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Summary of findings:

The effect of a United Fisheries Limited MCHC fish bone powder on osteoblast mineralisation

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(Sign)

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Lay Summary

Aims:

This study was designed to test the effect of a United Fisheries Limited MCHC fish bone powder on:

Mineralisation in osteoblast-like cells.

What effect does the United Fisheries Limited fish bone powder (MCHC) have on mineralisation of osteoblast bone cells? Osteoblasts are the cells which build bone. Does this powder stimulate or inhibit the production of bone mineral by these cells?

What we did:

We used osteoblast precursor cells grown in the lab. It is accepted practice to use mammalian cells, in this case a mouse cell line. We used a mouse osteoblast cell called MC3T3-E1 subclone 4. This cell line is a well established model used to demonstrate osteoblast functions and activities. We treated these cells with the MCHC powder to test the above aim. 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.

What we found:

The United Fisheries fish bone powder (MCHC) had a significant effect on bone cells in the lab, stimulating osteoblast mineralisation. This indicates a potential positive effect on bone cell function.

The fish bone powder MCHC had a significant positive effect on osteoblast mineralisation. This means that the powders stimulated the bone-making osteoblast cells to produce more bone mineral, although it should be noted that this effect was small. The powders did not inhibit and were therefore not toxic to this bone formation process in this model.

Recommendations:

The main findings of the latest study, along with the results from the previous reports appear to show:

- The United Fisheries fish bone powder (MCHC) stimulates osteoblast cell proliferation, differentiation, and bone mineral formation.

Based on the findings of these studies, it may be worth investigating the effect of the United Fisheries fish bone powder in a small growing rat model, to test if oral consumption of this powder has an effect on bone growth and apposition.

Scientific Summary

This study was designed to assess the effect of a United Fisheries fish bone powder MCHC on osteoblast function. We have previously tested and reported that this powder stimulates osteoblast differentiation, and this study assesses whether differentiation progresses to mineral nodule formation by these cells

The study used the mouse MC3T3-E1 cell line as an *in vitro* osteoblast model. This cell line is a well-established models used to demonstrate osteoblast phenotypic activities.

1. Mineral nodule quantity as assessed by Alizarin Red staining was increased in MC3T3-E1 cells after treatment with a United Fisheries MCHC fish bone powder after 17 and 25 days (Figure 1 and 2).
2. 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 proportion of powder which was insoluble).
3. The soluble portion of the powder had some effect on the bone cell function models used indicating bioactivity of some of the soluble components.
4. 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.
5. Subject the powder to a simulated gastric digest *in vitro*. Using a method compatible with the osteoblast model, 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.

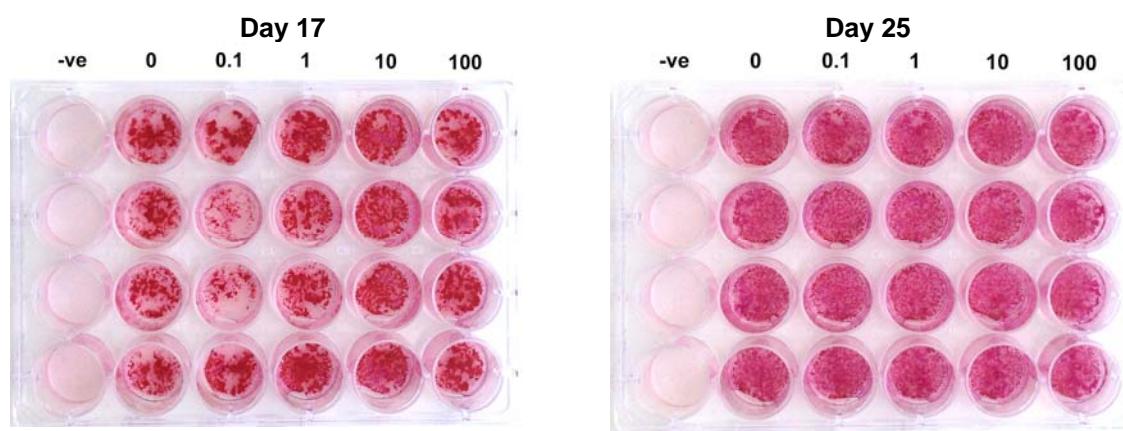


Figure 1. 24-well tissue culture plates showing mineral nodule formation by osteoblasts after 17 and 25 days of treatment with MCHC powder. Cells were incubated with solubilised powder samples on a protein concentration basis at 0.1, 1, 10 and 100 $\mu\text{g}/\text{ml}$ for 17 and 25 days in the presence of osteogenic cell culture media. Cells were also grown in the absence of osteogenic media to serve as a negative control (-ve). To assess mineralisation, each well was stained with Alizarin Red to stain mineralised nodules (pink/red staining).

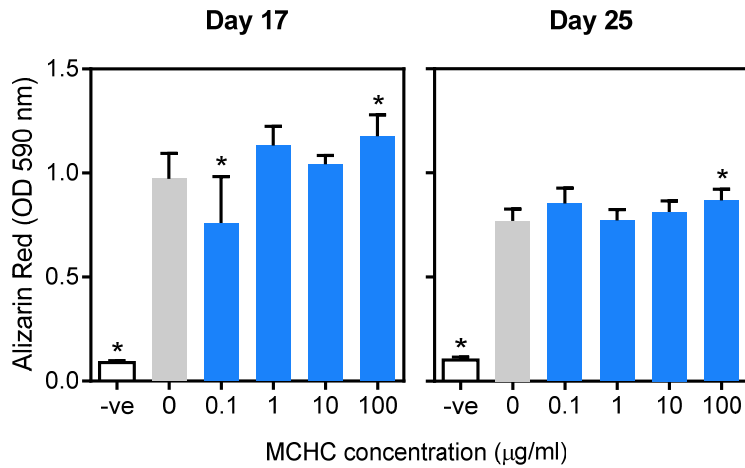


Figure 2. Alizarin red activity in Mc3t3-E1 subclone 4 osteoblast cells treated with MCHC. Cells were incubated with solubilised powder samples on a protein concentration basis at 0.1, 1, 10 and 100 µg/ml for 17 and 25 days in the presence of osteogenic cell culture media. Cells were also grown in the absence of osteogenic media to serve as a negative control (-ve). Alizarin red activity reflects the degree of mineralisation in each treatment. Results are shown as means ± 95% confidence intervals (CI). An asterisk is used to indicate statistical significant ($p < 0.05$) of the indicated treatment compared to 0.