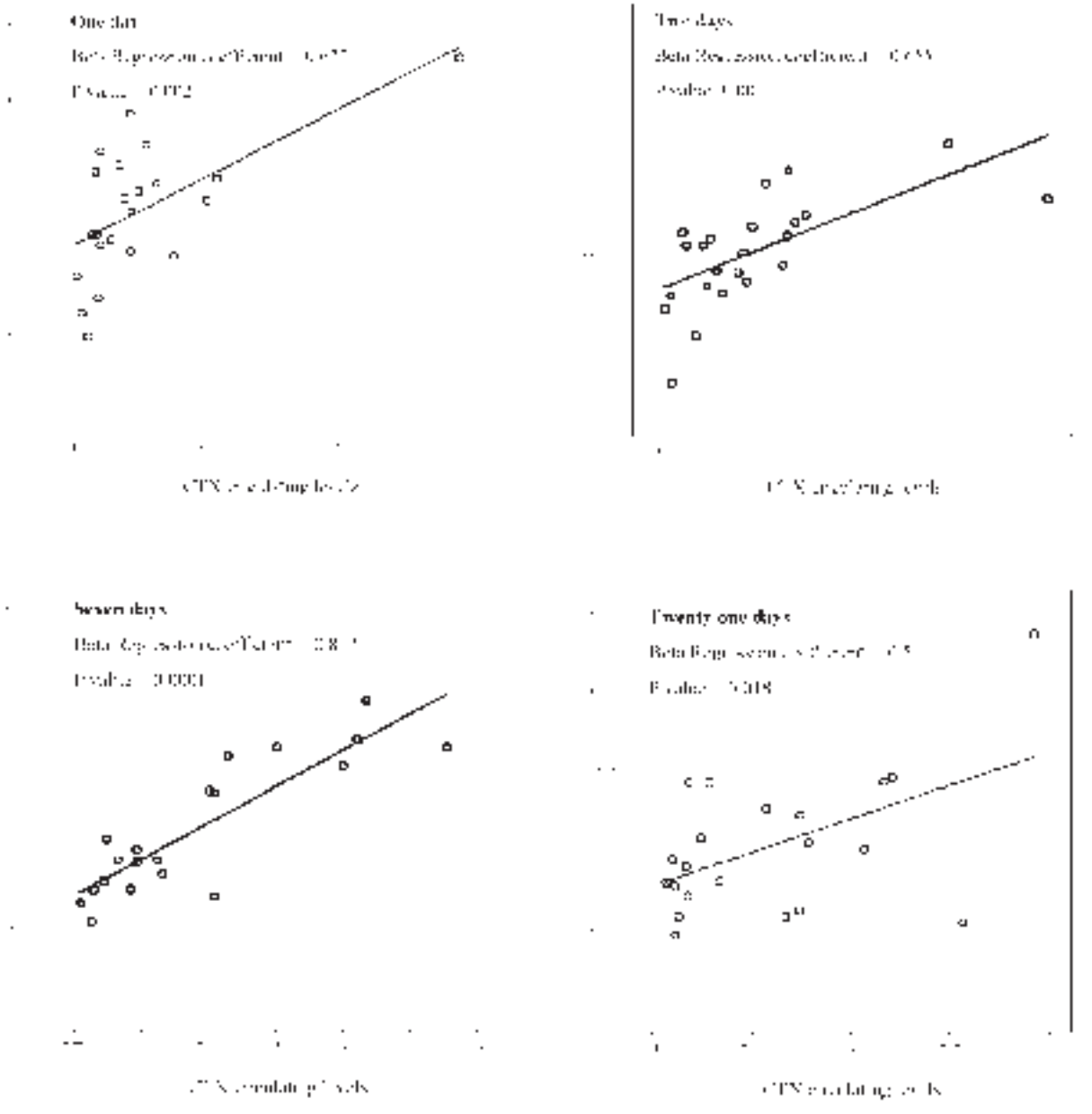

Figure 2. The figure shows the correlations between median VEGF and bCTX levels 1, 2, 7 and 21 days after Zoledronic Acid administration. A statically significant correlation between median VEGF and bCTX circulating levels was found at each of the evaluated time-points.

Conclusions

The present work demonstrates that a single infusion of zoledronic acid was able to induce a rapid and long lasting decrease of βCTX plasma levels in the majority (23/24) of the included cancer patients. Furthermore, we found that there is a correlation between the levels of VEGF and βCTX following zoledronic acid treatment. Future clinical trials should be designed to prospectively evaluate the prognostic role of reduction of βCTX and VEGF in response to zoledronic acid to predict clinical and skeletal outcome.

