PREVENTION OF APOPTOSIS REDUCES ARTHRITIS IN VIVO

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Introduction:
Cartilage injury has been shown to result in chondrocyte death. This study investigates the nature of cell death and whether inhibiting cell death affects the development of post-traumatic osteoarthritis in an animal model.

Methods:

In vitro
Full-thickness cartilage explants (5mm diameter disks) were harvested from fresh normal human cadaver donor patella. Explants were allowed to stabilize in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum, for 48 hours. Explants were then divided into 3 groups: Load, Load+CI and Control. Load and Load+CI groups were subjected to radially unconfined mechanical stress (30% strain applied for 500 msec). Loaded+CI groups were additionally cultured in media containing 100 µM z-VAD.fmk (a broad spectrum caspase inhibitor). Control explants were not loaded. At 96 hours after injury, explants underwent histologic examination and the number of apoptotic cells counted using TUNEL. Apoptosis was confirmed in select samples by electron microscopy and immunostaining of a neo-epitope of cytokeratin.

In vivo
After obtaining Institutional Animal Review Committee approval 8 New Zealand White rabbits were divided into ACLT and ACLT+CI groups. Both groups underwent bilateral anterior cruciate ligament transection. The ACLT+CI group was treated with intra-articular injections of 25 µg of z-VAD.fmk three times a week for six weeks while the control group received saline injections. Rabbits were euthanized at six weeks and femoral and tibial articular cartilage evaluated by India ink staining, and histologic Mankin grading after Safranin-O stain.

Results:

In vitro
Chondrocytes in Control explants demonstrated mean 8.7 (+6.3) % apoptosis while Loaded explants, mean 23.6 (+17.4) % (p<0.01). This was reduced to 12.5 (+4.8) % in Loaded+CI explants (p<0.05).

In vivo
Under India ink examination, all the ACLT rabbits demonstrated consistent cartilage lesions on both femoral condyles, lateral tibial condyles and posteromedial tibial condyles, ranging from grade III (overt fibrillation) to grade IVA (erosions < 5mm). The ACLT+CI rabbits demonstrated lesions that were smaller in area. The grade ranged from II (minimum fibrillation) to III, with only one rabbit having a Grade IVA lesion. Histologically, more knees from the ACLT group had higher Mankin grades. 7 out of 8 ACLT knees had grades 5 or higher, while 4 of the 8 ACLT+CI knees demonstrated grades 5 or higher.

Discussion:
We have previously shown that mechanical injury leads to chondrocyte apoptosis and that the percentage of cells undergoing apoptosis increases over time after injury.¹ This series of experiments was designed to determine whether certain agents could prevent this apoptosis. Apoptosis is mediated by a cascade of aspartate-specific cysteine proteases or caspases. z-VAD.fmk is a cell-permeable fluoromethylketone inhibitor of a broad spectrum of caspases, including the caspases 3, 8 and 9 that have been implicated in apoptosis. The in vitro study demonstrates that caspase inhibition can prevent apoptosis in a consistent manner in human cartilage. The in vivo study provides preliminary evidence that administration of z-VAD.fmk appeared to reduce the arthritic grades on both gross and histologic examination. This suggests a novel approach at chondroprotection targeting primarily the cells rather than the matrix.


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