Iron overload causes osteoporosis in thalassemia major patients through interaction with transient receptor potential vanilloid *type 1* (TRPV1) channels

C. Tortora[1], I. Manzo[2], G. Bellini[1], B. Nobili[2], S. Maione[1], F. Rossi[2]

[1]Dept. of Experimental Medicine, Second University of Naples, Naples, Italy
[2] Dept. of Women, Child and General and Specialistic Surgery, Second University of Naples, Italy

Osteoporosis (OP) is responsible for substantial morbidity in adult patients with -thalassemia major (TM). The pathogenesis of OP is multifactorial and our understanding of the underlying molecular and cellular mechanisms remains incomplete (Haidar et al., 2011*;* Toumba et al., 2010). The role of iron overload resulting to transfusion and iron chelation therapy are subject of increasing interest.

The endocannabinoid/endovanilloid (EC/EV) system has recently been considered a potential therapeutic target for bone disease. We have previously reported that human osteoclasts express the functional transient receptor potential vanilloid *type 1* (TRPV1) channel together with the cannabinoid receptors *type 1* and *2* (CB1/CB2), and metabolic enzymes for the two most studied endocannabinoids; anandamide (AEA) and 2 arachydonoylglycerol (2-AG). Cannabinoid/vanilloid agonists alone, or in combination with selective antagonists, are able to modulate osteoclast formation and activity (Rossi et al., 2013, 2011, 2009). Therefore, considering the emerging role of the EC/EV system in bone metabolism, and specifically in the pathophysiology of OP, we evaluated the possible influence of this system in the development of TM-induced OP and specifically its relationship with iron overload and chelation therapy, in TM patients with osteoporosis, through a multidisciplinary approach on TM patients-derived osteoclasts *in vitro*.

We find an increase of the expression of tartrate resistant acid phosphatase (TRAP) and cathepsin K, two specific osteoclast biomarkers and of TRPV1. Moreover, we find that TRAP expression levels inversely correlate with femoral and lumbar bone mineral density, and directly with ferritin levels and liver iron concentration, confirming a key role for iron overload in the pathogenesis of TM-associated bone disease.

TRPV1 stimulation, with the selective agonist resiniferatoxin (RTX) dramatically reduces cathepsin K levels, without affecting TRAP expression, that can be directly regulated by iron at the gene transcription level, through the presence of Iron Responsive Elements in the promoter region. Thus, the presence of iron overload in patients with TM may activate TRAP transcription *per se*.

Both TRPV1 stimulation with RTX or its selective blockade with and the 5-iodio-RTX (I-RTX) antagonist, significantly reduce osteoclast numbers *in vitro*, suggesting for a desensitization of the TRPV1 channel in presence of β-TM.

Moreover, TRPV1 stimulation is able to increase CB2 levels that has an important role in maintaining bone mass, confirming a functional cross talk between CB2 and TRPV1.

Iron chelators, deferoxamine, deferiprone and deferasirox decrease both TRAP and cathepsin K expression, as well as osteoclast number and activity, suggesting that chelation therapy is mandatory to reduce osteoclast activity.

Taken together, these data show that TRPV1 activation/desensitization influences TRAP expression and activity, and this effect is dependent on iron, suggesting a pivotal role for iron overload in the dysregulation of bone metabolism in patients with thalassemia major.

Our applied pharmacology provides evidence for the potential of iron chelators to abrogate these effects by reducing osteoclast activity and for the use of a combined therapy with hybrid molecules designed for, simultaneously, stimulating CB2 receptors and blocking TRPV1 channels to alleviate associated osteoporotic changes.

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