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Chondrocyte apoptosis following intraarticular fracture in humans

H. T. Kim*†, M. Y. Lo* and R. Pillarisetty†

*Department of Orthopaedic Surgery, University of California, San Francisco, California, U.S.A.

†Veterans Affairs Medical Center, San Francisco, California, U.S.A.

Summary

Objective: The primary objective of the present study was to establish the degree to which chondrocyte apoptosis occurs *in vivo* following intraarticular fractures in humans.

Design: Fracture cartilage specimens were obtained from patients undergoing surgical intervention for fractures of the articular surface of the proximal tibia. Normal proximal tibia cartilage specimens served as controls. Apoptotic chondrocytes were identified and quantified using TUNEL analysis.

Results: The percentage of TUNEL positive chondrocytes in the fractured articular cartilage specimens (mean 18.5%, range 1–44%) was found to be an order of magnitude higher than the percentage observed in control specimens (mean 1.9%, range 0–4%).

Conclusions: The percentage of TUNEL positive chondrocytes following intraarticular fracture is much higher than that reported for chronic degenerative conditions such as osteoarthritis and rheumatoid arthritis. These data provide strong evidence that chondrocyte apoptosis is a consequence of intraarticular fracture and suggest that chondrocyte apoptosis may play a particularly significant role in the subsequent development of post-traumatic arthritis. © 2002 Published by Elsevier Science Ltd on behalf of OsteoArthritis Research Society International

Key words: Chondrocyte, Apoptosis, Fracture, Arthritis.

Introduction

The development of post-traumatic arthritis following intraarticular fracture remains a major unsolved clinical problem. Of particular interest is the possibility that extensive chondrocyte apoptosis occurs following intraarticular fracture and may contribute to the development of post-traumatic arthritis. Chondrocyte apoptosis has been associated with degenerative cartilage diseases such as osteoarthritis (OA) and rheumatoid arthritis (RA)[1–3]. The acutely traumatized joint is an ideal microenvironment for the occurrence of chondrocyte apoptosis due to the convergence of several known mediators of chondrocyte programmed cell death, including matrix injury, the influx of inflammatory cells, the release of proinflammatory cytokines, the generation of reactive oxygen species, and the potential for continued joint injury from mechanical overload. The objective of the present study was to establish the degree to which chondrocyte apoptosis occurs *in vivo* following intraarticular fractures in humans.

Methods

Pieces of cartilage that otherwise would have been discarded were collected from patients undergoing surgery for intraarticular fractures of the proximal tibia. For this study, a single joint surface was selected for analysis based on preliminary data suggesting that cartilage from different joint surfaces may respond differently to mechanical injury. Exclusion criteria were: patient age >60, radiographic evidence of pre-existing joint pathology, or evidence of concurrent joint infection. A total of 15 specimens were obtained from 12 patients. The median age was 39 (range 21–57); 10 patients were male and two patients were female. Control samples of proximal tibial articular cartilage were collected from grossly normal knees at the time of limb amputation or joint resection as part of limb salvage surgery, and screened by light microscopy for degenerative changes. All tissue collection was performed following procedures approved by the Committee on Human Research and Institutional Review Board.

Specimens were fixed in 10% buffered formalin, decalcified, dehydrated, and embedded in paraffin for routine histology. The embedded specimens were sectioned at 5 μm thickness. DNA fragmentation analysis for apoptosis (a.k.a. TUNEL) was performed using the ApopTag Direct apoptosis detection kit (Oncor, MD). DAPI was used as a counter stain to label all nuclei. Labeled sections were examined using fluorescence microscopy with appropriate filters, and data was recorded using a Zeiss Axiocam digital camera. 'Percent positive cells' was defined as the number of TUNEL positive cells divided by the number of DAPI positive cells. Measurements for each specimen were taken from three near-adjacent sections, and then averaged. Each captured field measured 1.3×1.0 mm, and included the area of maximal apoptosis. Selected sections were subjected to a modified TUNEL technique as described by Aigner and colleagues to minimize non-specific labeling[4]. Independent confirmation of chondrocyte apoptosis was performed on selected specimens using

