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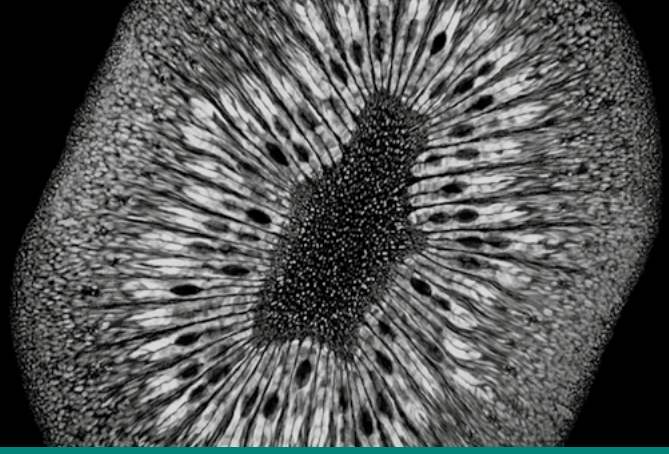
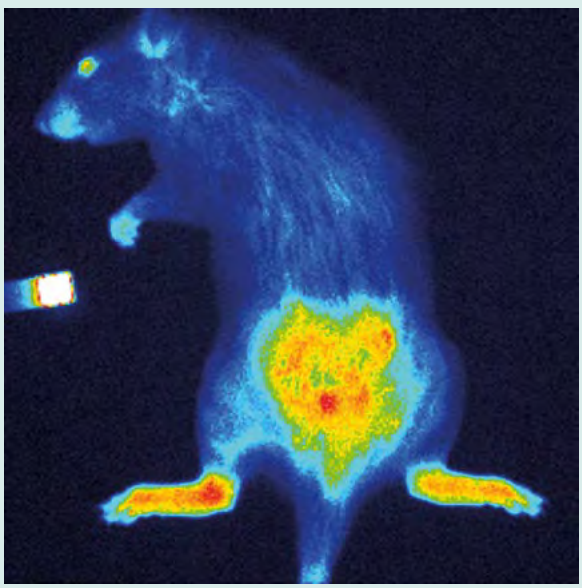


Optical imaging of inflammation



Longitudinal *in vivo* optical imaging of inflammation by NiraWave™ M

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Introduction

Since inflammation plays a major role in disease processes, diagnostic imaging of inflammation serves to establish the primary diagnosis and facilitate the outcome of therapeutic interventions. In this study we use *in vivo* fluorescence optical imaging (FOI) to non-invasively monitor inflammation in the collagen-induced arthritis (CIA) rat, a commonly utilized animal model of rheumatoid arthritis[1]. Rheumatoid arthritis is a progressive autoimmune disease that causes chronic joint inflammation resulting in pain and swelling and, ultimately, loss of joint function. Through implementation of FOI using the clinical Xiralite® system in an experimental setting, we show that the preclinical FOI agent, NiraWave™ M, allows improved detection of inflammation, potentially facilitating the monitoring of therapy.

Materials and methods

Experiments were performed on female CIA rats (n = 10), in comparison to healthy female Lewis rats (control, n = 10). Arthritis in CIA rats was induced by intradermal injection of an emulsion of bovine type II collagen (0.5 mg/rat) in Freund's incomplete adjuvant in the lower back of female Lewis rats, followed by administration of a collagen booster injection 7 days later[2]. On day 14, each individual rat paw was evaluated for arthritis and scored according to a macroscopic scoring system: *0* = paw with no swelling and focal redness; *1* = paw with swelling of digits; *2* = paw with swelling of digits and mild swelling of pad; *3* = severe swelling of the entire paw. After clinical scoring of the CIA rats, all animals underwent FOI using a fluorescence camera system (Xiralite®, Xiralite GmbH, Berlin, Germany). Imaging was performed on healthy animals (n = 5 per group) and CIA rats (n = 5 per group) over a period of 2 h after tail vein injection of the optical imaging agent NiraWave™ M (Viscover™, *nanoPET Pharma* GmbH, Berlin, Germany) or the standard clinical agent ICG-PULSION (PULSION Medical Systems AG, Munich, Germany). The agents were administered at a dose of 0.4 mg ICG/kg body weight, which is within the range recommended for standard clinical use (0.1 - 0.5 mg ICG/kg body weight). After completion of the imaging experiments, the rats were sacrificed and histological analysis was performed on formaldehyde-fixed and paraffin-embedded sections of the tibiotarsal articulation using Hematoxylin and Eosin (H&E) staining.

