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NIR-FLUORESCENCE IMAGING POINTS AT A ROLE FOR MATRIX-METALLOPROTEINASES IN CAUSING IRREVERSIBLE CARTILAGE DAMAGE DURING COLLAGEN-INDUCED ARTHRITIS

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Background During joint inflammation and destruction, a variety of proteases are upregulated, including different cathepsins and matrix-metalloproteinases (MMP). Enzyme activity and osteoblast activation can be quantitated in vivo by using activatable and targeting near-infrared probes. This also provides us with a tool to non-invasively monitor treatment efficacy on enzyme activity and bone remodelling. The authors investigated the role of MMPs in chondrocyte death and subsequent irreversible cartilage damage, using biophotonic imaging.

Methods Collagen-induced arthritis was induced in male DBA1/J mice by immunisation with bovine collagen-type II. Mice received either anti-IL-1 sera (200 l intravenously) or normal rabbit serum as a control at day 21. Ten days later, Sense 680 probes (PerkinElmer, Waltham, MA, USA) were injected intravenously and 6 h later mice were imaged using the IVIS Lumina (Caliper Life Sciences, Hopkinton, MA, USA). The ProSense 680 probe becomes activated upon enzymatic cleavage by cathepsins. The MMPsense 680 can be activated by different MMPs. OsteoSense 680 is a fluorescent biphosphonate that binds to hydroxyapatite, a biomarker for osteoblast activation. After imaging the ankle and knee joints were dissected and processed for histology or x-ray. Ankle joints were x-ray photographed and scored for bone destruction. Subsequently, serial sections were made and stained with H&E to study inflammatory cell influx, chondrocyte death, bone loss and cartilage destruction and with Safranin-O to study proteoglycan depletion. Sections were also stained for VDIPEN, a neoepitope of aggrecan cleaved by MMPs.

Results All the probes showed an increased fluorescent signal intensity during arthritis. Both the ProSense and MMPsense signal was reduced three times by the anti-IL-1 treatment, whereas the OsteoSense signal was reduced 1.5 times. On the other hand, OsteoSense signal was two times higher in mice showing mild bone loss, compared to no bone loss as measured by x-ray, suggesting an increased osteoblast activity to counterbalance bone loss. Correlations were found between MMP activity and chondrocyte death ($r=0.77$, $p<0.0001$), proteoglycan depletion ($r=0.65$, $p=0.0002$) and VDIPEN staining ($r=0.81$, $p<0.0001$), while less correlation was found between cathepsin activity and chondrocyte death (0.39 , $p=0.0064$).

Conclusion Imaging of these three probes is a sensitive method to measure joint inflammation, connective tissue destruction and repair, and overall is fluorescence imaging a valuable tool to monitor a treatment response. These findings also suggest that irreversible cartilage damage caused by chondrocyte death is a result of enhanced MMP activity rather than cathepsin activity.