INTRODUCTION

The development of post-traumatic arthritis following intra-articular fracture remains one of the major unsolved problems facing orthopaedic surgeons. Of particular interest is the possibility that chondrocyte programmed cell death, or apoptosis, occurs following intra-articular fracture and may contribute to the development of post-traumatic arthritis. Samples of cartilage obtained from patients with osteoarthritis and rheumatoid arthritis demonstrate increased levels of chondrocyte apoptosis. However, relatively little is known about chondrocyte apoptosis following acute cartilage injury in vivo. A comprehensive understanding of chondrocyte apoptosis following acute cartilage injury is critical in view of recent findings that inhibitors of caspases, key enzymes in the apoptotic pathway, can rescue chondrocytes from undergoing programmed cell death. The objective of the present study was to establish the extent to which chondrocyte apoptosis occurs in vivo following tibial plateau fractures in humans.

METHODS

Specimens were collected from patients undergoing surgery for repair of tibial plateau fractures. Exclusion criteria were: patient age > 60, radiographic evidence of underlying joint pathology, or evidence of concurrent joint infection. Any cartilage that was to be discarded at the time of surgery was placed in 10% buffered formalin and kept at 4°C overnight. A total of twelve specimens were obtained from ten patients. Control samples of tibial plateau cartilage were collected from normal knee joints at the time of normal amputation for trauma or malignancy. Collection of control specimens was restricted to areas distant from known pathology. Specimens were screened by light microscopy for pre-existing degenerative changes and excluded if abnormalities were noted. All tissue collection was performed following procedures approved by the Committee on Human Research.

Cartilage specimens were decalcified in 0.45M EDTA, pH 8.0, and processed for routine histology. The paraffin-embedded specimens were sectioned at 5μm thickness. DNA fragmentation analysis for apoptosis (aka TUNEL) was performed using the ApopTag Direct apoptosis detection kit (Oncor, MD), following the manufacturer's protocol with the addition of a hyaluronidase digestion step. DAPI was used as a counterstain 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 1.0 x 1.3 mm, and included the area of maximal apoptosis. Independent confirmation of chondrocyte apoptosis was performed on select specimens using an anti-PARP p85 fragment antibody (Promega, WI), a highly specific marker for caspase activation and apoptosis. Statistical analysis was performed using unpaired Student’s t-test as well as Mann-Whitney test for non-parametric analysis.

RESULTS

Markedly increased numbers of TUNEL positive chondrocytes were noted in the fracture specimens compared to normal controls. The mean percentage of TUNEL positive cells in fracture specimens was 19.1 (standard error 3.9; range 1-44); the mean percentage in control specimens was 2.7 (standard error 0.5; range 1-6) (fig.1). Increased numbers of TUNEL positive cells were not observed in two specimens collected on the day of injury. Specimens collected after the day of injury consistently demonstrated obvious increases in the number of TUNEL positive cells (Fig. 2). The distribution of TUNEL positive cells varied significantly among the different specimens. In many samples, TUNEL positive chondrocytes were concentrated at the fracture edge with extension into the superficial and middle zones of the cartilage (Fig. 3). In other samples, the distribution of TUNEL positive chondrocytes was more uniform throughout the entire section.

DISCUSSION

In spite of the limitations inherent in this type of retrieval study—including a relatively small number of specimens, the inability to control sampling location and timing, inconsistent specimen size and shape, and limitations of the TUNEL technique itself—the data clearly demonstrate that chondrocyte apoptosis is a real consequence of intra-articular fracture. The amount of apoptosis observed is extremely high, and suggests a possible link to the subsequent development of post-traumatic arthritis. The traumatized joint is an ideal microenvironment for chondrocyte apoptosis, bringing together several known mediators of chondrocyte programmed cell death including matrix injury, an influx of inflammatory cells, pro-inflammatory cytokines, reactive oxygen species, and potential mechanical overload. Fortunately, joints also are uniquely suitable for local administration of drugs targeted at apoptosis prevention. These agents could potentially limit chondrocyte loss following intra-articular fractures and improve patient outcomes.

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