Loss of Autophagy is Linked to Cell Death in Bovine Cartilage in Response to Mechanical Injury

INTRODUCTION
Apoptosis plays an important role in cartilage degradation in osteoarthritis (OA), and mechanical injury induces apoptosis in cartilage (1). Autophagy is a process for turnover of intracellular organelles and molecules that protects cells during stress and regulates cell survival (2). In this study we evaluated the potential role of ULK1, an inducer of autophagy, Beclin1, a regulator of autophagy and LC3 which executes autophagy, in the development of cartilage cell death in response to mechanical injury.

METHODS
Tissues: Full thickness articular cartilage explants were obtained from mature bovine knee joints (18-30 months).
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 platen. 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.
Caspase Inhibitor: IDN-6556 (Idun Pharmaceuticals; Pfizer, Inc.), an irreversible, cell-permeable pan-caspase inhibitor was used to determine the relationship between apoptotic cell death and autophagy.
Histology and Immunohistochemistry (IHC): Cartilage explants were fixed and paraffin embedded for safranin O – fast green staining. Antibodies to ULK1, Beclin1 (Santa Cruz Biotechnologies, CA) and LC3 (Abgent, San Diego, CA) were used for IHC.
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
Changes in ULK1, Beclin1 and LC3 expression in bovine cartilage after mechanical injury
Expression of the autophagy markers ULK1, Beclin1 and LC3 expression was decreased in superficial, middle and deep zones after mechanical stress at 48 hours (Fig. 1). This decrease was significant in all zones for ULK1 (P < 0.05) and in the superficial zone for Beclin1 and LC3 (P < 0.05).
Apoptosis inhibition increases autophagy expression in bovine cartilage.
After incubation with the caspase inhibitor IDN-6556 (1 uM), cell death decreased and expression of ULK1, Beclin1 and LC3 increased in all zones at 48 hours after mechanical injury (Fig. 2). This increase was significant in the middle zone for ULK1 (P < 0.01) and in the superficial zone for Beclin1 and LC3 (P < 0.01).

ACKNOWLEDGEMENTS: This study was supported by NIH grant AG07996 (ML) and a donation from Donald P. and Darlene V. Shiley (DDL). B. Caramés was supported by Postdoctoral Fellowship “Anxeles Alvariño”, Secretaría Xeral I+D+i, Xunta de Galicia, Spain.

REFERENCES

DISCUSSION
Autophagy, a protective cellular mechanism is decreased after mechanical injury in mature bovine articular cartilage, supporting the hypothesis that compromised autophagy represents a novel mechanism in the development of cartilage damage after mechanical injury. Furthermore, caspase inhibition increased autophagy expression, suggesting a potential link between autophagy and apoptosis. These results indicate that pharmacologic interventions that enhance autophagy and/or inhibit apoptosis may have chondroprotective activity after mechanical injury to articular cartilage.