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Address correspondence to: Hubert T. Kim, MD, PhD, SFVAMC (112), 4150 Clement St., San Francisco, CA 94121, U.S.A. Tel: 415-221-4810 ext: 2940; Fax: 415-750-2181; E-mail: hubert.kim@med.va.gov

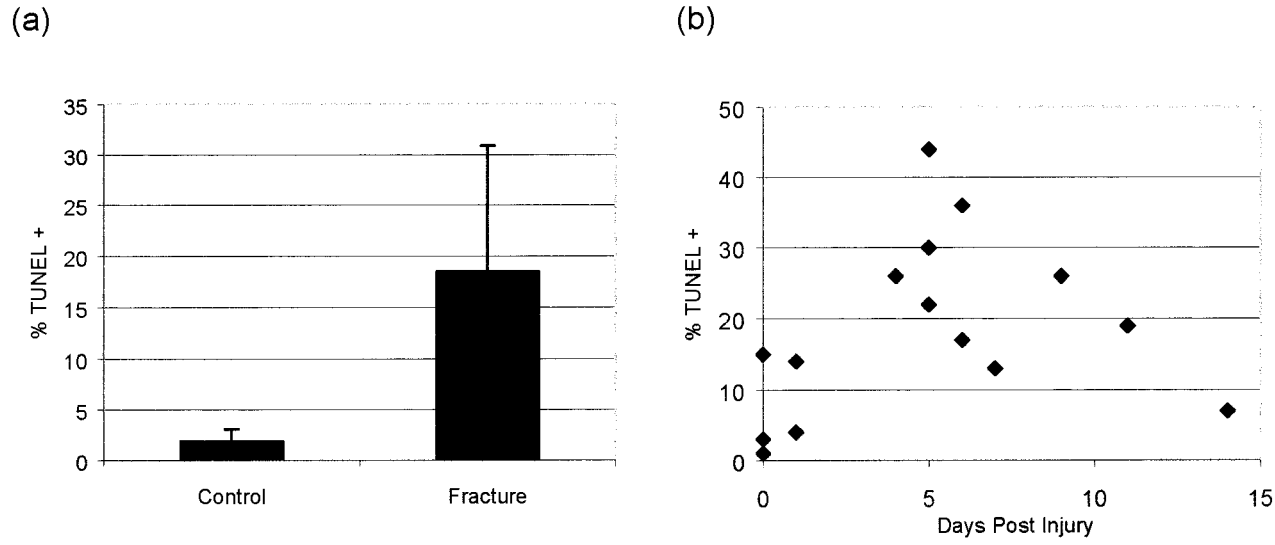

Fig. 1. (a) Percentage of TUNEL positive cells from cartilage specimens retrieved from patients with tibial plateau fractures and from normal controls (means with S.E.; $P<0.0001$). (b) Scatter plot of TUNEL positive cells vs time from injury to sample collection. Each data point represents a single specimen (mean of measurements obtained from three sections).