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Antiangiogenic activity of zoledronic acid: inhibition of the VEGF-VEGFR-2 autocrine loop in the endothelial cells of patients with multiple myeloma

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Blood vessels are an important component of bone marrow microenvironment in multiple myeloma (MM). Their formation (angiogenesis) parallels the transition from monoclonal gammopathy unassociated/unattributable (MG[u]) to MM, or from remission MM to relapse and the leukemic phase.[1,2] The new vessels convey oxygen and metabolites, while endothelial cells (EC) at their tips secrete growth and invasive factors for plasma cells.[3]

The mechanisms that induce formation and sprouting of new vessels, however, are not well established yet. Plasma cells are seen as primary inducers because they secrete major angiogenic factors, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and hepatocyte growth factor (HGF),[4-6] and their growth precedes the sprouting.[7] Stromal cells behave as secondary inducers following recruitment and activation by plasma cells. EC are themselves a vivid source of growth factors too.[8-12]

We have previously shown that bone marrow EC from patients with MM (MMEC) display a growth advantage over healthy human umbilical vein endothelial cells (HUVEC):[3] they secrete about 40 times larger amounts of VEGF into their culture medium, and express about 5 times higher levels of the cognate tyrosine kinase receptor VEGFR-2 (or kinase insert domain-containing gene KDR) suggesting the existence of a VEGF-dependent autocrine loop.

We have also shown the operativeness of this loop:[13] it mediates proliferation and capillarogenesis which are mandatory for the MM-associated angiogenesis. This loop exists in MMEC, but not MG(u)EC or HUVEC, and provides an amplification mechanism for the VEGF-driven angiogenesis in MM. Overall data support the view that efficacious antiangiogenesis could be achieved through VEGF-VEGFR-2 inhibition.

Zoledronic acid (ZA) is a bisphosphonate efficaciously used in MM for metastatic bone disease and hypercalcemia. Recent evidences indicate that it has a direct cytotoxic activity on tumor cells and suppresses angiogenesis,[14,15] but the associated molecular events have not been fully characterized. ZA inhibits the FCS-induced proliferation of HUVEC in a dose dependent manner (range 1-30 μM) and their capillary-like tubule formation on Matrigel *in vitro* (100 μM). Here, we have studied the antiangiogenic activity of ZA in MMEC of patients at diagnosis and compared it to that exerted on EA.hy926, used as control EC. We wondered to test the hypothesis that ZA directly targets the VEGF-dependent autocrine loop in MMEC.

MMEC from 8 patients were exposed on days 0, 2, 4, and 6 to both complete medium (10% fetal calf serum – FCS) and 1.5% FCS alone (positive controls) or added with ZA at different doses (1, 3, 10, 30, 50 μM), or to starvation serum-free medium (SFM - negative control). The EC proliferation rate was measured on day 8 by a colorimetric method.

ZA significantly inhibited proliferation at 3, 10, and 30 μM in a dose-dependent fashion: -25%, -45% and -52% of the positive control ($p<0.02$; Wilcoxon rank test), whereas 50 μM gave a plateau. ZA inhibited MMEC migration in a chemotaxis assay at 10 and 30 μM: -45%, and -61% of the positive control respectively ($p<0.01$; Wilcoxon rank test). The effect of ZA on capillarogenesis on the Matrigel surface was also investigated. After an 8-h incubation, unexposed MMEC gave a closely knit network whose filled areas were 27.5 ± 5.1, length 6314±708 μm, and branching points 48±6. In contrast, MMEC exposed to ZA 30 μM were less organized, and showed a lowering of all planimetric parameters, ranging from –38% to -58%. More evident inhibition was seen on mesh areas and vessel length (10±3 and 3110±290 μm respectively; $p<0.01$ or better; Student t-test for paired data).

By using RT-PCR and Real-Time RT-PCR we found that ZA down-regulates VEGF and VEGFR-2 expression in MMEC and EA.hy926, with maximum inhibition at 30

μM (-27% and –42%; $p< 0.05$ or better). The effect on MMEC seems to be specific addressed towards the VEGF- VEGFR-2 loop, since ZA does not produce any effect on the expression of both bFGF and its receptors FGFR-1/-2/-3/-4, and of HGF compared to EA.hy926, where it induces down-regulation of bFGF and HGF (-60% and –25%; $p<0.01$, respectively). Overall data provide a rationale for clinical employment of ZA in the antiangiogenic therapy of MM.

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