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ICG-enhanced imaging of arthritis with an integrated Optical Imaging/X-ray System

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Abstract

Background—Optical Imaging (OI) is a promising technique that is quick, inexpensive and, in combination with Indocyanine Green (ICG), an FDA-approved fluorescent dye, could provide early detection of rheumatoid arthritis.

Objective—The purpose of this study was to evaluate a combined X-ray/OI imaging system for ICG-enhanced detection of arthritic joints in a rat model of antigen induced arthritis.

Methods—Arthritis of the knee and ankle joints was induced in six Harlan rats with peptidoglycan polysaccharide polymers (PGPS). Three rats served as non-treated controls. Optical imaging of the knee and ankle joints was done with an integrated OI/X-ray system before and up to 24h post intravenous injection (p.i.) of 10mg/kg ICG. The fluorescence signal intensities of arthritic and normal joints were compared for significant differences using generalized estimation equation models. Specimen of knee and ankle joints were further processed and evaluated by histology.

Results—ICG provided a significant increase in fluorescence signal of arthritic joints compared to baseline values immediately after administration ($p < 0.05$). The fluorescence signal of arthritic joints was significantly higher compared to the non-arthritic control joints at 1 - 720 min p.i. ($p < 0.05$). Fusion of ICG-enhanced OI and X-rays allowed for anatomical co-registration of the inflamed tissue with the associated joint. H&E stains confirmed marked synovial inflammation of arthritic joints and absence of inflammation in control joints.

Conclusion—ICG-enhanced OI is a clinically applicable tool for detection of arthritic tissue. Using relatively high doses of ICG, a long term fluorescence enhancement of arthritic joints can be achieved. This may facilitate simultaneous evaluations of multiple joints in a clinical setting. Fusion of ICG-OI scans with X-ray imaging increases anatomical resolution.

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Introduction

Rheumatoid arthritis (RA) is the most common chronic inflammatory joint disease affecting approximately 40 million people worldwide and leading to substantial early disability and morbidity (1). Imaging plays only a secondary role in diagnosis of RA as clinical criteria are typically used for diagnosis and findings on X-rays are limited early in the course of the disease (2). An enhanced method for detection of inflamed joints is preferred for the evaluation of the extent of the disease and monitoring response to therapy.

Ultrasound and MR imaging are among the supplementary imaging modalities used in RA. While ultrasonography can provide information on inflammatory activity, it is time-consuming and operator dependent. MRI provides a high sensitivity for the diagnosis of early synovitis and joint effusions. However, MRI is complicated by long examination times and high costs (2,3).

Optical imaging (OI) is a relatively new, fast, inexpensive, non-invasive and non-ionizing imaging modality that offers a high sensitivity for the detection of inflammatory arthritis with image acquisition times of few seconds (4). The limited tissue penetration of this technique does not affect evaluation of the small joints of the hands that are typically involved by RA. While non contrast enhanced OI can yield high sensitivity and specificity in the detection of early hyperemia and joint inflammation (5), the addition of fluorescent contrast agents increases diagnostic utility. Among fluorescent agents used for OI, indocyanine green (ICG) is promising as it has been shown to enhance inflamed joints (6) and has been used extensively in the fields of ophthalmology and cardiology for over 40 years.

A significant drawback of current OI techniques is the limited anatomical orientation with regard to specific joint components. To overcome this problem, new integrated OI/X-ray imaging systems have been developed that acquire and fuse optical and X-ray images. Our hypothesis is that this fusion approach could combine the high sensitivity of OI and the anatomical resolution of plain film radiography for the detection of inflamed joints in ICG-enhanced imaging of RA in a rat model. To the best of our knowledge, this is the first investigation of the performance of an integrated OI/X-ray system for fluorescence-enhanced imaging of arthritis.

Materials and Methods

Contrast media

Indocyanine green (ICG) is an FDA approved hydrophilic anionic dye. The absorption and emission maximum wavelength of this near-infrared dye are 720 and 830 nm respectively (7). Within seconds of intravenous injection, ICG reversibly binds to up to 98% of all plasma proteins without extravasating. The protein-bound compound is taken up by hepatocytes and excreted via bile fluids without entering into the enterohepatic circle (6). For our study, ICG (Fisher Scientific, Pittsburgh, PA) was dissolved in 40% DMSO and 60% normal saline to yield a 10 mg/ml stock solution and was passed through a 0.2 µm nylon filter before intravenous injection (8).

