Instituto de Medicina Molecular João Lobo Antunes

Master Project Proposal

Title: Long term effect of tofacitinib on bone: friend or foe?

Synopsis: Rheumatoid arthritis (RA) is an immune mediated chronic inflammatory disease, of unknown etiology, which affects up to 1% of the world-population. It is associated with systemic inflammatory activity but the major burden occurs at the level of bone, leading to joint destruction and fragility fractures. This translates into major functional consequences: after 10 years more than 50% of the rheumatoid patients are impaired to perform professional activities. In a series of studies, using different animal models, human samples and several technical approaches we have unequivocally shown that chronic inflammation directly leads to the degradation of bone microstructural and mechanical properties.

More recently, we have evaluated the efficacy of tofacitinib (a drug that targets JAK1 and 3, down regulating STAT 1 and 3 of the JAK-STAT signaling pathway) to treat inflammation-induced bone damage in the adjuvant induced arthritis (AIA) rat model (submitted to publication). Tofacitinib was able to abrogate arthritis manifestations, synovial tissue inflammation and bone erosions, which was associated with lower serum CTX I, P1NP, RANKL and OPG levels. In addition, we have observed increased bone cortical and trabecular hardness, measured by nanoindentation in tofacitinib treated animals. Consistently, these animals presented more parallel-lamellae structures than untreated arthritic animals. This bone organization represents a mature bone structure, which is 10% harder than the concentric structures (related to a high rate of bone remodelling) more often observed in untreated arthritis animals. Finally, tofacitinib was also able to attenuate the reduction of osteocyte number and lacunae that occurred in arthritic animals. However, micro-CT and 3-point bending tests revealed that tofacitinib did not revert the negative effects of arthritis on cortical and trabecular bone structural and mechanical properties. The reasons for the apparent inefficacy of tofacitinib to prevent and revert inflammation induced bone fragility are unclear. Using the same animal model we were able to revert the structural and mechanical damage induced by arthritis using an experimental compound. However, the kinetics of the effects of tofacitinib might be different, needing longer exposure time to have an impact on bone quality. The effect at a tissue level might be an early sign of its delayed impact on bone. However, an increase in hardness is associated with a decrease in the relative ratio of elastic-to-plastic behaviour of the tissue and thus it is unclear if it represents ultimately a true improvement in mechanical properties. Another explanation for these observations might be related with the mechanism of action. Tofacitinib targets JAK1 and 3, downregulating STAT 1 and 3 of the JAK-STAT signaling pathway, and these intracellular molecules have complex interactions with bone. JAK1 is expressed in bone cells and is involved in bone formation. The depletion of JAK1 promotes bone growth delays, suggesting that JAK1 is critical for skeletal development. On the other hand, STAT1 inhibits Runx2 transcription in osteoblasts, the master transcription factor of osteoblast differentiation. Thus, STAT1 is an inhibitor of differentiation of osteoblasts and the inactivation of STAT1 leads to an osteopetrotic bone phenotype. Consistent with the higher bone mass in STAT1-deficient mice, inactivation of STAT1 can accelerate fracture repair. These data suggest that STAT1 negatively regulates bone formation in vivo. On the contrary, JAK-STAT3 signal transduction pathway promotes osteoblast differentiation. Inactivation of STAT3 in osteoblasts leads to lower bone mass due to inhibition of bone formation. In humans, STAT3 mutations reduce bone mass and increase incidence of minimal trauma fractures. Clinical studies indicate that STAT3 mutations increase osteoclast number and bone resorption, and are associated with recurrent fractures. It is conceivable that these intricate molecular interactions have an overall effect that might not translate into a

positive effect on bone remodelling. In addition, it is unclear if the high efficacy of this compound in the control of inflammation, will totally override any possible deleterious effects that tofacitinib might have on bone. To fully clarify these open questions, it will be relevant to test tofacitinib in longer duration arthritis models and in healthy animals. Our hypothesis, based on our preliminary observations, is that tofacitinib might have a relatively neutral or slightly negative influence on healthy bone but that the long-term exposure to an arthritic model will prevent bone fragility, mainly through its potent anti-inflammatory effects.
The main goal of this project is to verify how long term tofacitinib exposure will affect healthy and arthritic bone. We will specifically verify the influence of tofacitinib on healthy and arthritic animals using distinct techniques for assessing: 1) Histological evaluation of hind paws; 2) the microstructure of bone (micro-CT); 3) the mechanical properties at tissue level (mechanical tests and nanoindentation); 4) the cellular activity (RNA expression and functional tests); 5) the quantification of bone remodelling markers and cytokines.

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