been implicated as a biomarker of disease severity in established OA, further work is ongoing to establish whether these molecules are predictive of OA risk after acute knee injury.

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OSTEOARTHRITIC SCLEROTIC SUBCHONDRAL OSTEOBLASTS SECRETED ELEVATED CONCENTRATION OF FIBULIN-3 FRAGMENTS IN VITRO

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Purpose: To determine the expression of fibulin-3 gene by human osteoarthritic (OA) subchondral osteoblasts and the concentration of three fibulin-3 epitopes (Fib3-1, Fib3-2 and Fib3-3) in OA osteoblasts culture supernatants. The fibulin-3 neo-epitopes, originally discovered by proteomics studies, are recently shown to be diagnostic and prognostic biomarkers of OA.
Methods: OA osteoblasts were isolated from sclerotic (SC) or non-sclerotic (NSC) zones of OA subchondral bone of nine men undergoing total knee replacement. OA osteoblasts cultures were stopped at two different maturation stages, either pre-osteoblasts (after 3 days in 10% FBS) or mature osteoblasts (after further 14 days of culture in 2% ultroser G, proline 20 μg/ml, ascorbic acid 50 μg/ml and 1.25 dihydroxycholecalciferol 10−8 M). Maturation level was assessed by alkaline phosphatase activity and mineralization capacity. We evaluated the expression of fibulin-3 gene by RT-PCR in SC and NSC osteoblasts, and the concentration of three neo-epitopes, Fib3-1, Fib3-2 and Fib3-3 by specific competitive ELISAs.
Results: Fibulin-3 gene was 2.3-fold overexpressed in SC compared to NSC pre-osteoblasts ($p = 0.0255$ $n = 5$) and 1.3-fold in mature SC osteoblasts compared to NSC osteoblasts. The concentration of the three neo-epitopes were increased in SC pre-osteoblasts supernatants compared to NSC, but the difference was only significant for Fib3-3 [Fib3-1 (1.44-fold $p = 0.13$, $n = 4$) Fib3-2 (1.32-fold $p = 0.21$, $n = 7$) Fib3-3 (1.45-fold $p = 0.01$, $n = 9$)]. In contrast, the three fibulin-3 neo-epitopes levels were significantly higher in mature SC osteoblasts than in mature NSC osteoblasts culture supernatants [2.53- fold for Fib3-1 ($p = 0.0069$, $n = 7$), 2.41-fold for Fib3-2 ($p = 0.0364$, $n = 7$) and 1.37-fold for Fib3-3 ($p = 0.0105$ $n = 9$)].
Conclusions: Fibulin-3 fragments, further to be associated immunohistochemically with cartilage superficial layer degradation/fibrillation, could be a marker of the osteochondral plate remodeling in osteoarthritic joints. These findings contribute to explain the increased concentration of fibulin-3 neo-epitopes in sera and urines of OA patients.

