Imaging Macrophages During Post-traumatic Osteoarthritis With NIRF Labeled Nanoemulsions

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Introduction and background

Joint degeneration can be caused by trauma, leading to post-traumatic osteoarthritis (PTOA) within 10 years[1-2]. Systemic pharmacological treatments can cause serious side effects, therefore in this project we explored the ability of macrophages to be potential carriers of therapeutic agents into injured joints during post-traumatic OA (PTOA). We used a macrophage targeted nanoemulsion (NE) imaging agent (reagent source Janjic lab @ Duquesne University)[3]. Following the inflammatory response triggered by injury, monocytes, including their contents, are recruited to the injury site as macrophages. The NE contains a near-infrared fluorescent (NIRF) marker, so that macrophage recruitment to the site of inflammation can be monitored by *in-vivo* NIRF imaging[3]. We compared the NIRF optical imaging with the ^{18}F-Fluoro-deoxy-glucose (FDG) PET imaging, which has been shown to correlate with synovial inflammation and to accumulate in new bone formation, such as subchondral bone regions of new forming osteophytes, while showing poor uptake into articular cartilage. FDG PET has been recognized as useful for early detection of OA changes[4].

Objectives

- To establish macrophage localization using the drug-free NE (DF-NE) by NIRF and correlate it with disease severity (ACLT and DMM).
- To monitor ^{18}F-FDG uptake by PET and correlate with disease severity (ACLT and DMM).

Methods

C57BL/6 male mice were subjected to the destabilization of the medial meniscus (DMM, *n*=3) or the anterior cruciate ligament transection (ACLT, *n*=3). Unoperated mice were used as controls (*n*=3).

Optical Imaging: The DF-NE was injected by tail vein in both PTOA models one day before surgery, 4 weeks and 8 weeks post-surgery. The DF-NE localization was monitored by optical fluorescence imaging (EX= 745nm, EM=800nm) 24h, 48h, and every week up to 12 weeks post-surgery.

FDG/PET: We compared the novel NIRF optical imaging with the 18-fluorodeoxyglucose (FDG)/PET. ^{18}F-FDG is a positron emitting radiopharmaceutical that accumulates at sites of increased glucose metabolism. FDG was injected via tail vein in DMM or ACLT operated mice (~16 MBq in 0.1 ml), and PET imaging was conducted at 1, 2, 4, 8 and 12 weeks after surgery. FDG uptake was reported as the ratio between the operated leg (right) vs the unoperated leg (left).

OA Grading: Mice were euthanized at 12 weeks after DMM or ACLT, knees were prepared for histological evaluation (Safranin-O/Fast Green, H&E).

References

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Results

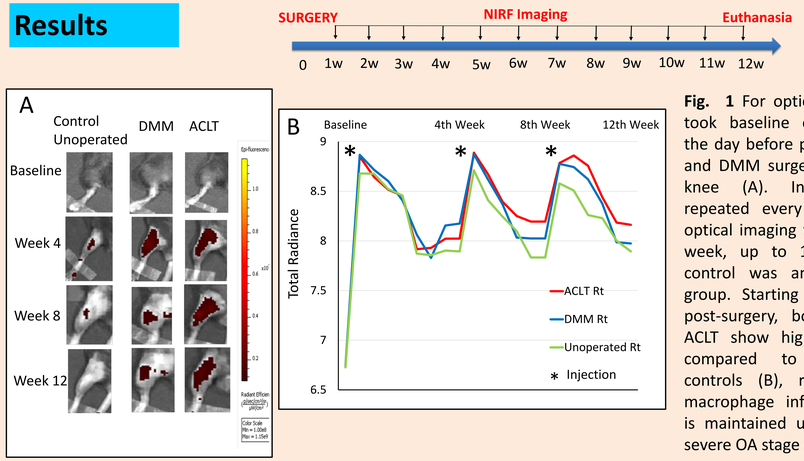

Fig. 1 For optical imaging, we took baseline optical imaging the day before performing ACLT and DMM surgery on the right knee (A). Injections were repeated every 4 weeks and optical imaging was done every week, up to 12 weeks. Our control was an unoperated group. Starting from 4 weeks post-surgery, both DMM and ACLT show higher NE uptake compared to non-operated controls (B), reflecting more macrophage infiltration, which is maintained up to the more severe OA stage (12 weeks).

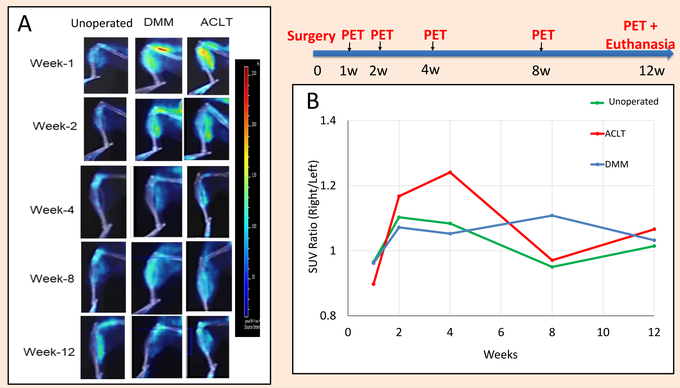

Fig. 2 For our PET analyses, we performed FDG injections at 1, 2, 4, 8 and 12 weeks post surgeries and measure FDG uptake at the same time points by PET (A). FDG uptake is higher in both DMM and ACLT models compared to unoperated (B), reflecting an higher glucose metabolism associated with new cell activity, such as inflammation and new bone formation; the ACLT group had higher FDG uptake, at the early stage, while the DMM group showed a higher uptake at the moderate stage. and more severe OA stages.

