CHONDROCYTE APOPTOSIS IS INHIBITED BY MINOCYCLINE FOLLOWING EXPERIMENTAL CARTILAGE INJURY IN VIVO

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INTRODUCTION:
Acute cartilage injury may contribute to the subsequent development of post-traumatic osteoarthritis by inducing chondrocyte apoptosis, or programmed cell death (PCD). Chondrocyte PCD is directly promoted by nitric oxide (NO), matrix degradation, and activation of caspase enzymes. In addition to their action as broad-spectrum antimicrobials, tetracyclines have recently been shown to inhibit NO synthetases, matrix metalloproteinases (MMPs), and caspas. In this study we tested the hypothesis that intra-articular treatment with minocycline could block chondrocyte apoptosis induced by acute osteochondral injury in vivo.

METHODS:
A cooled 2mm drill bit was used to create 2-3mm deep osteochondral injuries to the femoral condyles of 12 adult New Zealand White rabbits. To insure consistent drug delivery into the knee joints, an intra-articular catheter was placed prior to wound closure. Animals in the treatment group received daily intra-articular injections of minocycline (100um, 0.5cc). Animals in the control group received daily intra-articular injections of vehicle alone. Treatment was initiated immediately after wound closure and was continued for four consecutive days. Following the treatment period, animals were euthanized on post-op day 4. Paraffin embedded sagittal sections were stained for apoptosis by TUNEL. DAPI counterstain was used to determine the total cell count.

TUNEL images were captured at 5x at 5.2megapixel resolution, 1um/pixel. Each drill hole was treated as an independent specimen. The area of analysis included the full thickness of articular cartilage extending out to 2.0 mm away from both medial and lateral edges of the injury site. The area of analysis was further subdivided into 0.5mm zones radiating outwards from the injury site with each zone encompassing the full thickness of articular cartilage. For each 0.5mm zone, total cell count and TUNEL positive cell count were measured using semi-automated image analysis software. Statistical analysis was performed using two-tailed unpaired t-tests. The results are presented as the apoptotic index (TUNEL positive/total cells) +/- s.e.m.

Safranin-O staining of representative sections was performed to assess the effect of minocycline treatment on proteoglycan loss following osteochondral injury.

RESULTS:
Immediate treatment with 100um minocycline markedly reduced overall chondrocyte PCD compared to untreated controls, as measured by TUNEL analysis. Reduced PCD was statistically significant in areas adjacent to site of injury at 0-0.5mm (group A) [treated = 0.20 ± 0.02; controls = 0.37 ± 0.04; p<0.001], 1-1.5mm(group C) [treated = 0.04 ± 0.01; controls = 0.10 ± 0.02; p<0.02], and 1.5-2.0mm(group D) [treated = 0.03 ± 0.01; controls = 0.09 ± 0.02; p<0.05]. Although reduced PCD was also observed 0.5-1mm (group B) from the site of injury [treated = 0.06 ± 0.01; controls = 0.10 ± 0.02; p<0.05], the difference was not statistically significant.

DISCUSSION:
One possible therapeutic approach for the prevention of post-traumatic osteoarthritis is limiting chondrocyte loss by inhibiting apoptosis. Recently, we have reported that intra-articular administration of agents such as caspase inhibitors can block chondrocyte PCD in vivo. However, the toxicity of these agents may make them unsuitable for clinical application. The ideal anti-apoptotic agent must have good bioavailability and a favorable side-effect profile. Minocycline is widely used as a safe, effective antimicrobial agent. These data demonstrate reduction of chondrocyte apoptosis by minocycline delivered directly into the knee joint. Additional experiments will need to be performed to determine if similar effects can be obtained with oral administration and if treatment leads to long-term cartilage preservation.

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REFERENCES: