The efficacy of a caspase inhibitor in models of cartilage injury

INTRODUCTION

Patients who have been exposed to a traumatic joint injury are known to have an increased risk of osteoarthritis (OA). Recent studies have shown that acute cartilage injury induces cell death that may contribute to the development of posttraumatic OA. Chondrocyte death occurs in the form of apoptosis and necrosis. Apoptosis is mediated and executed by a cascade of caspases. Chemical caspase inhibitors have been developed both as research tools, and as clinically useful therapeutics in conditions that are associated with cell death-related tissue damage. In recent studies, it has been shown that IDN-6556 a novel broad-spectrum caspase inhibitor was potent in models of liver injury in both the mouse and rat, as well as in human clinical trials. In this study, we investigated the hypothesis that IDN-6556 can prevent chondrocyte death following mechanical injury in a bovine explant model.

MATERIALS AND METHODS

Tissues: Articular cartilage explants without subchondral bone were harvested from mature bovine knees (14-30 month of age).

Caspase inhibitors: IDN-6556 (Ibud Pharmaceuticals; Pfizer, Inc.) is an irreversible, cell-permeable pan-caspase inhibitor. ZV-AD-fmk (Sigma) is a research grade pan-caspase inhibitor that has been used extensively and served as a reference reagent.

Loading apparatus and mechanical injury: Mechanical injury was applied with an Instron 8511 mechanical testing device (Instron, Norwood, MA). Each explant was centralized on a loading platform and a radially unconfined compressive load was applied through an impermeable stainless steel plate. In this study, after a small preload (0.1MPa) was applied for two minutes, a 40% strain was applied to the explants for 500ms. Control explants were placed in the loading apparatus but not loaded.

Live/dead assay: Live and dead cells were simultaneously viewed in situ with a confocal microscope (LSM 510; Zeiss, Wetzlar, Germany), using a fluorescent double stain. Cartilage explants were cultured in DMEM supplemented with Calcein AM (1µM; Invitrogen, Carlsbad, CA) and ethidium homodine-1 (8µM; Invitrogen).

Image analysis: Confocal live/dead images (n=3 for each sample) were assessed for percentage live (green) and dead (red) cells using custom software (Matlab, version 7.1. The MathWorks, Inc, MA).

Statistical analysis: Statistically significant differences between two groups were determined with t tests. The results are reported as mean ± standard deviation. P values of less than 0.05 were considered significant.

RESULTS

Activity of the caspase inhibitor IDN-6556 and comparison of cytoprotective effects with ZVAD-fmk.

To test the chondroprotective activity of IDN-6556, we performed live/dead assay using explants which were incubated in IDN-6556 at 1µM for 48 hours. Chondrocyte death was induced by mechanical injury (Fig.1B; 56.0±0.7%) compared with non-injured explants (Fig.1A; 83.1±0.6%). IDN-6556 significantly prevented the induction of chondrocyte death following mechanical injury (Fig.1C; 74.2±7.2% = 0.006) compared with no treatment. IDN-6556 was significantly more effective in preventing cell death than ZVAD-fmk at the indicated concentrations for 48 hours. (B) Time dependent effect of IDN-6556 on cell viability. After mechanical loading, cartilage explants (n=40) were treated with inhibitors at the concentrations indicated after acute injury. The explants were cultured in media with inhibitor for 48 hours and analyzed by Live/Dead assay. Values represent mean±SD. * = p<0.005

DISCUSSION

The results of this study demonstrate that a mechanical injury to bovine articular cartilage resulted in cell death that progressed up to 7 days. IDN-6556 was significantly more effective in preventing cell death than ZVAD-fmk. IDN-6556 at 1µM could prevent significantly cell death on day 1 and this effect was maintained by day 7 although IDN-6556 was only present initially.

We have previously shown that pan-caspase inhibition and a combination of caspase-3 and -8 inhibition significantly reduced cartilage degeneration induced by anterior cruciate ligament transection in vivo. A pharmaceutical such as IDN-6556 can prove effective to preserve cartilage viability in a clinical setting after traumatic joint injury and may prevent or slow the development of post-traumatic OA.

REFERENCES