Myeloma-Induced Osteocyte Death Was Blunted By Proteasome Inhibitors Through The Modulation Of Autophagy

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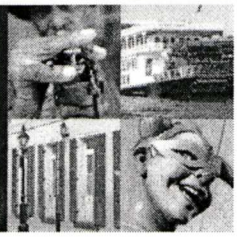
ABSTRACTS, PROGRAM, & PERSONAL SCHEDULER



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3096 Myeloma-Induced Osteocyte Death Was Blunted By Proteasome Inhibitors Through The Modulation Of Autophagy

Program: Oral and Poster Abstracts
Session: 651. Myeloma: Biology and Pathophysiology, excluding Therapy: Poster II

Sunday, December 8, 2013, 6:30 PM-8:30 PM
Hall G (Ernest N. Morial Convention Center)

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Osteocytes are critical in the maintenance of bone integrity regulating bone remodeling through the cell death and autophagy, a cellular process stress-induced to prolong cell survival but when induced excessively can cause cell death. Recently we have demonstrated that an increased osteocyte death is involved in multiple myeloma (MM)-induced osteolysis. However the mechanisms involved in this process as well as the effect of the proteasome inhibitors able to stimulate bone formation are not known and have been investigated in this study.

Firstly the effect of the proteasome inhibitors BOR and MG262 on osteocyte viability was evaluated *in vitro* in murine osteocytic cell line MLO-Y4 and in the human pre-osteocytic one HOB-01. Both cell lines were co-coltured for 48 hours in the presence or absence of the human myeloma cell lines (HMCLs) RPMI8226 and JJN3, placed in a traswell insert. The treatment for 12-24 hours with (BOR) (2nM) and MG262 (10nM) significantly blunted MLO-Y4 and HOB-01 cell death. In addition, dexamethasone (DEX)-induced MLO-Y4 apoptosis, obtained at high doses (10^{-5}-10^{-6} M), was reduced by the treatment with proteasome inhibitors. Interestingly, we found that PTH short-term treatment potentiated the *in vitro* effects of proteasome inhibitors on DEX-induced osteocyte death. To evaluate the presence of autophagy in osteocytes, we checked the expression of the autophagic marker LC3 both by confocal microscopy and western blot analysis in the co-colture system with MLO-Y4 and RPMI-8226. Prevalence of autophagic cell death and in a lesser extent apoptosis was observed in this system. BOR increased the basal level of LC3 indicating a pro-survival and protective function of autophagy against the BOR-induce stress. On the contrary, when cells undergo to a stronger stress such as in the presence of HMCLs or by treatment with high dose of DEX we found that both proteasome inhibitors BOR and MG262 blocked autophagic cell death in osteocytes.

To translate our *in vitro* evidence in a clinical perspective, thereafter we performed a histological evaluation on bone biopsies of a cohort of 37 newly diagnosis MM patients 31 of them with symptomatic MM and 6 with smoldering MM (SMM). The 55% of patients with MM have evidence of osteolytic lesions at the X-rays survey. Bone biopsies were obtained at the diagnosis and after an average time of 12 months of treatment or observation. Osteocyte viability was evaluated in a total of 500 *lacunae per* histological sections. A significant increase of the number of viable osteocytes was demonstrated in MM patients treated with BOR-based regimen as compared to those treated without BOR (% median increase: +6% *vs.* +1.30%; *p=0.017*). Patients treated with BOR alone showed the highest increase of osteocyte viability, as compared to those either treated without BOR (+11.6% *vs.* +1.3%, *p=0.0019*) or treated with BOR plus DEX (+11.6% *vs.* +4.4%, *p=0.01*). A reduction of both osteocyte apoptosis and autophagy was demonstrated by TUNEL assays and confocal microscopy. On the other hand, any significant difference was not observed in patients treated with Thalidomide (THAL) or Immunomodulatory drugs (IMiDs) than in those untreated with these drugs (*p= 0.7*). A multiple regression non-parametric analysis showed that BOR had a significant positive impact on osteocyte viability (*p=0.042)* whereas THAL/IMiDs as well as Zoledronic acid (ZOL) treatments have not (p=0.2). BOR also counterbalanced the negative effect of DEX treatment (*p=0.035*).

Our data suggest that proteasome inhibitors blunted osteocyte cell death induced by MM cells and DEX through the modulation of the autophagy supporting their use to improve bone integrity in MM patients.

Disclosures: Giuliani: *Celgene Italy:* Research Funding.

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