Author's response to reviews

Title: Age of donor alters the effect of cyclic hydrostatic pressure on production by human macrophages and osteoblasts of sRANKL, OPG and RANK

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Version: 7 Date: 9 February 2006

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BMC Musculoskeletal Disorders

9th February 2006

Dear Sir/Madam,

Re: Age of donor alters the effect of cyclic hydrostatic pressure on production by human macrophages and osteoblasts of sRANKL, OPG and RANK.

We are delighted that BMC Musculoskeletal Disorders has agreed to publish the above-named manuscript. We respond to the latest comments by the Reviewer below.

Reviewer: Gerald Atkins

Reviewer's report:

General
The manuscript has been simplified and improved by the modifications. The data show that hydrostatic pressure increases the ratio of OPG:RANKL in older patients and decreases this ratio in younger patients, where co-cultures of the patients' osteoblasts and macrophages are set up ex vivo. Some markers consistent with osteoclast formation were also upregulated, including RANK, TRAP and VnR. RANK expression by macrophages in particular was upregulated by pressure and was further upregulated by 1,25-vitamin D. Whether this represents an increase in functional osteoclast numbers remains untested.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

1) Page 3: A better description of the co-culture system in this paper is warranted. The paper refers to a previously published method [23], but that too lacks sufficient detail for the results to be replicated by others, for example the plating density of PBMC. The text has been altered and expanded to clarify the techniques used and cell numbers added.

2) Page 4: Under ‘Culture of macrophages’, the first two sentences are repetitious of the previous section – ‘prepared as above’ would be more concise. As suggested, the first two sentences have been shortened and conflated and the term ‘prepared as above’ has been added.

3) Page 10: Discussion, sentence 2: Osteoclastogenesis is initiated and maintained by <insert ‘at least’> two key factors...
This has been done on page 10.

4) Page 13: as stated, it is unlikely that the ELISAs employed are capable of measuring ‘total product’ of OPG and possibly also RANKL, as complexes are seldom detectable in the available assays. Therefore ‘total’ should be replaced by ‘detectable’.
The term “detectable” has replaced “total”.

5) Table 3: Please clarify the scoring – only “-”, “+/−” and “+++” appear in the legend. “-” does not appear in the table; what do “+” and “+/++” in the table mean?
The Legend under Table 3 has been altered and expanded to include definitions of the terms “+” and “+++”.

6) Fig 7 b) should be Fig 6 b).
The label on Fig 6 has been altered from 7 b) to 6 b).
I hope these changes now answer all the queries of the Reviewer to his satisfaction.
Yours sincerely,

C.E. Evans (non-clinical lecturer)
J.G. Andrew (consultant orthopaedic surgeon)
S. Mylchreest (research assistant)