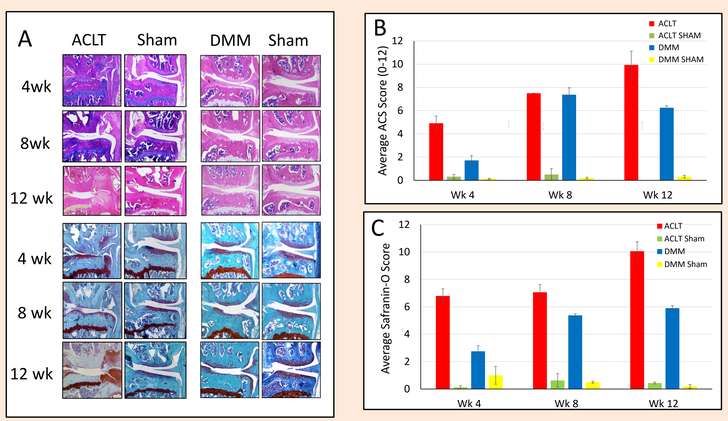

Fig. 3 Histological evaluation at all OA stages shows that ACLT surgery leads to more severe cartilage damage compared to DMM (A), indicated by higher articular cartilage structure scores (B, H&E staining) and extracellular matrix changes (C, Saf-O staining scores).

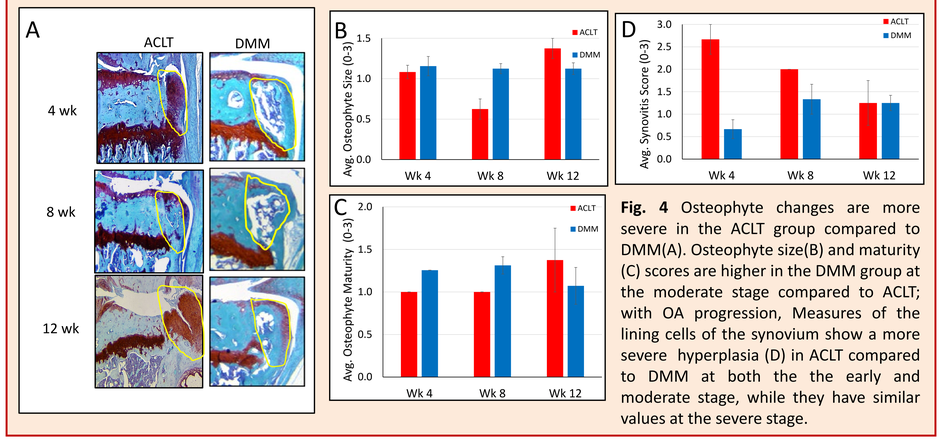

Fig. 4 Osteophyte changes are more severe in the ACLT group compared to DMM(A). Osteophyte size(B) and maturity (C) scores are higher in the DMM group at the moderate stage compared to ACLT; with OA progression, Measures of the lining cells of the synovium show a more severe hyperplasia (D) in ACLT compared to DMM at both the the early and moderate stage, while they have similar values at the severe stage.

Behavioral Testing : Pain sensitivity

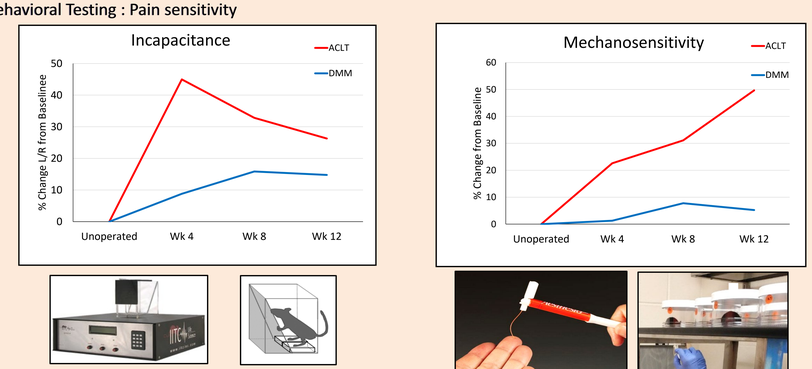

Fig. 5 Hindlimb weight distribution was measured with an incapacitance meter as an indicator of pain. Mice were placed in a restrainer with hindpaws resting on two separate platforms. As the mouse shifts their weight, the unit records the averaged weight in grams during a 2 sec. time period. Avoidance of weight bearing is an indication of sensitivity or discomfort as it relates to injury. ACLT mice were significantly more sensitive on the injured side as compared to the DMM surgery group.

Fig. 6 von Frey filaments were applied to the plantar surface of the hindpaw to determine a threshold for mechanosensitivity. A mouse responding to a filament of less force was more sensitive to the applied force. The ACLT surgery group (n=10) was more sensitive to applied force on the injured foot than the DMM surgery group (n=14).

Conclusions and Future directions

Our data show that ACLT mice show an higher level of macrophage infiltration and inflammation compared to DMM, as detected by NIRF optical imaging and PET analyses, and these changes are maintained during OA progression. These data are confirmed by histological evaluation and by an increased sensitivity to behavioral testing. However, the FDG uptake seems to follow a different temporal progression in the two models at the moderate OA stage (8-week post surgery), with DMM values higher than ACLT, suggesting that such differences might reflect a different bone metabolism during OA progression. Our study represents an important step to establish the potential use of macrophages as drug carriers in PTOA and to define critical windows for specific drug delivery in different PTOA models.

Supported by:

UNC Translational and Clinical Sciences Institute, #550KR252027

National Institutes of Health, NIAMS, #5R01AR070821-04