

Reviewer's report

Title: The Chondroprotective Actions of a Natural Product are Mediated by the Autocrine Activation of IGF-1 Production by Human Chondrocytes Despite the Presence of IL-1b.

Version: 1 **Date:** 16 January 2006

Reviewer: Ron Kooijman

Reviewer's report:

General

The manuscript of Miller et al. contains interesting data on the induction by natural plant products of IGF-I expression in chondrocytes which may be of interest for therapeutic applications. These products, RNI249 and vincaria, also inhibit NO production and the release of glycosaminoglycan from cartilage explants.

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

The title of the paper has to be changed, because it suggests that a mediating role of IGF-I in protection of chondrocytes has been proven. The authors have not tried to block the actions of the natural products by substances interfering with the IGF-system.

The Materials and Methods section can be improved by providing more details on the experimental design.

Where do the cartilage samples originate from? Healthy donors, males, females, age, certain patients?

How long after enzymatic digestion were the chondrocytes exposed to the stimuli?

What is the viability of this preparation at the start of the culture period?

Did IGF-I affect the viability or total cell number in chondrocyte culture?

Were cells exposed to vincaria or RNI249 at the same time as to IL-1beta?

Are the stimuli used at suboptimal or optimal concentrations for the effects tested for?

In the section Statistical Analysis the authors mention that "each experiment was repeated to ensure reproducibility of the data using cartilage samples from matched donors". However, the number of experiments has not been provided for Fig. 1. Also in Fig. 3, n is not given for the control.

Additional data on how the experiments have been designed are absolutely required. For instance, it should be made clear whether mean values \pm SEM are obtained from independent experiments using different donors (how many?) or from material derived from one donor cultured in n-fold.

Why is the number of experiments for different conditions in Fig. 3 different? Are explants from different donors used for the different conditions?

Has material from the same donor been used for experiments in different figures?

The authors also have to indicate how samples from donors were matched.

Discussion

The discussion is too long and contains a lot of speculation. For example, on page 13, the authors propose that IGF-I blocks the inhibitory effects of inflammatory mediators. However, there are no arguments for that from the data, because the effects of the natural products on IGF-I secretion have not been determined in the absence of IL-1beta. Also the literature on *Lepidium* (page 13 and 14) does not provide data that contribute directly to the elucidation of the working mechanism of vincaria.

I agree that it is true and of significance that the natural products are still effective in augmentation of IGF-I gene expression in the presence of IL-1beta.

The authors state (page 15) that IGF-I silences pro-inflammatory genes and refer to refs 54, 61-63. Please make clear which pro-inflammatory factors are meant.

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

Discretionary Revisions (which the author can choose to ignore)

For researchers outside the field of bone research it may be questionable whether the release of GAG is a good indicator for degradation of cartilage. It could be worthwhile to make this clear in the discussion.

Am I right to say that a mediating role of IGF-I is unlikely, because the experiments have been performed in serum-containing media? When yes, it means that the natural products exert IGF-I-independent effects in the culture system applied. In that case, the effects of the natural product on cells cultured in serum-free may even be stronger.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:

I declare that I have no competing interests.