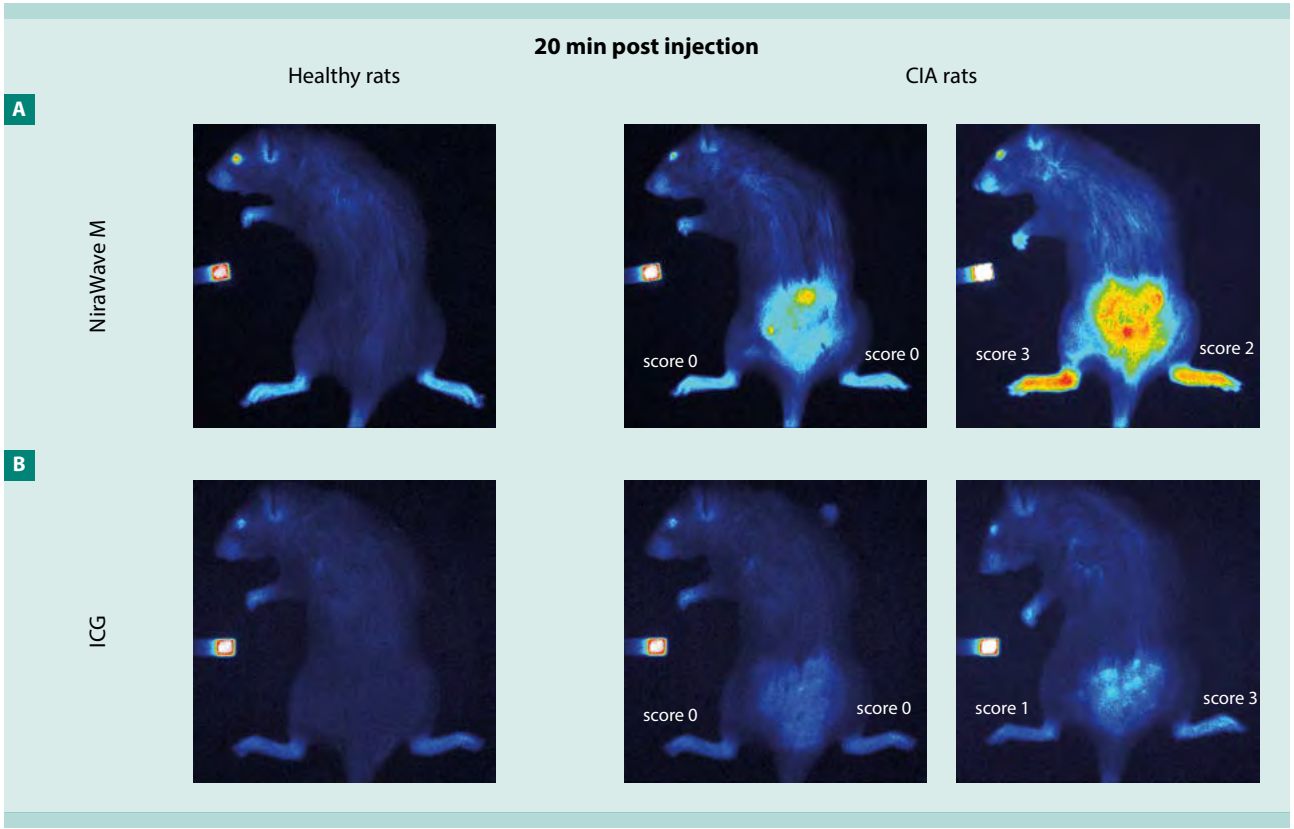

Figure 1: FOI images of healthy and CIA rats at 20 min post injection of imaging agent.
Application of **A.** NiraWave M and **B.** ICG show that the fluorescent signals in inflamed areas have intensities that are proportional to the disease severity score. Compared to conventional ICG, NiraWave M provides significantly higher fluorescent signals in healthy vessels as well as in areas of inflammation.

