Multiple Myeloma

The effect of zoledronic acid on serum osteoprotegerin in early stage multiple myeloma

We evaluated the effect of zoledronic acid (ZA) on serum levels of osteoprotegerin (OPG) and the ligand for receptor activator of nuclear factor κB (RANKL) in patients with smoldering myeloma. In treated subjects we found an increase of OPG accounting for an effect of ZA on osteoblast and/or bone marrow stromal cells together with the direct effect on osteoclasts.

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Multiple myeloma (MM) is characterized by the presence of lytic bone lesions, reflecting an increase in osteoclastic bone resorption.[1] The ligand for receptor activator of nuclear factor-κB (RANKL) has been identified and associated with the increase in osteoclast-induced bone resorption in MM. By contrast, osteoprotegerin (OPG) acts as a soluble neutralizing receptor for RANKL and inhibits osteoclast formation.[2-3] Several data have demonstrated that myeloma cells are able to induce an imbalance in the OPG/RANKL system in bone environment.[4] Bisphosphonates are potent inhibitors of osteoclast activity and bone resorption. However, it has been recently demonstrated that bisphosphonate treatment can increase OPG production both *in vitro* from primary human osteoblasts and *in vivo* in patients with Paget's disease of bone.[5-6] A recent study in 43 MM patients with stage IA diseases showed that prophylactic administration of the bisphosphonate pamidronate does not improve overall progression-free survival but could limit the development of bone disease, expressed as skeletal related events (SRE), at progression.[7]

The aims of our study were to evaluate whether the inhibition of osteoclast activity in smoldering MM, obtained with the potent aminobisphosphonate zoledronic acid (ZA) without other pharmacological interference, can be related to changes in circulating levels of RANKL and OPG.

Twenty-six consecutive patients (14 males/12 females; median age 71.4±7.8, range 49-82 years) with smoldering MM (14 IgG/κ, 2 IgG/λ, 8 IgA/κ, 2 IgA/λ) were recruited between October 2004 and March 2005 from the Department of Hematology of the University of Siena. Fifty-eight healthy age- and sex-matched subjects were used as controls. After baseline evaluation, the patients with smoldering MM were randomly assigned to receive no treatment (10 patients) or ZA (16 patients), 4 mg administered as a 15 min infusion at baseline and at months 1, 2, 4 and 6.

Basal biochemical and densitometric data of the patients and controls are shown in Table 1. Basal OPG levels were significantly lower in patients with smoldering MM than in controls. An increase of the RANKL/OPG ratio, an expression of osteoclast activity, was also observed. Biochemical variations at different times in treated and untreated patients are shown in Table 2. As expected, an early and significant reduction of serum C-terminal telopeptides (CTX) was found in treated patients, while bone alkaline phosphatase decreased later but not significantly.[8] The significant correlation between RANKL/OPG ratio and serum of type-1 collagen (CTX) (r=0.45; $p<0.05$) confirmed the influence of the RANKL/OPG system on osteoclast activity. Remarkably, ZA caused a progressive and significant increase of serum OPG while serum RANKL resulted unchanged. The decrease of the RANKL/OPG ratio in treated patients was not significant. The positive effect of ZA on bone tissue was confirmed by an increased bone mineral density of the lumbar spine at 6 months (+3.8%±2.6 vs baseline; $p<0.05$). Moreover, we did not observe significant differences in monoclonal protein variations in treated or untreated patients and no disease progression.

Table 1. Biochemical characteristics of patients at baseline and controls.

	Smoldering myeloma n=26	*Controls n=58*	*Significance*
Calcium (mg/dL)	9.2±0.4	9.3±0.5	ns
Phosphorus (mg/dL)	3.3±0.5	4.0±0.7	$p<0.01$
Creatinine (mg/dL)	1.0±0.2	0.8±0.3	$p<0.01$
UCalcium (mg/24h)	140±60	135±58	ns
Bone ALP (U/L)	10.9±4	12.1±5	ns
CTX (ng/mL)	0.46±0.20	0.39 ± 0.17	ns
MIP-1α (pg/mL)	25.3±13	22.5±10	ns
OPG (pmol/L)	2.8±1.7	9.2±6.1	$p<0.01$
RANKL (pmol/L)	0.27±0.18	0.28±0.23	ns
RANKL:OPG	0.20±0.39	0.06±0.11	$p<0.05$
LSpineBMD (g/cm^2)	1.07±0.24	0.862±0.16	$p<0.01$
T-score LSpine	-0.26±1.95	-2.21±1.44	$p< 0.01$
FNeck BMD (g/cm^2)	0.84±0.16	0.724±0.09	$p<0.01$
T-score Fneck	-1.29±1.09	-1.93±0.82	$p<0.01$
THip BMD (g/cm^2)	0.93±0.16	0.829±0.10	$p<0.01$
T-score THip	-0.87±1.13	-1.45±0.80	$p<0.01$

UCalcium: urinary calcium; Bone ALP: bone alkaline phosphatase; CTX: C-terminal telopeptides of Type-I collagen; MIP: macrophage inflammatory protein; OPG: osteoprotegerin; BMD: bone mineral density; LSpine: lumbar spine; FNeck: femoral neck; THip: total hip. T-score is the difference in standard deviation from the mean value in healthy young people.

