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TITLE:
Photoacoustic/ultrasonic Dual-mode Imaging For Monitoring Angiogenesis And Synovial Erosion In Rheumatoid Arthritis

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ABSTRACT BODY:

ABSTRACT INTRODUCTION: **Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by the formation of new vessels, synovial proliferation and destruction of articular cartilage. However, characteristic early diagnostic and thera- peutic monitoring methods are still lacking. We report a study using a photoacoustic/ultrasound (PA/US) dual- mode imaging for RA disease. By establishing a collagen-induced (CIA) RA mouse model to classify disease states based on a subjective grading system, PA/US imaging allows real-time assessment of synovial erosion and vascular opacification within the knee joint in different disease states at high spatial resolution. The system also quantitatively monitors subcutaneous vascular physiology and morphology in the hind paw of mice, measuring the area and photoacoustic signal intensity of vascular proliferation and showing a positive correlation with disease grading. Compared to traditional subjective scoring of arthritis severity, the PA/US imaging is more sensitive i.e., vascular signals and synovial erosion can be observed early in the course of arthritis.**
METHODS: The general procedure was to add an equal amount of type II collagen (4 mg/ml) drop by drop to complete Freund's adjuvant (CFA) (4 mg/ml), which was completely mixed and emulsified in a tissue homogenizer. It is important to ensure that the procedure is performed at low temperature. Mice anesthetized with 2.5 % isoflurane were slowly injected intradermall (i.d.) with 50 μL of emulsion at a distance of 1.5 cm from the tail root. The same concentration of emul- sion was re-injected in the tail 14 days after the first immunization to enhance immunity and ensure a high incidence of CIA. Based on the subjective scoring system reported in the literature , the mice were evaluated 21 days after injection of the modeling drug, and different disease scores were given to the onset mice for the experiments based on the degree and extent of hind paw swelling. In this process, we ensured that there were at least three mice with a similar disease score for each disease score class (0-4). In dual-modal PA/US imaging, three mice in each grade (0-4) were scanned 3 times to ensure the reliability and reproducibility of the imaging data. Before the experiment, mice were anesthetized with 3 % isoflurane and kept at rest, and the knee imaging area of the mice to be imaged was removed with commercial hair removal cream. During the experiment, the mice were secured in a special immobilization holder (used 3D printing technology to specially make a groove for mice to lie in.) on a heating pad to ensure proper imaging body position (to ensure that the imaging positions of different groups of mice were similar as far as possible) and to maintain their body temperature, and the hind limbs were secured and bent approximately 60 degrees to expose the imaging area as much as possible during the experiment. Ultrasound gel and deionized water were used to couple the PA signal between the mouse skin and the transducer. Mice with normal and different RA scores were imaged at least three times in each group. Except for the mice that died during the experiment, the remaining mice with different disease scores were imaged. [1, 2]

RESULTS SECTION: On the bimodal imaging results, localization by B-mode de- lineates the TTF region, within which the photoacoustic signal is visible. In photoacoustic image of the normal mouse, a very small amount of punctate photoacoustic signal can be seen within the TTF region. In the RA mouse, two to three larger areas of punctate photoacoustic signal attached to the femoral and tibial joint surfaces can be seen within the TTF region in mice with a score of 1. In mice with a score of 2, more areas of dotted lamellar photoacoustic signals can be seen in the TTF area attached to the femoral and tibial articular surfaces. In mice with a score of 3, linear areas of photoacoustic signal along the femoral and tibial joint surfaces can be seen in the TTF region. In mice with a score of 4, a patchy area of photoacoustic signal extending toward the center of the joint cavity can be seen in the TTF area. Compared with normal mice, the photoacoustic signal region area of RA mice increases with the increase of disease score. We calculated the area of the pho- toacoustic signals, which reflects the extent of pannus in the knee joint, also shows the intensity of the photoacoustic signal, which reflects the density, diameter, and flow of blood vessels. The area and intensity of the photoacoustic signal are positively correlated with disease score. Compared with the photoacoustic signal area ($r^2 = 0.82$), the photoacoustic signal intensity seems to be able to better predict the severity of disease ($r^2 = 0.98$).

DISCUSSION: This study focused on the photoacoustic imaging of pannus and ultrasonic imaging of joint structure of the knee joint. The synovial blood vessels proliferating in the knee joint of RA mice are rich in hemoglobin. Hemoglobin, a natural photoacoustic imaging agent, has a high absorption peak near 532 nm, which enables us to render the signal of vascular opacities well without additional contrast agent. In the RA model, pannus persists and develops through the supply of inflammatory cells, cytokines and nutrients in the synovium. After CIA induction, the number of new blood vessels in the synovium will increase with time. Vascularization is also associated with the severity of arthritis and can be assessed by subjective assessment scoring system or histological scores. However, the strength of the PA signal mainly depends on the concentration of hemoglobin in the blood vessels. The PA signal represents the difference in the number and density of synovial hyperplasia and new blood vessels, the range of blood flow signals detected in photoacoustic imaging will reflect the severity of the disease which is verified on the HE slices.

SIGNIFICANCE/CLINICAL RELEVANCE: PA offers the advantage of visualizing and analyzing the neovascularization within the RA knee, and US can also quantify erosive vascular opacities within the knee joint. This study has not only provided more tools and possibilities for the research of RA mouse models, but also assisted the evaluation of potential drug.
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