In vivo SPECT imaging of liver inflammation in a murine model of nonalcoholic steatohepatitis.

A. Broisat1,2, R. Clerc1,2, C. Montemagno1,2, P. Perret1,2, M. Ahmadi1,2, S. Bacot1,2, N. Devoogdt4, T. Lahoutte4, D. Fagret1,2,5, F. Briand3, T. Sulpic3, C. Ghezzi1,2.

1 Université Grenoble Alpes, F-38000, France
2 Inserm, U1039, F-38000, France
3 Physiogenex, Labège, France
4 ICMI Laboratory, VUB, Brussels, Belgium
5 CHU Grenoble Alpes, F-38000, France

Introduction. We recently developed 99mTc-cAbVCAM1-5, a single domain antibody (sdAb) derived radiotracer directed against the inflammatory marker Vascular Cell Adhesion Molecule 1 (VCAM-1). This imaging agent has been fully validated for the non-invasive imaging of murine atherosclerotic lesions (1-2), and is currently under clinical translation for this application. However, VCAM-1 is considered as a potent inflammatory marker not only in the field of atherosclerosis, but in several other clinically relevant pathophysiological settings such as chronic liver inflammation. Thereby, 99mTc-cAbVCAM1-5 imaging was here evaluated in a nutritional mouse model of nonalcoholic steatohepatitis (NASH).

Methods. Thirty 12-wks old male C57Bl/6J mice were used (n=10/group). Mice were either fed a standard diet (STD) or methionine and choline deficient diet (MCD) to induce NASH. Longitudinal SPECT imaging was performed at baseline and at 4 wks and 8 wks following diet onset using either the anti VCAM-1 (V) or an irrelevant control sdab (C). Results were expressed as standardized uptake values (SUV). Following the 8 wks time point mice were euthanized and post mortem analysis were performed.

Results. MCD diet induced a 10-fold increase in serum alanine transaminase (ALT) levels both at 4 and 8 wks in comparison to STD diet (p<0.001). Moreover Oil Red O staining showed large lipid vacuoles in the liver of MCD fed mice. Finally, as demonstrated by ELISA, a significant 5-fold increase in the level of hepatic VCAM-1 expression was observed in MCD in comparison to STD fed group (MCD: 5.6±1.7 vs STD: 1.1±0.5 ng/mg, P<0.001).

Robust 99mTc-cAbVCAM1-5 uptake in the liver of MCD fed mice (V-MCD) was readily observable by SPECT at 4 wks and remained unchanged at 8 wks. In comparison, modest liver uptake was found in control group fed a standard diet (V-STD) and in control group injected with the irrelevant control sdab (C-MCD) at all investigated time points. Image quantification further confirmed these results. Indeed 99mTc-cAbVCAM1-5 hepatic uptake significantly increased in MCD fed mice between baseline and 4 wks, and then remained unchanged at 8 wks (SUV = 0.25±0.04; 0.48±0.11 and 0.45±0.10 respectively, p<0.001 vs baseline at 4 and 8 wks), whereas no significant change was found in control groups. Moreover, SPECT imaging quantification performed at 8 wks significantly correlated with ex vivo biodistribution and with the level of VCAM-1 expression (p<0.001 for both).

Conclusions. The present data demonstrate that MCD diet increases VCAM-1 hepatic levels in mice. Robust and specific uptake of 99mTc-cAbVCAM1-5 was visible as early as 4 wks following MCD diet onset. 99mTc-cAbVCAM1-5 can therefore be employed for the non-invasive longitudinal imaging of
NASH in mice, and further studies are ongoing in order to evaluate the sensitivity of this technique for the monitoring of NASH therapy.


Representative transversal sections obtained at the level of the liver at baseline, 4 wks and 8 wks together with image quantifications. No change in hepatic uptake was found in control diet nor in control sdab groups (V-STD and C-MCD) whereas robust $^{99m}$Tc-cAbVCAM1-5 uptake was found in MCD fed mice at 4 and 8 wks (V-MCD). * p<0.05 and *** p<0.001 vs V-STD; ### p<0.001 vs basal.