Animal model of arthritis

The study was approved by the Committee for Animal Research at our Institution and was performed in accordance with the guidelines of the National Institutes of Health for the care and use of laboratory animals. Nine athymic Harlan rats (Harlan Bioproducts Inc, Indianapolis, IN) aged 5-6 weeks were randomly divided into two groups, the induced arthritis animals (group A, n=6) and the non-treated control animals (group B, n=3).

Immune mediated arthritis in group A was induced by injection of streptococcal cell wall (SCW) component peptidoglycan polysaccharide polymers (25 mg/kg PGPS 10S, Lee Laboratories, Grayson, GA) (9). Half of the dose was injected intraperitoneally while the remaining dose was equally distributed among the bilateral knees and ankles. Development of arthritis in the knee and ankle joints was determined by manifestation of clinical signs (limping) and measurement of joint diameters.

Imaging

OI scans were obtained once animals in group A showed clinical symptoms of arthritis. Animals were anaesthetized with Isoflurane (Draeger Medical, Inc., Telford, PA, USA) and placed prone into the integrated OI/X-ray scanner (Imaging Station In-Vivo FX, Eastman Kodak Company, New Haven, CT). This OI system is equipped with a high-intensity halogen illuminator, exchangeable excitation wavelength filters and a thermoelectrically cooled CCD camera. Co registration of both imaging modalities is facilitated by the optical and X-ray focal planes being equal. The following imaging parameters were used for optical imaging with ICG: exposure time: 5 sec; f-stop: 0.0; FOV: 160×160 mm; focal plane: 5, excitation filter 755 nm, emission filter 830 nm. X-rays were obtained with an exposure time of 60 sec, f-stop at 2.8, a field of view (FOV) of 160×160 mm, focal plane: 5, 35 kV. Using the Kodak software (Kodak Molecular Imaging Software 4.5) the data sets of the two imaging modalities were fused by superimposing the false color optical image to the greyscale X-ray image on a pixel by pixel basis.

Animals were imaged as follows: (1) pre-contrast OI scan followed by injection of ICG, (2) 11 repetitive OI scans at 1, 2, 3, 4, 5, 8, 10, 15, 20, 25 min post injection (p.i.), (3) X-ray at 25 min p.i., (4) OI and X-ray at 60 min p.i., (5) at 90 min p.i., (6) at 12h p.i., (7) and at 24h p.i.. Following the last imaging session, rats were sacrificed and ankle and knee joints were processed for histology.

Image analysis

Image analysis was independently performed by two observers (RM and CK). The optical images of the in vivo study were evaluated qualitatively by assessing the presence or absence of visibly increased fluorescence in the region of the inflamed joints compared with the fluorescence of normal muscle. Quantitative analysis of the OI scans was performed by measuring the fluorescence signal intensity (SI) of the knee and ankle joints via operator defined regions of interest and subtracting the SI from the background noise measured in the outlying air (ΔSI).

Histopathology

Knee and ankle joints were fixed in 10% paraformaldehyde in phosphate-buffered saline and decalcified with anhydrous EDTA buffer. Specimen were dehydrated in ethanol and embedded in paraffin. 2 μ m thick slices were stained with standard hematoxylin and eosin. Two pathologists (GP, 16 years of experience; MR, 8 years of experience) who were blinded to the OI data determined the presence or absence of inflammatory changes in the joints.

Statistical Analysis

Statistical analysis was performed by the statistician at our institution using SPSS software (version 16.0, SPSS Inc., Chicago, IL). Generalized estimation equation models (GEE) were used to evaluate differences between arthritic joints and normal joints and to compare signal intensities at different time points. The GEE approach properly reflects the structure of repeated data and takes correlation within same individuals into consideration. Because the changes in signal intensity over time were different between arthritic and normal joints, an

interaction between time and joints was also included in the model. As correlation structure within subjects a first-order autoregressive relationship was assumed. This implies that two observations close to each other over time are more highly correlated than two observations spread further apart. Statistical comparisons were made using a two sided 0.05 level of significance.

