TOWARDS AN ANIMAL MODEL FOR JOINT REGENERATION

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Purpose: Osteoarthritis is a disease of progressive joint failure, leading to pain and disability. Only symptomatic treatment options exist. A key prerequisite is the absence of endogenous repair capacity of adult mammalian joint structures. Amphibians are able to regenerate lost limbs, thus we hypothesized that endogenous repair may be possible in amphibians after local joint injury.

Methods: Knee joints of adult newts (nototusitalamus viridescens viridescens) were treated intraarticularly with collagenase in analogy to murine models of osteoarthritis. The clinical and histological course was analysed.

Results: Clear cut joint injury was observed by inspection, with a clinical score (incorporating swelling, spontaneous joint use, deformity, and range of motion), and with histologic analysis after treatment. The severity of joint damage increased over the first three weeks and then abated. Disruption of joint anatomy with cartilage loss was confirmed by magnetic resonance imaging. Histologically, loss of proteoglycan and collagen II staining was observed in addition to thinning of cartilage. Chondrocytes of the femoral and tibial joint underwent cell death. Beginning after three and five weeks, evidence for mesenchymal progenitor cell recruitment with chondrogenic differentiation was observed. Clinical use of joints was normal within five weeks, histological healing still continued after 12 weeks of observation. Currently, the observation period is extended and molecular factors involved are determined by RT-PCR of joint extracts.

Conclusions: Joint injury can be induced in the newt with approaches that are used to induce arthritis in murine models. In contrast to murine models, newts are able to recruit progenitor cells in order to induce the regeneration of joint structures. Further studies will help to elucidate cellular and molecular mechanisms.

SYNOVIAL TISSUE; A CANINE IN VIVO MODEL OF OSTEOARTHRITIS

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Purpose: Joint bleeds lead to joint destruction. In vitro exposure of human and canine cartilage to blood results in long lasting severe adverse changes in cartilage. An in vivo joint haemorrhage in the canine knee joint demonstrates similar adverse effects although less outspoken and long-lasting. We investigated the clearance rate of blood from canine knee joints as a possible explanation for this discrepancy.

Methods: Blood was injected into the knee joint of Beagle dogs, either 48h, 24h or 15 m before termination. The amount of red and white blood cells present in the joint cavity was determined. Chondrocyte activity and cartilage matrix integrity as well as cartilage destructive activity of synovial tissue were determined biochemically. Additionally, synovial tissue was analyzed by use of histochemistry.

Results: Fifteen minutes after the injection of autologous blood, the red blood cell count was 5.7 × 1012/L, comparable to the amount present in whole blood, and gradually decreased (1.6 × 1012/L at 24 hours) to 0.3 × 1012/L within 48 hours (less than 5%). The amount of white blood cells increased in the first 24 hours, and was still increased after 48 hours, although less than after 24 hours. The proteoglycan synthesis rate and -release were adversely affected already within 24 hours (~22% and +24% respectively), and these effects were more severe 48 hours post-injection (~34% and +53% resp.). Synovial tissue culture supernatants demonstrate cartilage destructive properties as expressed by an increased release, a decreased synthesis rate, and decreased content of cartilage proteoglycans; increasing with time after the experimental haemorrhage (+207%+/−247%; −58%–62%; −8%–28% respectively, for 24/48 hours).

A NOVEL AGE- AND STRAIN-DEPENDENT IN VIVO MODEL OF ARTICULAR CARTILAGE HEALING IN MICE

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Purpose: To optimize and validate an in vivo model of mechanical cartilage injury and regeneration in mice. Chondral injuries are frequent, and can either improve spontaneously or acquire a chronic symptomatic course, which may require surgical intervention, and may require chondrocyte transplantation. Research in this field is hampered by the lack of mouse models of joint surface regeneration which would allow the use of mouse genetics to study molecular function. In this study we have validated a mouse model of joint surface injury and repair with a strain and age-dependent outcome.

Methods: Full thickness defects were generated in the patellar groove of adult C57BL/6 and DBA/1 mice by microsurgery. Control knees were either sham-operated or non operated. Outcome was evaluated by histological scoring systems. Apoptosis and proliferation were assessed using TUNEL and Phospho-Histone H3 staining. Type II collagen neo-differentiation and degradations were evaluated by immunostaining using antibodies to the CII telopeptide and C1,2G (Co1,2G/3,4G) respectively. Aggrecanase and MMPs activity were assessed by immunostaining for TEGE373 and VDIPEN neoepitope.

Results: Eight weeks following surgery, adult eight weeks old DBA/1 mice displayed consistent repair of the defects with safranin-O positive cartilage tissue. Age matched C57BL/6 mice repaired poorly and developed osteoarthritic (OA) features. Cartilage injury induced apoptosis and matrix remodelling in both strains. However, compared to C57BL/6, DBA1 mice displayed a progressive decline of chondrocyte apoptosis, persistent cell proliferation within the repair tissue, persistent type II collagen neo-deposition, less type II collagen degradation, less aggrecanases-induced aggrecan degradation, and OA, and MMP-induced aggrecan degradation. Aged eight months old DBA/1 mice failed to repair, but, contrary to age-matched C57BL/6 mice, developed no signs of OA.

Conclusions: We have generated a murine model of cartilage regeneration in which the outcome of joint surface injury is strain and age dependent. This model will allow testing the function of different molecules in the context of joint surface regeneration in adult mammals using genetic models.