INTRODUCTION

Increased chondrocyte apoptosis has been observed in samples of cartilage obtained from patients with rheumatoid arthritis. Reactive oxygen species such as hydrogen peroxide (H$_2$O$_2$) are produced by inflammatory cells and synoviocytes. H$_2$O$_2$ may mediate cartilage degeneration associated with inflammatory joint diseases by inducing chondrocyte apoptosis. The mechanisms by which H$_2$O$_2$ induces chondrocyte apoptosis are not well understood; however, in other cell types, H$_2$O$_2$ increases mitochondrial membrane permeability that allows translocation of cytochrome C into the cytoplasm and formation of an “apoptosome” complex. This apoptosome complex activates proteolytic enzymes termed caspases that are key mediators of apoptosis. Agents such as cyclosporin A (CsA) and aristolochic acid (Ara A) stabilize the mitochondrial membrane and can prevent apoptosis in some cell types. Recent studies have also shown that caspase inhibitors can block chondrocyte apoptosis in vitro. Our goals were: (1) to establish a model of human chondrocyte apoptosis induced by H$_2$O$_2$; and (2) to use this model to test candidate inhibitors of chondrocyte apoptosis including mitochondrial membrane stabilizers and caspase inhibitors.

METHODS

Primary human chondrocytes were isolated from normal cartilage by enzymatic digestion and grown in monolayer at high density. Cells were split into 96-well plates, and H$_2$O$_2$ was added to final concentrations ranging from 0.4 µM to 50 µM. Cells were treated for 16 hours under standard tissue culture conditions. Chondrocyte apoptosis was quantified using the Cell Death ELISA Plus for nucleosome formation, a quantitative and highly specific measure of apoptosis (Roche, IN). Confirmation of apoptosis was obtained using TUNEL analysis (not shown). This model of H$_2$O$_2$-induced chondrocyte apoptosis was then used to test the effects of mitochondrial membrane stabilizers and caspase inhibitors. Chondrocytes were pretreated with CsA (1, 10 µM) and AraA (5, 50 µM) for one hour, and then incubated with H$_2$O$_2$ (4 µM). Caspase inhibitors were tested at a final concentration of 10 µM. The caspase inhibitors tested were: (1) a non-selective caspase inhibitor, Z-VAD; (2) a selective inhibitor of the caspase 3 family, Z-DEVD, and (3) a selective inhibitor of the caspase 1 family, Z-YVAD. Statistical analysis was performed using non-paired Student’s t-test.

RESULTS

H$_2$O$_2$-induced apoptosis in primary human chondrocytes in a dose dependent manner as measured by nucleosome ELISA (fig. 1). DNA fragmentation was confirmed using TUNEL analysis (not shown). Human chondrocytes were extremely sensitive to H$_2$O$_2$ with apoptosis occurring at concentrations as low as 2 µM. Pre-treatment with CsA and AraA did not inhibit apoptosis induced by H$_2$O$_2$. The non-selective caspase inhibitor, the caspase 3-selective inhibitor, and the caspase 1-selective inhibitor all blocked chondrocyte apoptosis induced by H$_2$O$_2$ (fig. 2b).

DISCUSSION

Hydrogen peroxide induces apoptosis in primary human chondrocytes at concentrations one to two orders of magnitude lower than those reported for bovine chondrocytes. Therefore, H$_2$O$_2$ may play a particularly important role in human joint diseases including inflammatory arthritis. In contrast to results reported in cell types such as fibroblasts, we find that mitochondrial membrane stabilizers do not inhibit H$_2$O$_2$-induced apoptosis in primary human chondrocytes. In contrast, caspase inhibitors were very effective in blocking apoptosis in this model system. Therefore, H$_2$O$_2$ appears to trigger chondrocyte apoptosis through caspase activation independent of mitochondrial membrane permeability transition. Of particular interest is the finding that the caspase 1-selective inhibitor also blocked apoptosis. Although caspase 1 is known to be expressed by chondrocytes, little is known about its role in the execution of apoptosis in this cell type. Previous studies failed to show activation of caspase 1 during apoptosis induced by agents such as topoisomerase inhibitors. These data suggest that caspase 1 may have a specific role in chondrocyte responses to H$_2$O$_2$, and that multiple caspases may be targets for therapeutic intervention.

INHIBITION OF CHONDROCYTE APOPTOSIS INDUCED BY HYDROGEN PEROXIDE

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Fig. 1. Primary human chondrocytes were treated with H$_2$O$_2$ at final concentrations of 0.4 to 50 µM. The amount of apoptosis was quantified using nucleosome ELISA. Results show means +/- S.D. (n=3).

Fig. 2. Primary human chondrocytes were treated with H$_2$O$_2$ (final concentration 4µM) in combination with candidate inhibitors of chondrocyte apoptosis. Apoptosis was quantified using nucleosome ELISA. Results show means +/- S.D. (n=3).

a) Effects of mitochondrial membrane stabilizers CsA and AraA
b) Effects of caspase inhibitors ZVAD, ZDEVD, and ZYVAD