Results

All 6 animals of the experimental group A developed a PGPS-induced arthritis with marked swelling of the knee and ankle joints 3-5 days after induction (9).

Imaging

Pre-contrast versus post-contrast scans—All rats of the arthritic animal group A showed signal enhancement in bilateral knee and ankle joints following intravenous administration of 10 mg/kg of ICG (Figure 1A). During the initial post contrast phase, increasing ΔSI in the knees and ankles was measured with a plateau effect noted between 4 minutes and 25 minutes after contrast injection (Figure 2). Increased signal from these joints persisted for 12 hours (Figure 2). Corresponding quantitative ΔSI data of the arthritic knee and ankle joints of the animal group A was significantly higher on post-contrast images compared to pre-contrast images ($p < 0.05$; Figure 2). In rats of the control group B, OI images showed a minor enhancement of the joints at 1 - 25 minutes after intravenous injection of ICG, presumably due to a non-specific perfusion effect (Figure 1A). Delayed optical images at 60, 90, 720 and 1440 minutes showed no substantial joint enhancement after ICG injection. ΔSI data between pre- and post-contrast images were significantly different in the knee and ankle joints of the normal animal group B ($p < 0.05$, Figure 2).

Comparisons between group A and B—The fluorescence signal of the joints on post-contrast images was markedly higher in the arthritic animals in group A compared to animals in the control group B. Corresponding quantitative ΔSI data of arthritic knee and ankle joints of the animal group A was significantly higher compared to the control joints of group B at all time points between 1 minute and 12 hours p.i. ($p < 0.05$, Figure 2). At 12 and 24 hours p.i. an increased fluorescence signal was noted in the abdomen in both animal groups, presumably representing biliary excretion of ICG into bowel loops.

Fusion—Fusion imaging with OI and X-ray allowed co-localization of areas of increased OI signal to anatomical structures (Figure 1B). In the arthritic animal group, the enhancing areas could be associated with the knee and ankle joints (Figure 1B). Furthermore, these enhancing areas corresponded to the area of inflammation seen on histopathology (Figure 3C, 3D). In the non-arthritic animal group, the enhancing areas could not be associated with the joints, but with the lower abdomen, presumably representing biliary excretion of ICG into bowel loops (Figure 1B).

Histology

Corresponding H&E stains of the knee and ankle joints of the arthritic group A showed a markedly thickened synovium with variable infiltrations by leukocytes and macrophages (Figure 3C, 3D), while the control group B did not show any signs of inflammation (Figure 3A, 3B). Fluorescence microscopy of the samples to visualize ICG was not possible as the ICG fluorescence was presumably bleached by the time the samples were processed and was thus not detectable.

Discussion

Our data shows that ICG enhanced optical imaging can detect inflamed joints. The relatively high dose of ICG used provided a contrast effect that lasted for almost half an hour. Fusion of OI and X-ray images leads to an improved anatomical localization of the inflammatory process.

Different approaches have been described for the evaluation of arthritis with optical imaging. Imaging of laser light scattering without any contrast agent can detect inflammation of the PIP joint with a sensitivity of 80% and a specificity of 89% (5). However, analysis of these data requires the use of non trivial algorithms and changes between normal and inflamed joints were not evident on qualitative imaging. The addition of fluorescent contrast agents has the potential to improve both sensitivity and specificity of optical imaging (4,6,15). The fluorescent probe can either be injected intravenously and depict the hypervascularity of inflamed joints or the fluorescent probe can be used to label leukocytes and depict the accumulation of these cells in inflamed joints, similar to radiotracer-based leukocyte scans (10).