Results and discussion

To monitor inflammation associated with rheumatoid arthritis, CIA rats were injected with NiraWave M or ICG on day 14 after the induction of arthritis and thereafter imaged over a period of 2 h. In comparison to healthy rats, CIA rats injected with NiraWave M showed strong fluorescent signals in arthritic limbs as well as at the collagen injection sites on their lower backs (Fig. 1A).These signals appeared readily after contrast agent injection and the signal intensities were found to be dependent on the severity of the disease. Indeed, CIA rats having a clinical score of 0 showed fluorescent intensities in the limbs that were similar to those of healthy rats. With increasing clinical score the fluorescent signals in the limbs of the CIA rats were found to increase in intensity and most certainly pertain to increased perfusion and vascular leakage in areas of inflammation due to the enhanced permeability and retention (EPR) effect[3]. CIA rats injected with the standard clinical ICG agent also showed fluorescent signals in inflamed regions (Fig. 1B), but these signals were markedly lower in intensity than those obtained with NiraWave M. This is due, in part, to the increased quantum yield and higher blood half-life of NiraWave M, resulting in improved detection of the agent by fluorescence optical imaging[4,5].

At 2 h post injection of NiraWave M, fluorescent signals could still be observed in both healthy and CIA rats but these were highly reduced in intensity (Fig. 2A). At this later timepoint most of the imaging agent has been cleared from the blood, as can be observed by the minimal fluorescence in the paws and eyes of the healthy rat. As a result, the signals observed in arthritic limbs and the lower backs of CIA rats at this later timepoint are mainly due to the EPR effect alone. Again, the fluorescent signal intensity in inflamed regions was found to be proportional to the disease severity score, proving that the EPR effect can be successfully detected using NiraWave M. In the case of ICG, healthy rats showed no fluorescence at 2 h post injection indicating complete blood clearance of the contrast agent at this timepoint (Fig. 2B). Furthermore, the fluorescent signals obtained in the CIA rats were considerably lower than those obtained with NiraWave M, confirming improved detection of the innovative agent in areas of inflammation.

The inflammatory areas associated with rheumatoid arthritis detected via FOI after injection of NiraWave M were corroborated by histopathological analysis of the tibiotarsal articulation. In comparison to the joints of healthy rats (Fig. 3A), the joints of arthritic rats showed not only damage of the articular cartilage but also a high degree of cellular infiltrates in the synovial cavity, associated with inflammation in rheumatoid arthritis (Fig. 3B).

Conclusion

In this study we use fluorescence optical imaging and NiraWave M to monitor inflammation *in vivo* in a rat model of rheumatoid arthritis. With its high fluorescence intensity and prolonged blood half-life, the innovative imaging agent enables effective monitoring of inflammatory areas through its EPR-based accumulation at sites of vascular leakage. As a result, NiraWave M presents researchers with an innovative tool for detection and monitoring of leaky vessels as found in inflammatory areas and certain types of tumors.

