**Folate Mediated Imaging of Activated Macrophages in Experimental Osteoarthritis Using SPECT/CT**

+Piscaer, T.M. 1; Müller, C 2; Mindt T. L. 2; Lubberts E. 1; Verhaar J.A.N. 1; van Osch G.J.V.M. 1; Schibli R. 2; De Jong M. 1; Weinans H. 1; 2Erasmus Medical Center, Rotterdam, Netherlands, 2Eidgenössische Technische Hochschule, Zürich, Switzerland

**Introduction**

It is unclear what exact role macrophages play in the development of osteoarthritis (OA). Macrophages can produce a large variety of enzymes and pro-inflammatory cytokines. These mediators are known to be involved in the degradation as well as repair of joint tissues in osteoarthritis. Evaluation of macrophage involvement in the osteoarthritis process may provide essential information about aetiology, progression rate as well as potential treatment options for osteoarthritis. Not resting, but activated macrophages express the functional form of the folate receptor beta (FR-β), which binds the vitamin folic acid with high affinity. Folic acid can be labelled with radioactivity and provides the opportunity to image activated macrophage in-vivo in real-time. After binding to the folate receptor, the radioactive labelled folic acid is rapidly endocytosed by the macrophage. Using a high resolution 3D nuclear imaging technique such as multipinhole single photon computed emission tomography (MPH-SPECT) in combination with a high resolution anatomical imaging technique such as micro-CT, macrophage activation can be detected and located in a small animal model for osteoarthritis.

The aim of this study was to image and monitor macrophages and to investigate their involvement in experimental osteoarthritis.

**Method**

**Animal models**

Experimental OA was induced in male Wistar rats by intra-articular injection of 3 mg mono-iodoacetate into one knee (MIA, n=4), the contra-lateral knee served as a control and was injected with saline. In a second model OA was induced at one side by means of anterior ligament transaction (ACLT, n=2), this was used to verify model specific macrophage activity. Since macrophages are not abundantly involved in osteoarthritis we used a rheumatoid arthritis mouse model as a positive control condition (RA, n=2). RA was induced into the right knee of two C57/Bl6 mice by injection of methylated bovine serum albumin (mBSA) after immunisation. In this RA model, activated macrophages are known to be involved abundantly.

**Monitoring macrophage activity**

The animals were intravenously injected with radioactive labeled folic acid (111 In-DOTA-FA) 24 hours prior to imaging. 50 mega-Becquerel of radioactivity was injected in the rats, 25 mega-Becquerel in the mice. Images were obtained at 3, 27 and 52 days after induction of experimental OA. After reconstruction the SPECT and µCT images were fused using dedicated fusion software (invivoscope, Bioscan inc.) to locate the position of the radioactive tracer.

**Results**

All three models (MIA, ACLT, RA) showed increased uptake of the 111In-DOTA-FA on the affected side, not only in the synovial region of the knee, but also in the plopliteal and inguinal lymph node regions at 15, 27 and 52 days. However, the uptake of activity was less in the experimental OA models than in the RA model. The MIA model showed a higher activity rate in the lymph node regions than was seen in the ACLT-model, in which activated macrophages seemed to play a more prominent role in the synovium, though macrophage activity was seen in both models in the affected inguinal lymph node regions up to 52 days after induction of the experimental OA.

**Discussion**

Macrophage activation in experimental OA could clearly be monitored by SPECT/CT in combination with a folate tracer. As expected we found increased activation around the knee area, probably the synovium. Interestingly, there was also an increased amount of activity of macrophages in the lymphoid system up to the inguinal nodes. This shows that macrophages play not only a local role but are also involved in a more systemic response to the affected knee. This response is known in RA, in which macrophages have primarily an antigen presenting role in the lymph-nodes. Though, in osteoarthritis, it is not exactly known what role the macrophages play in the lymphoid tissue, it can be an antigen presenting role or a role of uptake of degraded matrix molecules and tissue in the draining lymph node areas of the affected joints. By investigating a surgical as well as a biochemical OA-model it becomes likely that macrophage activity is a part of the osteoarthritis process, in the synovium as well as in the lymph node regions and not as a side-effect to the specific model. As the macrophage activation was high in the lymph nodes well as in the synovial regions in both models till the end of the experiment (7.5wks).

To conclude, macrophages play a role in the osteoarthritic synovium and in the surrounding lymph node areas of the affected joint. Folate targeted imaging of these macrophages provides the opportunity to get more insight in the disease process and can potentially be used as a new imaging biomarker.

**References**