For the scope of RA diagnosis and monitoring, direct injections of fluorescent probes followed by optical imaging appear to be most practical (4). Several investigators developed cell specific fluorescent dyes in order to optimize the specificity of the optical imaging approach. Chen et al developed a fluorescent contrast agent bound to folate that was preferentially taken up by activated macrophages in arthritic joints of experimental animals. The amount of detected activated macrophages correlated with articular destruction and poor disease prognosis (11). Unfortunately, such specialized dyes are not FDA approved and therefore have limited clinical applicability. FDA approved contrast agents would facilitate clinical translation. A recent study by Fischer et al (6) showed the feasibility of ICG for arthritis imaging in a Lyme arthritis based mouse model. Following injection of ICG at a dose of 1-2 $\mu\text{mol/kg}$ a relatively short lived signal enhancement of inflamed joints was noted at 60-120 seconds post injection. For imaging of multiple joints, a lengthier enhancement would be preferred.

ICG has been used extensively for over four decades in the fields of ophthalmology for retinal imaging and cardiology for detection of cardiac output. ICG has a well documented safety profile (12). It may very rarely ($<1/10,000$) cause anaphylactoid and anaphylactic effects such as hyperthermia, nausea, pruritus, urticaria, tachycardia, hypotension, dyspnea and bronchospasm (12). We used a higher dose for rodents as described for patients in order to compensate for the shorter blood half life of ICG in rodents (1.5 - 2.3 min) as opposed to patients (3 - 4 min) (6,8). The relatively high ICG dose provided a prolonged contrast enhancement and allowed for sequential imaging of multiple joints without additional contrast injection.

Several translational optical imaging devices have been recently introduced to the clinics, such as optical mammography (custom device, Taroni et al) (13) and optical arthritis scanners (Xiralite, Mivenion Ltd, Germany) (14). A custom built ICG enhanced OI device for hand imaging has recently been shown by Fischer et al to correlate well to enhanced MRI in detection of rheumatoid arthritis in patients (15).

We acknowledge various limitations of our study. Our study protocol includes the acquisition of multiple X-rays since standardized positioning of the animals in the scanner for all time points was not possible. In a clinical setting, however, where a patient's hands and feet are imaged in a standardized position only one X-ray per examination would be required.

Furthermore OI does not require ionizing radiation whereas the combination with X-ray does. However, nearly every patient with RA will receive an initial X-ray and the addition of this imaging modality compensates for the lack of anatomical information in OI. Thus, the combination of both modalities would not increase radiation exposure for patients; it will, however, improve the sensitivity of the diagnostic test and the sensitivity of follow-up exams that monitor disease burden and progression. While X-rays might not be required to localize optical enhancement to the investigated knee and ankle joints of rats, X-rays will be helpful to localize areas of enhancement to more complex structures such as a hand of a patient.

We also acknowledge the limitations of our animal model of acute arthritis which is self-limited and resolves spontaneously within 4-5 days. Future studies will need to assess the value of our diagnostic approach for the detection of early stages of arthritis and for long term therapy monitoring, as this would be more pertinent to patient applications.

In summary, we have shown that the FDA-approved ICG can be used to detect joint inflammation in an animal model of arthritis. The short examination time coupled with the patient-friendly imaging modality could easily allow for detection of early inflammatory changes in rheumatoid arthritis and facilitate therapy monitoring.

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Figure 1.

A Representative false-color optical images of arthritic (top row) and non-arthritic control animals (bottom row) before and 1 to 90 minutes after intravenous injection of 10 mg/kg ICG. B Representative optical and X-ray images of a rat of the arthritic animal group with subsequent fusion of imaging data (Kodak Molecular Imaging 4.5 software) and a fused OI/X-ray image of a rat of the control animal group, each 60 min after injection of 10 mg/kg ICG. From the OI data, only pixels with signal intensities above 6500 units were used for fusion images.

Figure 2.

Fluorescence signal intensity of arthritic (triangles) and normal control knee and ankle joints (squares) at different time points before and after intravenous injection of 10 mg/kg ICG. Data are displayed as means and standard deviations. (*) indicates significantly higher SI of the arthritic joints when compared to normal control joints ($p < 0.05$).

Figure 3.

Representative H&E stains of a normal knee (A, B) and a knee with induced arthritis (C, D). Magnification: 10 $\times$ (A, C); 100 $\times$ (B, D). Normal knees show a thin synovial lining (arrows in A, 100 $\times$ magnification in B). The synovial membrane of the arthritic knee is markedly thickened (arrows in C) and reveals numerous leukocytes (D).