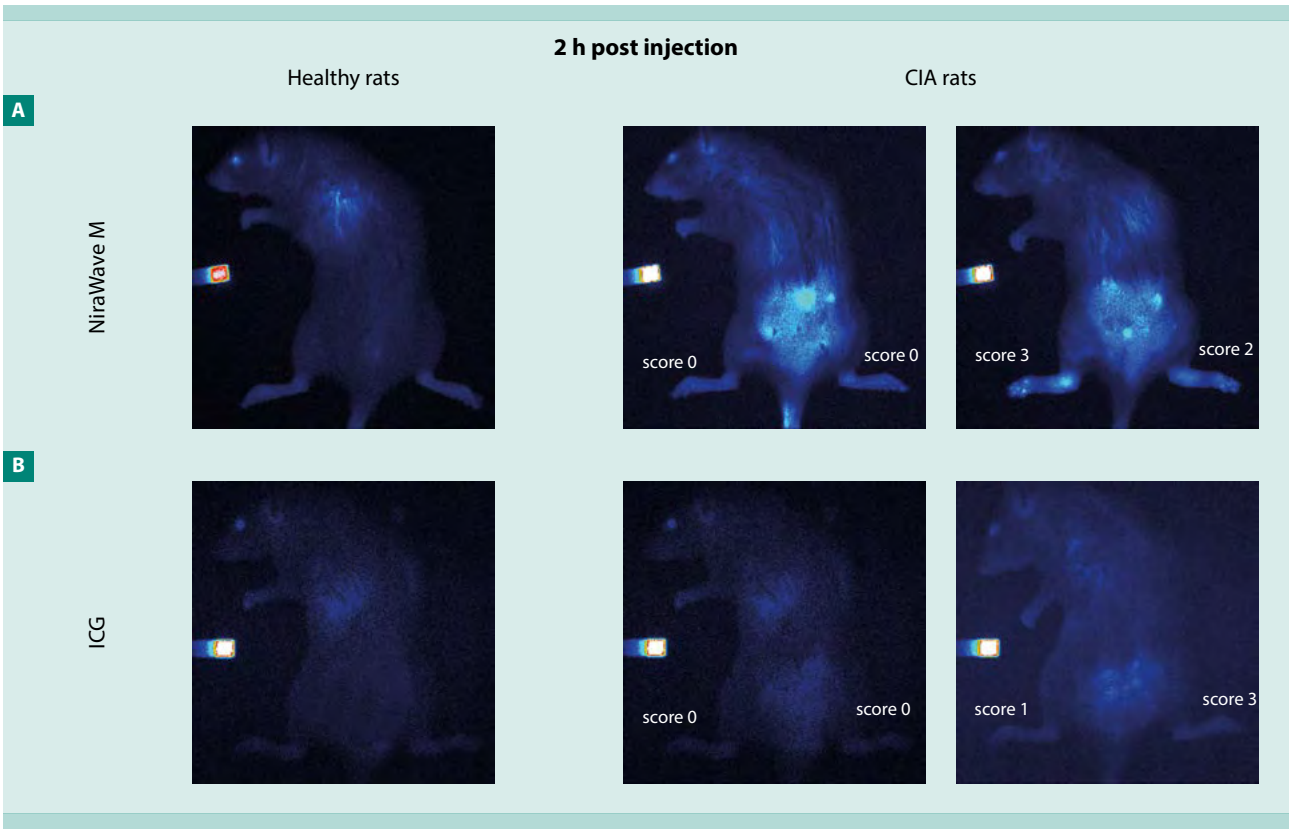

Figure 2: FOI images of healthy and CIA rats at 2 h post injection of imaging agent.
At this timepoint, signal intensities obtained after injection of **A.** NiraWave M and **B.** ICG show fluorescent signals that are highly reduced in intensity. The imaging agents have been practically cleared from the blood pool and fluorescent intensities arise mainly from the EPR effect. The fluorescent signals obtained with NiraWave M are considerably higher than those obtained with ICG, allowing for improved detection of inflammation.

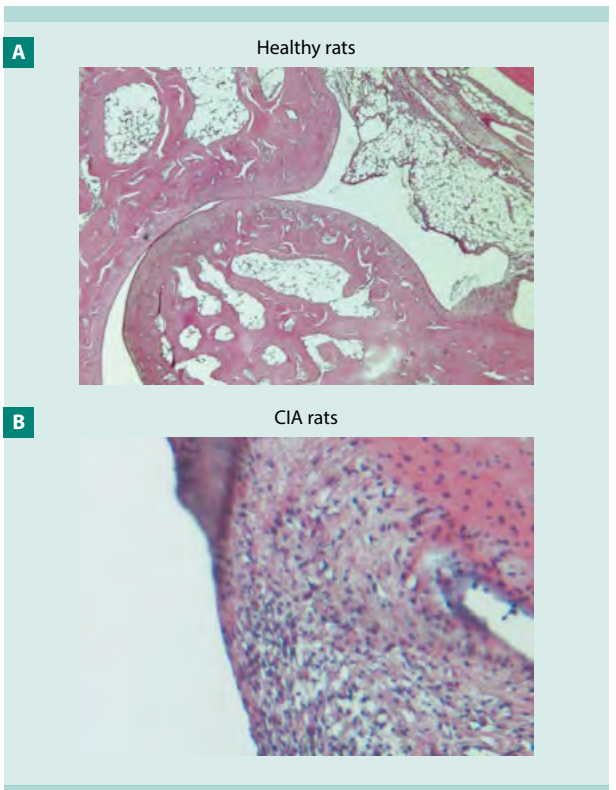

Figure 3: Histopathological analysis of the tibiotarsal articulation at approx. 3 h post injection of NiraWave M.
Comparison of **A.** healthy and **B.** CIA rats clearly reveals the presence of inflammatory cell infiltration associated with rheumatoid arthritis.

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