The BMP7-Derived Peptide p[63-82] Reduces Cartilage Degeneration in the Rat ACLT–pMMx Model for Post-Traumatic Osteoarthritis

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INTRODUCTION:
Osteoarthritis (OA) is characterized by articular cartilage erosion, pathological subchondral bone changes, signs of synovial inflammation and pain. We previously identified p[63-82], a bone morphogenetic protein 7 (BMP7)-derived bioactive peptide, that attenuates structural cartilage degeneration in the rat medial meniscal tear-model for post-traumatic OA[1]. The present study aimed to broaden the scope by evaluating the cartilage erosion-attenuating activity of p[63-82] in a different less progressive pre-clinical model for OA (anterior cruciate ligament transection - partial medial meniscectomy (ACLT-pMMx)), and include a longer follow-up time of 10 weeks to determine the disease-modifying action of the p[63-82]. Additionally, potential disease-modifying activity in the subchondral bone compartment of the knee joint, as well as uncovering any p[63-82]-dependent functional improvement in weight-bearing and gait were evaluated. We hypothesized that frequent intra-articular administration of p[63-82] is able to structurally and functionally attenuate the course of OA development in the rat ACLT-pMMx model for post-traumatic OA.

METHODS:
The animal study was reviewed and approved by the Maastricht University Animal Ethics Committee (Protocol No. WP2018-004-001). Sample size was calculated according to the formula of L. Sachs n=(sigma/delta)2 * 15.7) and corrected for potential drop-out, resulting in 7 animals per group for the 5-week follow-up and 10 animals per group for the 10-week follow-up. Skeletally mature male Lewis rats (mean age 3 months, average weight 360,6 ± 14.2 g) underwent ACLT-pMMx surgery. One week post-surgery, rats received weekly intra-articular injections with either saline or 500 ng p[63-82]. Five and 10 weeks post-surgery, rats were sacrificed. Histopathological evaluation of cartilage degradation and OARSI-scoring was performed following Safranin-O/Fast Green staining. Pain-related behavior was measured by incapacitance testing and footprint analysis pre-surgery, one week post-surgery and at 5 or 10 weeks post-surgery. Subchondral bone characteristics were determined using µCT (µCT 100, Scanco Medical, resolution of 10 µm). Statistical significances were determined using GraphPad PRISM 5.0 (La Jolla, California, USA). Statistical significance was determined by a 2-tailed Mann-Whitney U test. The statistical significance of all tests was set at $p \leq 0.05$.

RESULTS SECTION:
Histopathological evaluation at 5 and 10 weeks post-surgery showed reduced cartilage degeneration and a significantly reduced OARSI-score. For the saline treated joints more enlarged (hypertrophic) chondrocytes were detected, as well as more fibrillation and in general less Safranin-O positivity compared to the peptide treated group. While ACLT-pMMx surgery induced significant changes in the tibia plateau subchondral bone compartment (µCT), no significant changes were observed in subchondral bone characteristics of the p[63-82]-treated rats compared to the saline-treated rats. ACLT-pMMx-induced imbalance of static weight-bearing capacity in the p[63-82] group was significantly improved compared to the saline-treated rats at weeks 5 post-surgery. Footprint analysis scores in the p[63-82]-treated rats demonstrated improvement in gait at week 10 post-surgery.

DISCUSSION:
In the current study, we aimed to investigate whether the protective effect of p[63-82] on cartilage degeneration could be translated to other pre-clinical models for OA. The rat ACLT-pMMx model is less progressive than the rat MMT model and involves an anterior cruciate ligament-dependent biomechanical destabilization of the knee joint. We showed that weekly intra-articular injections of p[63-82] in the rat ACLT-pMMx post-traumatic OA model resulted in a functional improvement in static weight-bearing capacity during follow-up, and specifically reduced tissue degenerative changes in the articular cartilage layer at both 5 and 10 weeks follow-up. This strengthens our previously conducted study[1], enhancing the clinical translatability of p[63-82].

SIGNIFICANCE/CLINICAL RELEVANCE: The disease-modifying outcomes of the current p[63-82] study strengthen the conclusions of our previously conducted p[63-82] study[1]. The BMP7-derived peptide p[63-82] provides potential novel disease-modifying treatment options for OA.

REFERENCES:
[1] Caron MMJ, Ripmeester EGJ, *et al.* TJM. Discovery of bone morphogenetic protein 7-derived peptide sequences that attenuate the human osteoarthritic chondrocyte phenotype. Mol Ther Methods Clin Dev. 2021 Mar 17;21:247-261. doi: 10.1016/j.omtm.2021.03.009. PMID: 33850953; PMCID: PMC8022858.