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COMMENTARY

Purinergic ADP receptors: from block buster blood drugs to bone-disease busters

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Purinergic signalling is a primitive and ubiquitous cell to cell signalling system that involves extracellular nucleotides, such as adenosine triphosphate (ATP) and adenosine diphosphate (ADP), being used outside the cell to bind specific receptors called purinoceptors. There have been numerous advances in the field of purinergic signalling since its inception in the early 1970s, including the discovery that ADP-mediated platelet aggregation occurs via activation of specific purinoceptors. As well as being important in platelet aggregation, ADP was shown to be a powerful modulator of osteoclast activity in 2001 by Hoebertz *et al.*,[1] and the effect attributed to the well-characterized P2Y1 receptor. Around the same time, the elusive ADP platelet receptor (previously known as the P2T receptor[2]) was cloned and identified as the $P2Y_{12}$ receptor.[3] It was subsequently found that the $P2Y_{12}$ receptor is the target of Clopidogrel.[4] Like many of the purinoceptors, expression of $P2Y_{12}$ was thought to have a restricted cellular expression pattern (in this case to immune cells and platelets); however, the $P2Y_{12}$ receptor was first noted to be expressed by bone cells by Buckley *et al.*[5] in 2003. Since then very little has been reported, other than in abstract form,[6,7] as to the role of the $P2Y_{12}$ receptor in bone, despite the increased interest and available data on purinergic signalling in bone (for a recent review see Rumney *et al.*[8], and for a historical review see Bower *et al.*[9]) as well as the availability of the $P2Y_{12}$ receptor knockout mice.[10] The paucity in data on the $P2Y_{12}$ receptor in bone has been redressed with the recent publication by the group of Katherine Weilbaecher.[11] Su *et al.*[11] in their study perform an extensive in-depth analysis of the bone phenotype of the *P2ry12*$^{-/-}$ (knockout) mice under normal and pathological conditions, consolidating their findings with use of the clinical inhibitor of $P2Y_{12}$, clopidogrel. The major findings of this paper are summarized as follows:

(1) Deletion of the *P2ry12* Gene Leads to an Age-Related Bone Phenotype

Comparison of the bones of *P2ry12*$^{-/-}$ and wild-type (WT) mice at 2 months of age revealed no significant difference in the typical static and dynamic bone measurements. Bone turnover (both formation and resorption) markers were both significantly reduced at this age compared with WT, despite the lack of effect on the structural indices of bone. A different pattern emerges once the mice reach the age of 8 months—the *P2ry12*$^{-/-}$ mice now had significantly increased bone mineral density and trabecular bone volume in the primary and secondary spongiosa of the tibia with trabecular bone extending further into the diaphysis compared to WT. Osteoclast surface was reduced, while osteoblast surface, number and bone formation rate was unaffected. At this age the bone turnover markers reflect the change in the architecture—with CTX being decreased and P1NP equivalent to that of the WT controls. These observations led the authors to conclude that the *P2ry12*$^{-/-}$ animals were partially protected against age-related bone loss. From the data provided, 2-month-old mice had a BV/TV of ~24%, while at 8 months this had reduced to ~12% in WT and 15% in *P2ry12*$^{-/-}$, meaning a loss of trabecular bone volume of approximately 50% and 38%, respectively, over 6 months.

(2) ADP Modulates Osteoclast Activity Predominantly via the $P2Y_{12}$ Receptor Upstream of $\alpha_v\beta_3$ and RAP1 Activation

Decreased osteoclast activity *in vivo* in the *P2ry12*$^{-/-}$ mice led the group to investigate the cell autonomous $P2Y_{12}$ receptor expression and function. They found that the expression of *P2ry12*, but not *P2yr1*, increased during osteoclastogenesis in WT cells *in vitro*, and that the level of *P2yr1* was not altered in *P2ry12*$^{-/-}$ osteoclasts. Decreased resorption pit area was evident in the *P2ry12*$^{-/-}$ osteoclast cultures, while ADP stimulation significantly increased osteoclast number and resorption in both WT and *P2yr1*$^{-/-}$, but not *P2ry12*$^{-/-}$ osteoclasts. They identified $\alpha_v\beta_3$ and RAP1 activation as being downstream of $P2Y_{12}$ receptor activation using WT, *P2ry12*$^{-/-}$ and integrin β3 gene knockout (*Itgb3*$^{-/-}$) pre-osteoclasts. The authors concluded that the $P2Y_{12}$ receptor is the predominant ADP receptor modulating osteoclast formation and function.

