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Summary of findings:

The effect of a United Fisheries Limited shark cartilage powder on osteoblast function and osteoclast-precursor differentiation

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Lay Summary

This study was designed to test the effect of the United Fisheries shark cartilage powder on:

Aims:

The proliferation and differentiation of osteoblast-like cells.

> *What effect does the United Fisheries shark cartilage powder have on the osteoblast? Osteoblasts are the cells which build bone. Does this powder cause the cells to grow and multiply (proliferation) and develop the characteristics of cells which could make bone (differentiate) or do the powders prevent this from happening?*

The differentiation of osteoclast precursors into osteoclasts.

> *What effect does the United Fisheries shark cartilage powder have on osteoclast precursor cells? Osteoclasts are the cells that break down bone, these cells are absolutely necessary for healthy bones, but if their activity exceeds the amount of bone made by the osteoblast then we can lose bone mass (eg. osteopenia or worse osteoporosis). "Osteoclast precursor cells" are the "early stage" version of the osteoclast, they are like the hull of a boat, but without further work they can't do anything useful. A mature osteoclast which can resorb (breakdown) bone doesn't form from the precursor until "called into duty" by a range of stimuli. In this study we want to test if the United Fisheries powder encourage or inhibit this process.*

What we did:

We used osteoblast and osteoclast precursor cells grown in the lab. It is accepted practice to use mammal cells, in this case mouse cells. We used a mouse osteoblast cell called MC3T3-E1 subclone 4, and a mouse osteoclast precursor cell celled the RAW 264.7 macrophage. Both cells, or cell lines, are well established models used to demonstrate osteoblast and osteoclast functions and activities. We treated these cells with the powders to test the above aims. This is a similar process as would be used with the first steps of testing factors or drugs that might have a positive effect on bone growth or a protective effect from bone breakdown.

What we found:

The shark cartilage powder had a significant effect on bone cells in the lab, indicating potential positive effects on bone cell function.

The shark cartilage powder increased osteoblast growth and differentiation, this means that the powder causes bone-making osteoblast cells to grow and show characteristics of cells that could produce bone matrix. This also means that the powder is not toxic to bone cells at the concentrations it was tested at.

The shark cartilage powder reduced and inhibited the formation of osteoclast cells.

The shark cartilage powder had to be dissolved into solutions before cells were treated. The powder did not dissolve completely in the cell growth solutions.

Recommendations:

Recommendation 1:

The powder didn't dissolve completely in the cell culture media which the cells were grown in (i.e. part of the powder remained as a solid powder and therefore couldn't be put on the cells). Because of this, we recommend that we repeat the process of dissolving the powders in cell culture media and then submit the dissolved portion for chemical analysis for

i. Protein
ii. Calcium
iii. Magnesium
iv. Phosphorus
v. Collagen
vi. Glycosaminoglycan
vii. Chondroitin sulphate
viii. Fat

There will be two samples total, the United Fisheries shark cartilage powder solution, and a cell culture solution by itself.

Why do this? This will tell us what proportions of each of the above analysed compounds were dissolved and are present in the solutions which the cells were treated with.

Recommendation 2

Test to see if the United Fisheries powder is able to enhance osteoblast mineralization. The development of the osteoblast bone cell is a three step process, proliferation followed by differentiation, and then final the production of mineral. The current study has looked at the first two steps but not the last which is a more prolonged experiment. Treating the cells with the powder(s) will test to see if they enhance the production of mineral by the osteoblasts.

Recommendation 3

The United Fisheries powder was partially soluble in cell culture media (a balanced salt solution compatible with cells). Powders such as this are likely to be dissolved or digested better in the stomach as there are digestive factors in the digestive tract that break down products better.

To replicate the form that this powder might take when they have been through the stomach, it is recommended that the powder is subjected to a simulated gastric digestion in the lab and then treating the cells with this digested form of the powder. Using a digestion method compatible with the osteoblast and osteoclast models, this could completely digest the entire powder sample (instead of the soluble part only) in a form similar to what would be presented to the gut. This may give you a better representation of the form that the powder might take when they enter the bloodstream when absorbed from the gut.

Cons: The technique is more complicated and timeconsuming, but could give you more meaningful results.

Scientific Summary

This study was designed to assess the effect of a United Fisheries shark cartilage powder on osteoblast function and the differentiation of osteoclast precursors into osteoclasts.

The study used the mouse MC3T3-E1 cell line as an in vitro osteoblast model, and the mouse RAW 264.7 macrophage cell line as a model of an osteoclast precursor cell. Both cell lines are well established models used to demonstrate osteoblast and osteoclast phenotypic activities respectively.

1. The United Fisheries shark cartilage powder increased MC3T3-E1 osteoblast cell proliferation and differentiation indicating an osteogenic effect (anabolic) of the soluble component of the powders *in vitro*.
2. The shark cartilage powder inhibited osteoclast formation in RANKL-stimulated RAW 264.7 cells indicating a possible role for controlling bone resorption *in vitro*.
3. While the powder had a significant effect on bone cells in vitro, the powder was only partially soluble in solution (i.e. there was a significant amount of powder which was insoluble).
4. The soluble portion of the powder had some effect on some of the bone cell function models used indicating bioactivity of some of the soluble components.
5. To further investigate the effects of this powder, it is recommended that the powder solubilisation process is replicated and samples of the soluble fractions are submitted for chemical analysis for (i) protein, (ii) calcium, (iii) magnesium, (iv) phosphorus, (v) collagen, (vi) glycosaminoglycan, (vii) chondroitin sulphate, and (viii) fat. Including respective blank controls for each of the cell culture medias used.
6. Subject the powder to a simulated gastric digest *in vitro*. Using a method compatible with the osteoblast and osteoclast models, this could completely digest the entire powder sample (instead of the soluble part only) in a form similar to what would be presented to the gut.