Reviewer's report

Title: Impregnation of bone chips with alendronate and cefazolin, combined with demineralized bone matrix: a bone chamber study in goats.

Version: 1 Date: 19 December 2011

Reviewer: Magnus Tägil

Reviewer's report:

I have read the article "Impregnation of bone chips with alendronate and cefazolin, combined with demineralized bone matrix: a bone chamber study in goats. It is a clinically oriented experimental study where the authors investigate if locally applied antibiotics, DBM and bisphosphonates influence the bone resorption part of allograft bone graft remodeling."

General comments,

1 Methodologically I miss evidence that the antibiotics ever would stay in the graft after rinsing and impaction. It should not be that difficult to repeat the impregnating procedure and measure the amount of remaining antibiotics.

2 The other major concern is that measuring the amount of new bone that remains after remodeling, not only reflects how much new bone have formed but rather how effective the antiresorptive treatment is to prevent the new-formed bone to resorb. So you cannot claim you measure anabolism but rather catabolism. Rewrite section on p 15

Specific points

Abstract

The aims could be sharper defined. That antibiotics prevent infection might be generally known but I am not sure every reader understands why inhibiting the osteoclastic response prevent instability.

Background

Also here the hypothesis and the aims are missing. I found them partly in the beginning of the discussion and the first sentences there could be moved to the introduction forming the aims.

The hypothesis of bisphosphonates stalling resorption thereby providing better support during healing of a hip prosthesis is not very easy to understand as it is written now.

Further there is no mechanistic hypothesis why Cef would influence bone healing, whether this could be stopped by the proposed drugs and how this could be measured.

Further there is no mechanistic hypothesis why DBM is used in the experiment more than "This critical period after reconstruction with bone impaction grafting might be shortened when demineralized bone matrix (DBM) is used." Please
state what do you expect DBM to do and how you will measure it?
I suggest the introduction is partly rewritten to include hypothese and aims
Minor things- I dislike the phrase catabolic effect on osteoblasts. Decreased
anabolism or toxic effect?
"Although bisphosphonates efficiently block resorption" Do BPS really block
resorption or rather postpone it?

Methods
I cannot find what dosis you used when combining DBM and bisphosphonate.
Please describe briefly the random order of the chambers so the readers
understand how you randomized the sites.
Please mention shortly the results of the sample size estimation/power analysis-
what effect that was expected, the measurement accuracy and how many
animals that were needed.
Bisph bind tightly to bone- what about cefazolin? Please expand. Do we know
whether there are any bone-bound cef or does it go away when rinsing?
References? Or what is your hypothesis?
Does the bisphosphonate concentration matter most or is it the soaking time?
References ?
In rats twelve weeks is a long time and the differences are greater at six weeks.
Why 12 weeks and why only 12 weeks and not for example 6 and 12 weeks.
With 200 chambers and 25 samples in each maybe you could have done two
time points?
Being the author that once described the compaction method I hope you are
aware that it is a very dense bone graft of 65%. The density of impacted
morsellized bone graft in hip revision is about 35%.
Why doing the impaction after giving the doses? Especially the Cef will be
squeezed out?

Results
Only 8/200 loose. Good surgery.
I would like to see better blow ups of Fig 1 to be able to see if it is living bone at
the pop? In the rat chambers we have never seen such a thick fibrous tissue as
in fig 1a . Interesting to see bone formation at the top due to the DBM?

Discussion
Move purpose to aims?
P 14 lin 3-9 Here you write it is important with a balance between formation and
resorption but you only measure one side- the resorption. So we do not know
where this balance is?
P 14 line 10-22 Jacobsen did not rinse like you do. Is that important do you
think? Or is it what is bound to the bone that matters like Agholme thinks?
Jacobsen has a mechanically loaded situation and you an unloaded (even
stress-shielded) hence the difference I believe. You could comment on that in the text

P 14 line 23 Agholme- as you both are evaluating the remodeled area it should be equivalent regardless of study time- even if it would be same species. But ingrowth distance i.e. the anabolic equivalent of the chamber would differ!

P 14 last line Zolendronate has also been used for a decade (2002 in Sweden). Alendronate was introduced in 1995.

P 15 Just because you find more bone it does not imply anabolism. This is important! It simply means that more or less of the new-formed bone has been resorbed- due to the BP concentration. It has nothing to do with osteoblasts- at least not until a too high toxic dose !!!!!You have to delete this passage about the amount of new bone.

P 15 line 6 from the end. As written above, I would suggest you measure the amount of Cef, in the graft with or without rinsing. It is rather simple to do and would add so much to the interpretation. There is simply too much guessing as it is.

Here are two examples of conclusions you cannot draw

“Also, this concentration did not cause any side effects and is well above the minimal inhibitory concentration (MIC) for S. epidermidis.

How could you tell without knowing the concentration?

“No subinhibitory amount of the drug is left behind which can induce resistancies”

How would you know if you do not know the concentration?

“Cefazolin is completely eluted from the bone chips after three days.”

Please add reference or report your own data

P 17-line 3 why would cement matter?

v P 17-line 3 osteoclasts resorb old AND NEW formed bone- which explains why you get more new bone when BP dose increases.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests'