INTRODUCTION

Chondrocyte apoptosis is associated with cartilage degeneration, and increased numbers of apoptotic chondrocytes have been observed in samples of cartilage obtained from patients with osteoarthritis. Consequently, apoptosis inhibition may prove to be a novel therapeutic approach for the treatment of degenerative joint disease. Chondrocyte survival is dependent upon intact extracellular matrix (ECM). Recent work suggests that disruption of integrin-mediated “survival signals” is a consequence of collagen degradation, and may play an important role in chondrocyte loss from cartilage degeneration. Therefore, an experimental system in which chondrocyte apoptosis is triggered by collagen degradation would be a useful and physiologically relevant model for testing potential apoptosis inhibitors. Our goals were: 1) to establish an in vitro model of human chondrocyte apoptosis induced by collagen degradation; and 2) to use this model to test candidate inhibitors of chondrocyte apoptosis.

METHODS

Primary human chondrocytes isolated from normal cartilage, and the immortalized human chondrocyte cell line C-28/I2, were grown in monolayer at high density. Cells were split into 96-well plates, and collagenase (Sigma, MO) was added to final concentrations of 0.02% to 0.08%. Chondrocyte apoptosis was quantified using a commercial ELISA assay for nucleosomes present in the cytoplasm of cells undergoing apoptosis (Roche, IN). Apoptosis was confirmed using TUNEL staining for DNA fragmentation following the manufacturer’s protocol (Intergen, NY). This model of collagen degradation-induced chondrocyte apoptosis was then used to test the effects of: (1) a non-selective caspase inhibitor, Z-VAD(OMe)-FMK (Z-VAD); (2) a selective inhibitor of the caspase 3 family, Z-D(OMe)E(OMe)VD(OMe)-FMK (Z-DEVD); (3) a selective inhibitor of the caspase 1 family, Z-YVAD(OMe)-FMK (Z-YVAD); and (4) insulin-like growth factor 1 (IGF-1). Statistical analysis was performed using Student’s t-test with p<0.05 considered significant.

RESULTS

Collagenase treatment induced chondrocyte apoptosis in a time and dose dependent manner as measured by an ELISA test for nucleosomes (fig. 1). TUNEL staining of collagenase treated chondrocytes was similar to the staining observed for chondrocytes treated with the topoisomerase inhibitor camptothecin, a well-described inducer of apoptosis (not shown). The non-selective caspase inhibitor, the caspase 3-selective inhibitor, the caspase 1-selective inhibitor, and IGF-1 all were effective in blocking apoptosis induced by collagenase treatment (fig. 2; p<0.05 for all inhibitors compared with collagenase treatment alone).

Fig. 1. Primary human chondrocytes in monolayer culture were treated with collagenase (final concentration 0.08%) in combination with potential inhibitors of apoptosis. The non-selective caspase inhibitor Z-VAD-FMK, the caspase 3-selective inhibitor Z-DEVD-FMK, and the caspase 1-selective inhibitor Z-YVAD-FMK were used at a final concentration of 10µM. IGF-1 was used at a final concentration of 200ng/ml. Cells were treated for 16 hours under standard tissue culture conditions. Apoptosis was quantified using an ELISA assay for nucleosomes. Results show means +/- S.D. (n=3).

Fig. 2. Primary human chondrocytes in monolayer culture were treated with collagenase (final concentration 0.08%) in combination with potential inhibitors of apoptosis. The non-selective caspase inhibitor Z-VAD-FMK, the caspase 3-selective inhibitor Z-DEVD-FMK, and the caspase 1-selective inhibitor Z-YVAD-FMK were used at a final concentration of 10µM. IGF-1 was used at a final concentration of 200ng/ml. Cells were treated for 16 hours under standard tissue culture conditions. Apoptosis was quantified using an ELISA assay for nucleosomes. Results show means +/- S.D. (n=3).

DISCUSSION

Collagenase induces apoptosis in primary and immortalized human chondrocytes grown in monolayer. Compared to previous studies of chondrocytes grown in suspension culture, higher concentrations of collagenase and a longer duration of treatment are required for apoptosis to occur. This model of collagen degradation-induced apoptosis may have certain advantages over other models that use inducers of apoptosis, such as topoisomerase inhibitors, that may be less physiologically relevant. The anti-apoptotic effect of the caspase inhibitors Z-VAD and Z-DEVD is consistent with recent reports that non-selective caspase inhibitors and inhibitors of caspase 3 can block chondrocyte apoptosis in vitro. The ability of the caspase 1-selective inhibitor to block chondrocyte apoptosis is novel and may be uniquely relevant to apoptosis induced by ECM disruption. To our knowledge, caspase 1 has not previously been linked directly to chondrocyte apoptosis, but its expression by chondrocytes has been confirmed. The ability of IGF-1 to decrease chondrocyte apoptosis suggests that growth factors can initiate a response that may promote chondrocyte survival after matrix disruption. Taken together, these results demonstrate that multiple pathways may be potential targets for therapies aimed at reducing chondrocyte apoptosis to prevent or delay the progression of joint degeneration.

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