Introduction: The treatment of grade I chondrosarcoma (CSI) is still controversial. Either wide resection or intralesional curettage with an adjuvant can be used. Patients treated with curettage and bone grafting had a 4% local recurrence rate for enchondroma and 9% for CSI(1). With the use of adjuvant cryosurgery local recurrence rate was 0% (2). Other adjuvant therapies include polymethylmethacrylate cement, phenol, and hydrogen peroxide (H₂O₂). In cell culture, 0.1M H₂O₂ kills giant cells from giant cell tumors(3), however its efficacy has not been studied in the treatment of chondrosarcoma. As a first step in the assessment of H₂O₂ as an adjuvant treatment for CSI, we studied the ability of H₂O₂ to kill chondrosarcoma cell lines in vitro.

Methods: Five human chondrosarcoma cell lines derived from grades II and III tumors, and chondrocytes from an enchondroma and articular cartilage chondrocytes were cultured to confluence, then transferred to 96-well assay plates at a density of 3.5 x 10⁴ cells/well. Each well was then treated with H₂O₂ for two minutes at concentrations from 0.1 to 1M (“standard” strength 3% H₂O₂ is 0.9M).

Cell viability was ascertained with a colorimetric assay based on the ability of cells to metabolize a tetrazolium compound to formazan by measuring UV absorbance at 490 nm.

Cytotoxicity was measured by release of lactic dehydrogenase (LDH) into the medium and was normalized to the amount of LDH released by an equivalent number of cells after freeze-thaw. These analyses were performed immediately after exposure and at 24 and 48 hour time-points. Photographs of separate 12-well plates treated identically were also taken.

Results: Figures 1 and 2 show typical cell cytotoxicity and viability curves immediately after treatment. There was minimal effect on cell viability below 0.8M, and then a dose response effect was seen. The two assays gave similar results and the results at all 3 time points were the same.

The five chondrosarcoma cell lines and chondrocytes had a viability of 0-2% after 0.9M H₂O₂ exposure and consistently 0% after 1M H₂O₂ using the formazan assay. Cytotoxicity (based on LDH release) was less than 60% below 0.7M and averaged 96% at 0.9M and 100% at 1M.

Photographs taken after exposure to 1M H₂O₂ showed dead cells floating in the media and a few cells attached to the plate with a necrotic appearance. One week of culture failed to result in any viable appearing cells.

Discussion: Our results show that H₂O₂ is cytotoxic to chondrosarcoma and chondrocytes in vitro. H₂O₂ at a concentration of 1M consistently killed 100% of chondrosarcoma cells whereas the standard 3% concentration (.9M) was only 96-98% effective. Therefore, a higher concentration is needed than is routinely available. Of note, a higher concentration is required to kill chondrosarcoma cells than giant cells.

While cryosurgery is an effective adjuvant in the treatment of benign, aggressive and low grade tumors, complications include fractures of the treated bone which sometimes do not heal, nerve palsies, and soft tissue damage. Thus H₂O₂ shows promise as an effective alternative adjuvant for the treatment of CSI and has the advantages of low cost, ease of use, and no morbidity. Further study is necessary to assess in vivo efficacy and degree of penetration into bone.

References

Figure 1. Graph of cytotoxicity (LDH release) of hydrogen peroxide on chondrosarcoma cells.

Figure 2. Graph of chondrosarcoma cell viability after exposure to hydrogen peroxide.