INHIBITION OF OSTEOCYTE APOPTOSIS PREVENTS ACTIVATION OF BONE REMODELING AFTER FATIGUE IN VIVO

Leni & Peter W. May Department of Orthopaedics, Mount Sinai School of Medicine, New York, NY, USA
Luis.cardoso@mssm.edu

**Introduction:** Osteocytes surrounding fatigue microcracks in bone undergo apoptosis. Furthermore, bone regions containing apoptotic osteocytes co-localize with the areas of bone that are subsequently resorbed by osteoclasts. These spatial and temporal correlations between microdamage, osteocyte apoptosis and remodeling have led to the hypothesis that osteocyte apoptosis is a key controlling step in the activation and/or targeting of osteoclastic resorption after bone fatigue.

Here, we tested this hypothesis by pharmacological inhibition of osteocyte apoptosis after fatigue loading in vivo.

**Materials and Methods:** Twenty adult female Sprague-Dawley rats (120 days old) were randomly distributed into four groups of analysis. In the first group of animals (FAT), in vivo fatigue loading of the left ulna was used performed to initiate intracortical resorption, following the protocol reported by Verborgt et al. Briefly, ulnae in rats were cyclically loaded in vivo (18N maximal load, 4 Hz) to a predetermined increased in whole bone compliance using a small servohydraulic loading system. All tests were conducted under isoflurane anesthesia. A second group of animals (FAT+Casp Inh) were fatigue and treated with a pan-caspase inhibitor (Q-VD-OPh, MP Biomedicals, Livermore, CA, 20mg/kg/day with half dose given IP every 12 hrs) to prevent osteocyte apoptosis, since previous studies showed that this treatment prevents fatigue-induced osteocyte apoptosis in this system. Administration of the caspase inhibitor was started 2 hours before fatigue loading. Non-loaded groups of animals received either Q-VD or vehicle (DMSO) to assess baseline effects of the treatment regimen. Ulnae were harvested at 14 days after fatigue loading and treatment. All procedures were conducted with approval from the Institutional Animal Care and Use Committee of Mount Sinai School of Medicine.

At necropsy, ulnae were manually dissected free of soft tissues, fixed in formalin, decalcified in EDTA for 10 weeks and paraffin embedded cross sections of the ulnar diaphysis were cut at 5µm thickness. Approximately five hundred sections per specimen were obtained spanning the diaphyseal region where remodeling occurs in this system. The number of resorption spaces (Rs.N/B.Ar., #/mm²) and their areas were measured from Toluidine Blue stained sections. Osteocyte apoptosis was assessed by immunohistochemical staining for the cleaved form of caspase-3 (Asp175, Cell Signaling Technology, Beverly, MA), an effector caspase required for regulated cell death; detection was performed using a streptavidin-biotin conjugated system and developed with a DAB substrate chromogen system (Dako, Carpinteria, CA). The numbers of caspase positive and caspase negative osteocytes were counted at 40X magnification for entire ulnar cross-sections. Differences in resorption spaces and apoptotic osteocytes among groups were tested using ANOVA with the Tamhane test for post-hoc testing (SPSS 12.0).

**Results:** Osteocyte apoptosis: Treatment with the caspase inhibitor in fatigue-loaded animals prevented the increase in osteocyte apoptosis seen in loaded, non-treated animals (Fig 1,2), with the number of caspase positive cells in Fatigue+Q-VD treated bones comparable of baseline control levels.

Osteoclastic resorption: Intracortical resorption was activated in fatigue-loaded non-treated animals. In contrast, administration of the Q-VD completely inhibited the activation of intracortical resorption in ulnae to animals receiving treatment (Figure 3).

**Discussion** Previous studies led to the hypothesis that osteocyte apoptosis is a controlling step in the tissue level signaling and/or targeting of osteoclastic resorption of fatigue-induced microdamage. The current studies support such a causal relationship. When osteocyte apoptosis was prevented in fatigue loaded bone, resorption was not activated – despite the presence of matrix damage. The nature of the signals linking osteocyte death to osteoclast recruitment remain to be identified, as do the roles played by both the dying and surviving cells in the region of bone to be remodeled. However, these pathways may be analogous to those seen in other focal injuries, such as ischemic brain and heart injuries.

**Conclusion** Prevention of osteocyte apoptosis by a pan-caspase inhibitor completely blocked bone resorption in response to fatigue-induced microdamage. This finding supports a causal relationship between osteocyte death and the initiation of targeted bone remodeling.

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**References:**