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SYNOVIAL FLUID LEVELS OF IL-6 AND MMP'S IN A MILD EQUINE LPS MODEL

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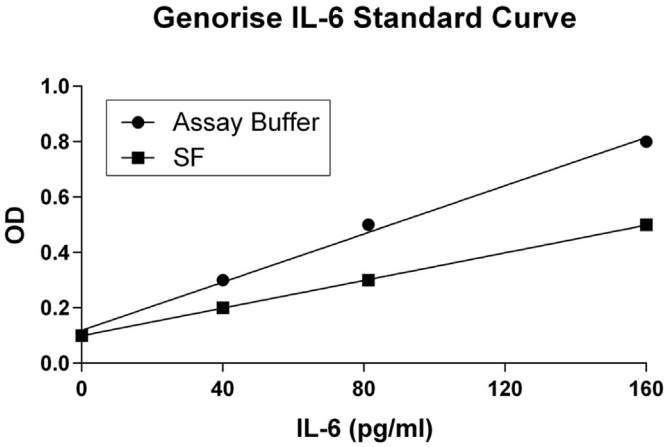
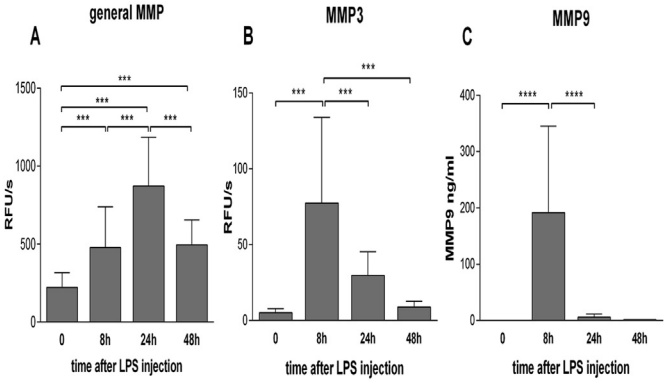
Purpose: The intra-articular lipopolysaccharide (LPS) model is well established in horses to investigate the inflammatory component of joint disease and to evaluate analgesic and anti-inflammatory medication. Time-dependent upregulation of inflammatory mediators and markers of cartilage turnover can be determined in sequential synovial fluid (SF) samples. In this study we aimed to optimize quantitative measurements of interleukin-6 (IL-6) and matrix metalloproteinaeses (MMPs) in equine synovial fluid.
Methods: Inflammation was induced in the right middle carpal joint of 24 healthy Standardbred horses by intra-articular challenge with 0.5 ng lipopolysaccharide of E. Coli (L5418 Sigma-Aldrich). Synovial fluid samples were taken immediately prior to LPS-injection, and 8, 24 and 48 hours post-injection and stored at −80 degrees until biochemical assays were performed. IL-6 was measured using commercially available ELISA kits from Cusabio (SEA079Eq for equine IL-6), Genorise Scientific (106001-SF for equine synovial fluid) and Invitrogen (CHC1264 human cytoset), as well as a homemade ELISA using R&D horse-specific antibodies (AF1886, BAF1886) and recombinant equine IL-6 (R&D 1886-EL). Endogenous general MMP activity and MMP3 activity were measured using the fluorescent substrates FS-6 (Bachem M-2350) or MMP3green (Abcam, 112148), respectively and results were expressed as relative fluorescent units/second (RFU/s). MMP9 was measured using the Quickzyme human MMP9 activity colorimetric assay with and without APMA activation of pro-MMP9. Samples were tested according to assay instructions. Because hyaluronidase treatment is often advised for SF samples, both hyaluronidase treated (10 ng/ml) and untreated samples were tested at different dilutions. Standard curves were made in de appropriate assay buffer and in synovial fluid.
Results: Equine IL-6 could not be detected using the Invitrogen or Cusabio ELISA kits and the homemade (R&D) ELISA was not sensitive enough (detection limit 1 ng/ml). Using the Genorise equine SF ELISA the highest IL-6 levels were measured at 8 hours after LPS injection (73.2 pg/ml) compared to 19.1 pg/ml at $t = 0$ ($p<0.001$). SF was found to interfere with detection, which is most likely due to matrix components in the SF. Therefore the standard curve was made in SF instead of assay buffer (figure 1). We observed no effect of hyaluronidase on IL-6 measurements.



General (endogenous) MMP measurements using the FS-6 substrate showed a high detection range and sensitivity. Samples were diluted at least ten times in assay buffer and EDTA was added to obtain negative controls. Highest levels were observed 24 hours after LPS injection, and MMP activity was still elevated after 48 hours (figure 2A). The MMP3-specific fluorescent substrate showed less sensitivity and required 2-20x dilution of SF samples. Interestingly, highest MMP3 activity was observed 8 hours after LPS injection and levels were almost back to baseline after 48 hours (figure 2B). Hyaluronidase had no effect on FS-6 fluorescence but caused a small increase in MMP3green fluorescent activity. The MMP9 assay showed high interference of SF and required SF to be diluted at least 10 times to obtain accurate results and the standard curve was made in SF. Endogenous MMP9 levels were very low and therefore pro-MMP9 was activated using APMA (1mM). At baseline and at $t = 48$ hours the majority of samples were below the assay's lowest detection limit 0.005 ng/ml. Highest levels of activated MMP9 were measured 8 hours after LPS injection (191.9 ± 153.4 ng/ml) and by 24 hours levels had dropped to 5.7 ± 6 ng/ml (figure 2C).



Conclusions: Intra-articular injections with 0.5 ng LPS caused the highest increase in IL-6, MMP3 and MMP9 levels after 8 hours while