Reviewer's report

Title: Alterations in Osteoclast Function and Phenotype Induced by Different Inhibitors of Bone Resorption - Implications for Osteoclast Quality

Version: 1 Date: 19 October 2009

Reviewer: Anne Gingery

Reviewer's report:

The manuscript by Neutzsky-Wulff and colleagues proposes that inhibition of acidification, proteolysis and/or general osteoclast inhibition in terms of resorptive phenotypes of osteoclasts is differentially regulated dependent on inhibition targets. They propose that different bone resorption inhibitors result in distinct osteoclast phenotypes that can each have a particular effect on bone quality. The discussion of osteoclast signaling between osteoclast and osteoblast is an important aspect of this research and points to the significance of resorption versus other paracrine/autocrine interactions. This manuscript has some substantial information regarding the effects on inhibitors on osteoclasts and can advance the knowledge of bone regulation as well as therapeutic strategies.

Major Revisions:

In general the abstract should indicate the conclusions of the research concerning each type of inhibition and its effects.

While discussing osteoclast viability make sure to clearly note that TRACP measure is not a viability assay rather it is a measure of maturity or differentiation. The viability assay should be discussed as well as noted below.

Please note in the text the concentrations that you suggests inhibit activity. For instance “E64 potently reduced organic resorption by 80% ...” - should indicate at what concentration it inhibits resorption at 80%. This additional information should be noted throughout the manuscript. Further a discussion of your EC50’, IC50’s and the effective doses that are listed would provide more clarity on to the discussion of inhibition of osteoclast resorptive activity.

“MMP’s appear to compensate for the absence of cathepsin K ...” – this section notes the evaluation was done at selected concentrations. Please indicate why you selected these concentrations – effective dose, EC50’s, etc.

“Ibandronate potently inhibits both organic and inorganic.....” - in this section you claim that osteoclast specific cell death has occurred. Reduction in TRACP activity does not necessarily correlate with osteoclast specific cell death. In addition if you think that monocytes are involved in your cell viability assay this should be addressed. If you had actual cell counts to add to these data that would be helpful, however if that data is not available addressing this issue is
important. In addition there is some indication of bisphosphonates uptake in monocytes. The claim that monocytes are not able to release ibandronate in an environment where it is being released does not adequately address this issue of their potential uptake. It is not accurate to claim that TRACP activity reveals that osteoclast number is reduced. This is especially important since your viability assay does not necessarily measure cell number, and specifically osteoclast cell number. It is unlikely that the number of monocytes is so greatly increased in your ibandronate treatment versus the control to make the viability essentially unchanged or increased. Your conclusion that ibandronate specifically targets and kills mature osteoclast is not shown in your data and is an over-reaching conclusion. Perhaps this statement could be explained in more detail or related to osteoclast activity versus cell-death.

Minor Revisions:

Overarching labels for the figures would be helpful. Figure 1. Inhibition of Acidification, Figure 3. Inhibition of cathepsin K, etc. would be helpful.

Ibandronate potently inhibits both organic and inorganic…. in this section you claim that osteoclast specific cell death has occurred. Reduction in TRACP activity does not necessarily correlate with osteoclast specific cell death. In addition if you think that monocytes are involved in your cell viability assay this should be addressed. If you had actual cell counts to add to these data that would be helpful, however if that data is not available addressing this issue is important. In addition there is some indication of bisphosphonates uptake in monocytes. The claim that they are not able to release ibandronate in an environment where it is being released does not adequately address this issue of their potential uptake. It is not accurate to claim that TRACP activity reveals that osteoclast number is reduced. This is especially important since your viability assay does not necessarily measure cell number, and specifically osteoclast cell number. It is unlikely that the number of monocytes is so greatly increased in your ibandronate treatment versus the control to make the viability essentially unchanged or increased. Your conclusion that ibandronate specifically targets and kills mature osteoclast is not shown in your data and is an over-reaching conclusion.

Accept after adequate response to revisions.

An article of importance to its field.
Acceptable quality of written English
Statistical Review – good
Reviewer has no competing interests