Abstract Submission

13. Myeloma and other monoclonal gammopathies - Biology

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PROTEASOME INHIBITORS MODULATE OSTEOCYTE DEATH AND AUTOPHAGY IN MULTIPLE MYELOMA.

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Background: Cell death and autophagy are the main cellular processes involved in the regulation of bone remodeling by osteocytes. Recently we have demonstrated that an increased osteocyte death is involved in multiple myeloma (MM)-induced osteolysis through the upregulation of osteoclast recruitment.

Aims: Because proteasome inhibitors including Bortezomib (BOR) are known to be able to target osteoblasts in this study we have investigated the potential effect of these drugs on osteocytes and their cell death and autophagy.

Methods: 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 transwell insert in the presence or the absence of BOR or MG262. Moreover the effect of proteasome inhibitors on dexamethasone (DEX)-induced MLO-Y4 death, obtained at high doses (10^{-5}-10^{-6}M), was checked in combination with PTH(1-34). To evaluate the presence of autophagy and apoptosis in osteocytes, we checked the expression of both autophagic marker LC3 and apoptotic marker APAF-1 by confocal microscopy in the co-colture system with MLO-Y4 and RPMI-8226. Finally we performed a retrospective histological evaluation on bone biopsies of a cohort of 31 newly diagnosis MM underwent to different treatments including BOR-based regimen. Bone biopsies were obtained at the diagnosis and after an average time of 12 months of treatment. Osteocyte viability was evaluated in a total of 500 lacunae per histological sections.

Results: The *in vitro* treatment with BOR or MG262 significantly blunted MLO-Y4 and HOB-01 cell death. Similarly, DEX-induced MLO-Y4 death was reduced by proteasome inhibitors. Interestingly, we found that both proteasome inhibitors potentiated the PTH (1-34) short-term effects on DEX-induced osteocyte death. Prevalence of autophagic cell death compared to apoptosis was observed in this system. In line with these data, we showed that neither the HMCLs nor treatment with DEX increase the apoptotic death and caspase 3 activation in both MLO-Y4 and HOB-01 cell lines. BOR treatment increased the basal level of LC3 indicating a pro-survival and protective function of autophagy against the BOR-induce stress. On the contrary, when the 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 blocked autophagic cell death in osteocytes. In the *in vivo* study we found a significant increase of the number of viable osteocytes 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). 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).

Summary/Conclusion: Our data suggest that proteasome inhibitors blunted osteocyte cell death induced by MM cells and DEX through the modulation of the autophagy and potentiated the effect of PTH. Overall our *in vitro* and *in vivo* data support the use of BOR to improve bone integrity in MM patients.

Keywords: Bone disease, Bortezomib, Myeloma