(3) *P2ry12*$^{-/-}$ Mice were Protected from Pathological Bone Loss

Finally, the *P2ry12*$^{-/-}$ mice were subject to different pathological situations including serum transfer-induced arthritis, primary bone cancer and oestrogen deficiency-induced osteoporosis. In the first two situations the primary disease was unaffected by deletion of *P2ry12*, however in all situations the amount of disease-associated bone loss was significantly reduced in the *P2ry12*$^{-/-}$ mice. Use of clopidogrel in a tumour metastasis model resulted in no effect on tumour burden, but a significant inhibition of tumour-associated bone loss. Similarly, treatment of OVX WT mice with clopidogrel reduced the extent of bone loss over 5 weeks by blunting osteoclast-mediated resorption.

Taking these key observations into account the authors concluded that the $P2Y_{12}$ receptor exerts most of its effects under pathological conditions, in which extracellular ATP, and thus ADP, would be increased. They also commented that 'clopidogrel or other novel ADP and P2RY12-targeted therapies could have a role as in the treatment of disease states characterized by excessive bone resorption'.

This extensive report highlights a number of significant issues in bone research. The first is the importance of experimental design—of performing careful, in-depth characterization of knockout models. It highlights the reason for assessing the bone phenotype at more than one age and stresses the use of challenging the model beyond normal physiology.

The second is the importance of purinergic signalling in bone and, in particular, ADP-mediated effects on osteoclasts. This particular report does a convincing job of attributing the effect of ADP to the $P2Y_{12}$ receptor, despite previous evidence suggesting that the effect of osteoclasts was via P2Y1 receptors. A number of studies relevant to this current work were published around the same time this paper was originally submitted and subsequent to its acceptance for publication. The first is the report by Wang *et al.*[12] that the $P2Y_{13}$ receptor, a G_i-coupled receptor that shares 45–48% amino-acid sequence identity with the $P2Y_{12}$ receptor and has high affinity for ADP responding in the nanomolar range,[13,14] also has a significant role in bone homeostasis.[12] He demonstrated that $P2Y_{13}$ receptor knockout mice had reduced bone turnover rates in normal physiology due to effects on both osteoblasts and osteoclasts, and a protective effect of up to 65% in the increased resorption situation of oestrogen deficiency-induced osteoporosis.[12] The second was a report by Jorgensen *et al.*[15] demonstrating that use of the clopidogrel in the recommended dose range is associated with an increased risk of fractures. The third paper from Syberg *et al.* details the effect of clopidogrel treatment on bone cells *in vitro* and bone *in vivo*.[16] In agreement with Su *et al.*,[11] they demonstrated that expression of the $P2Y_{12}$ receptors increased in osteoclasts culture over time and that clopidogrel treatment reduced osteoclast formation and resorption if added continually, but had no effect on mature osteoclasts. In addition, they found that clopidogrel similarly affected osteoblasts in a negative way—reducing cell numbers and viability and inhibiting bone formation *in vitro*. However, in contrast to Su *et al.*,[11] they demonstrated that clopidogrel treatment significantly exacerbated OVX-induced bone loss. The difference between the results of these two reports is probably due to study design—Su *et al.*[11] used 14-week-old mice and started clopidogrel treatment 2 days after OVX for up to 35 days, in essence a preventative model with effects driven mainly by osteoclastic activity. While Syberg *et al.*[16] used 16-week-old mice and started clopidogrel treatment 28 days after OVX and continued for a further 28 days—in essence a rescue model with effects mainly driven by osteoblasts.

Clopidogrel is already an approved drug, widely used against stroke and thrombosis, and when marketed as Plavix was one of the best selling pharmaceutical drugs in the world of all time, with worldwide sales of 6.8 billion $US for 2012. Plavix has gone off patent in the United States, and the availability of generic clopidogrel may increase its use in treating cardiovascular diseases worldwide. As such, any unwanted negative side effects such as increased fracture risk and exacerbation of post-menopausal bone loss need to be considered. Clearly, with the observations from Su *et al.*,[11] the opportunity for its use in treating the painful, debilitating bone loss associated with arthritis and cancer should not be missed and needs further investigation.

Conflict of Interest

The author declares no conflict of interest.

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