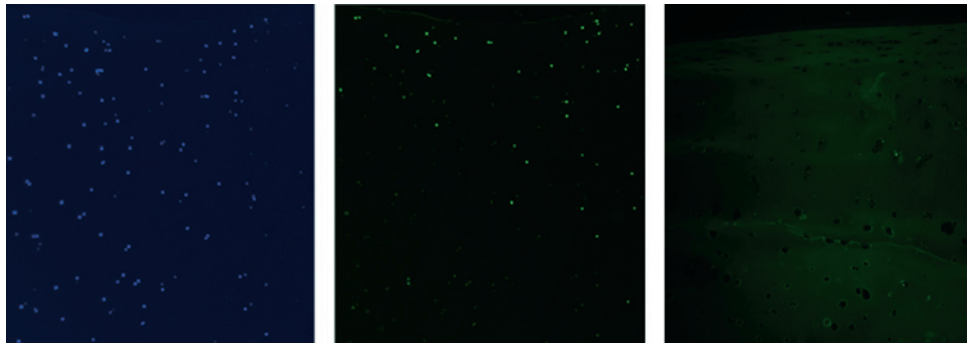

Fig. 2. DAPI staining (left panel) and TUNEL analysis (middle panel) of cartilage retrieved from a 32-year-old patient with an intraarticular fracture of the proximal tibia. TUNEL identifies cells with DNA fragmentation that is a hallmark of apoptosis. Fracture surface is on the right; articular surface is at the top. TUNEL analysis of control cartilage is shown in the right panel.

Hoechst 33342 staining for the analysis of nuclear morphology[5] and immunohistochemistry using antiPARP p85 fragment antibody(Promega, WI), a highly specific marker for apoptosis[6]. Statistical analysis was performed using Mann–Whitney test for non-parametric analysis.

Results

Markedly increased numbers of TUNEL positive chondrocytes were noted in the fracture specimens compared with normal controls. The mean percentage of TUNEL positive cells in fracture specimens was 18.5 (standard deviation 12.4; range 1–44); the mean percentage in control specimens was 1.9 (standard deviation 1.2; range 1–4) [Fig. 1(a)]. The highest percentage of TUNEL positive cells was observed in samples collected approximately five days after injury [Fig. 1(b)]. The distribution of TUNEL positive cells varied significantly among the different specimens. In a majority of samples, TUNEL positive chondrocytes were concentrated at the fracture edge with extension into the superficial and middle zones of the cartilage (Fig. 2). In other samples, the distribution of TUNEL positive chondrocytes was more uniform throughout the entire section. Modifications to the TUNEL technique designed to maximize specificity for apoptotic cells[4] resulted in a slight decrease in the observed percentage of positive cells, but this difference was not statistically significant. We noted good correlation between TUNEL positive cells and nuclear morphology consistent with apoptosis including nuclear condensation and blebbing. Positive staining with anti-PARP p85 provided independent confirmation of chondrocyte apoptosis.

Discussion

In spite of limitations inherent in this type of retrieval study—including a relatively modest number of specimens and the inability to control the precise location of sampled tissues—the data provide strong evidence that abundant chondrocyte apoptosis is a consequence of intraarticular fracture. These findings are consistent with a recent report documenting increased levels of apoptosis in biopsies of human cartilage obtained one to six months after acute

knee injury[7]. The signals that mediate chondrocyte apoptosis following cartilage injury are not clear. A major contributing factor may be the loss of pro-survival signals resulting from extracellular matrix degradation. Chondrocyte survival is known to be dependent upon intact matrix, and chondrocyte apoptosis has been linked to matrix degeneration as measured by Safranin O staining of proteoglycans[1]. The relationship between proteoglycan depletion and apoptosis was not studied in a systematic fashion in this study; however, we have observed a similar association in an *in vivo* rabbit model of acute cartilage injury (data not shown).

The general increase in the percentage of TUNEL positive cells over the first five to six days following fracture is similar to that observed in recent *in vitro* studies of cartilage explants subjected to mechanical injury[8–9]. This delay in peak apoptosis may represent a window of opportunity for intervention as suggested by D'Lima and colleagues[9]. Recent studies have demonstrated that inhibitors of caspases, key enzymatic mediators of apoptosis, can inhibit chondrocyte death and maintain chondrocyte functionality *in vitro*[9–12]. Given the potential toxicity of long-term treatment with caspase inhibitors, short-term use following intraarticular injury may prove to be the optimal application for these anti-apoptotic agents. Furthermore, the high levels of chondrocyte apoptosis observed following intra-articular fracture in humans suggest that interventions aimed at limiting chondrocyte loss through apoptosis inhibition may be particularly effective in the setting of acute trauma.

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