8. Summary

Detection of inflammatory joint processes based on laser-induced fluorescence: Implementation of a new method using a mouse model

The aim of the present study was to investigate the detection of inflammatory joint processes using unspecific near-infrared (NIR) dyes. The experiments were conducted in an established murine model of Lyme arthritis. Fluorescence imaging of the ankle joints was performed after intravenous administration of NIR dyes. Results were analyzed to determine whether fluorescence imaging is a suitable method for the detection of arthritis and for assignment of the grade of inflammation.

Preliminary experiments were performed to test the potential of two unspecific NIR dyes, indocyanine green (ICG) and NIR-1, to detection arthritis and to optimize the imaging procedure and technique. While ICG with its pronounced plasma protein binding only enables visualization of contrast medium arrival in the body and of higher perfusion in inflamed areas, the hydrophilic NIR-1 dye shows extravasation and accumulation in areas of inflammation.

The NIR-1 dye was therefore used in the further experiments where it was tested at a dose of 1 µmol/kg in seven control animals and ten arthritic animals and at a dose of 2 µmol/kg in eight control animals and ten arthritic animals. Laser-induced fluorescence was excited with a solid-state laser system at an excitation wavelength of λ = 740 nm. Fluorescence was recorded using an intensified CCD camera. A series of 60 images was acquired over a period of about three minutes starting immediately with the administration of the dye. A final fluorescence image was obtained five minutes after dye injection.

The acquired images were analyzed using a Windows-based Visual Basic Evaluation Software (Petzelt, D., PTB). Regions of interest (ROIs) of identical size were placed in the area of the left and right ankle joint as well as outside the joints as a reference. For these three ROIs the mean fluorescence intensity of all pixels was calculated. Next, the normalized fluorescence intensity was calculated for each joint as the mean intensity of the joint divided by the fluorescence intensity of the reference ROI in order to adjust for possible variations in laser excitation.

Quantitative analysis demonstrated significant differences between control animals and arthritic mice in the fluorescence intensity curves (steepness) and the normalized fluorescence intensities three and five minutes after dye injection at a dose of 1 µmol/kg. No such differences were seen between arthritic mice and control animals after dye injection at a dose of 2 µmol/kg.

Qualitative analysis of the NIR images by two investigators evaluating the distribution of fluorescence intensity revealed preliminary information on the presence of arthritis in Borrelia-infected mice after administration of 2 µmol/kg. For the lower dose of 1 µmol/kg the results of the qualitative analysis were inconclusive.

The histological examination of H&E-stained paraffinsections demonstrated pathological changes of the synovial area, Achilles' tendon, surrounding tissue, and occasionally also of the cartilage and bone in the mice with Lyme arthritis.

NIR imaging with administration of unspecific dyes enables the detection of arthritis of the ankle joints in mice. Good results in the documentation of arthritic changes are achieved with the NIR-1 dye administered at a dose of 1 µmol/kg. A higher signal intensity was found in animals with a moderate arthritis compared to those with mild or severe signs of inflammation. The results suggest that dye-enhanced NIR imaging may be used as a diagnostic tool in early identification of inflammatory changes, for instance in the diagnosis of rheumatoid arthritis. It is an easy to use and fairly inexpensive modality that requires affordable equipment and does not involve exposure to ionizing radiation.