**Type II Collagen Degradation After Anterior Cruciate Ligament Transection In The Rat**

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**Introduction:**
Degradation of type II collagen is thought to be a pivotal process in articular cartilage destruction during osteoarthritis (OA) but little is known about the kinetics of this process during the early phase of the disease. Therefore type II collagen destruction was studied at different time points after induction of experimental OA in the rat. Damage to the collagen network was investigated by analysis of type II collagen neoepitope expression. Cleavage of type II collagen by collagenases (MMP's) was detected by the Col2-3/4C-short antibody and collagen denaturation by Col2-3/4m.

**Methods:**
Osteoarthritis was induced in male Wistar rats by transection of the ACL in the left knee joint. The right knee joint underwent a sham operation without cutting the ACL. Animals were sacrificed after 2, 7, 14, 28 and 70 days whereafter both knee joints were dissected. The animal welfare committee approved all animal procedures. Knee joints were decalcified using EDTA-PVP and coronal cryosections were made. Sections were stained by HE and immunostaining was performed using the Col2-3/4C (Collagenase-cleavage site) or the Col2-3/4m antibody (denatured type II collagen).

**Results:**
The first changes after the ACL-transection were chondrocyte death at the margins of the articular cartilage of the tibia and femur. At day seven a pannus-like tissue protruded from the synovial tissue over the dead cartilage. Underneath the pannus-like tissue a marked staining for the collagenase cleavage neoepitope was observed (figure 1). The dead cartilage was replaced by fibrocartilage within 4 weeks after which the staining for the collagenase cleavage neoepitope had completely disappeared.

**Discussion:**
Experimental OA in rats after ACL transection can be divided in two stages. An early phase lasting about 4 weeks, in which chondrocyte death at the cartilage margins is followed by remodeling of the dead cartilage. In this phase marked degradation of type II collagen by collagenases occurs. The second phase, characterised by cartilage damage in the central tibia and femur, shows increased staining for denatured type II collagen but little staining for the collagenase cleavage neoepitope. This observation can be explained by fast removal of the collagenase cleavage site staining or might indicate deterioration of the collagen network due to mechanical overload.

**Figure 1:** Intensive staining for the collagenase cleavage neoepitope (Col2-3/4C) in tibial cartilage of rats after ACL-transsection (day 7).

In contrast with the peripheral cartilage, in the central part of the medial tibia and femur dead chondrocytes were found on week 2 until the last time point examined (week 10). On these sites the dead cartilage was not replaced by fibrocartilage in this time span. In these areas, severe loss of proteoglycans, fibrillation of superficial cartilage and pronounced staining for denatured type II collagen was found (figure 2). Both cartilage damage and staining for denatured collagen increased with time. In the central cartilage areas only light collagenase cleavage site staining was observed on all time points.

**Figure 2:** Central cartilage after ACL-transsection showing extensive staining for denatured type II collagen in location with chondrocyte death and cloning.

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