In this study ZA administration was found to modify the biochemical abnormalities occurring in smoldering MM, characterized at baseline by low levels of serum OPG and increased RANKL/OPG ratio. To our knowledge, this is the first time that an *in vivo* increase of serum OPG after ZA administration in MM has been demonstrated. Our data are in keeping with *in vitro* observations showing that ZA both stimulates OPG production and inhibits RANKL as well as myeloma cell growth.[5] These data could be the biochemical explanation for the potential benefit of ZA treatment in early stage asymptomatic myeloma patients.

In fact the reduction of SRE in patients with smoldering MM treated with pamidronate in the study by Musto *et al.* could potentially be explained by these changes in RANKL/OPG balance.[9] There are questions about whether the increase in OPG is correlated with disease progression. RANKL/OPG ratio showed a not significant decrease after the first month from the beginning of therapy, as did serum RANKL. There are, however, major concerns about the interpretation of serum RANKL levels. In fact RANKL is produced by both osteoblasts and T-cells and these cells can express a cell-specific membrane-bound RANKL that is cleaved by metalloproteinase in a soluble form.

There may be some functional differences between membrane-bound and soluble RANKL, with the cell-bound form being more effective mediators of osteoclastogenesis when measured by *in vitro* assays. Available

Table 2. Biochemical variations at different times iin patients treated or not treated with zoledronic acid.

	Baseline		*1 Months*		*3 Months*		*6 Months*	
	Treated n=16	*Untreated n=10*	*Treated n=16*	*Untreated n=10*	*Treated n=16*	*Untreated n=10*	*Treated n=16*	*Untreated n=10*
Calcium (mg/dL)	9,2±0,4	9,0±0,2	9,0±0,8	8,9±0,3	8,9±0,7	9,0±0,4	9,0±0,7	9,0±0,4
UCalcium (mg/24h)	137±77	157±78	99±67	135±41	112±76	157±75	138±74	161±96
Creatinine (mg/dL)	1,00±0,2	1,02±0,1	0,9±02	0,92±0,1	0,9±02	0,9±0,2	0,9±0,2	0,9±0,1
Bone ALP (U/L)	11,3±3,3	10,7±2,3	11,4±4,2	10,5±2,6	9,6±3,4	9,8±2,5	8,6±2,5	10,2±2,5
CTX (ng/m)	0,47±0,22*	0,38±0,1	0,22±0,07	0,48±0,12§	0,29±0,18	0,48±0,13§	0,26±0,12	0,52±0,12§
MPI-1α (pg/mL)	25,3±1,5	25,4±0,8	25,2±0,8	25,6±0,8	25,3±0,7	25,0±0,6	26,9±1,1	26,2±1,4
OPG (pmol/L)	2,55±1,8*	3,0±1,2	4,0±2,4	3,0±1,1	4,8±2,6	2,9±0,8§	5,4±2,6	3,0±0,4§
RANKL (pmol/L)	0,26±0,19	0,28±0,14	0,27±0,17	0,30±0,13	0,25±0,16	0,28±0,12	0,28±0,16	0,32±0,11
RANKL/OPG	0,23±0,44	0,14±0,22	0,09±0,07	0,13±0,1	0,07±0,05	0,11±0,11	0,08±0,10	0,11±0,09

**p<0.05 treatment effect (repeated measures ANOVA); §p<0.05 untreated vs. treated (T-test for independent data). UCalcium: urinary calcium; Bone ALP: bone alkaline phosphatase; CTX: C-terminal telopeptides of type-I collagen; MIP-1α: macrophage inflammatory protein-1α; OPG: osteoprotegerin.*

ELISA assays measure only the soluble form and this could explain the lack of statistical significance of our results. In conclusion, the most important finding of this study is that the administration of ZA without other pharmacological treatment is able to increase the serum level of OPG in patients with smoldering MM.

Giuseppe Martini, Alessandro Gozzetti,° Luigi Gennari,* Annalisa Avanzati,* Ranuccio Nuti,* Francesco Lauria°*

**Department of Internal Medicine, University of Siena, Siena, Italy; °Division of Hematology and Transplants, University of Siena, Siena, Italy*

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Correspondence: Giuseppe Martini, Policlinico "Santa Maria Le Scotte" 53100 Siena, Italy. Phone: international +39.057.7586453. E-mail: martinig@unisi.it

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