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INTRODUCTION

The lack of an accurate, reproducible and easily applied method for liver fibrosis assessment has been a major limitation in both the clinical management and liver disease research. Therefore, the carbon tetrachloride (CCl_4) induced liver fibrosis model was used to evaluate the possibility to detect the onset of liver fibrosis in rats, in particular the activation and proliferation of hepatic stellate cells (HSCs).

Vitamin A-functionalized magnetoliposomes (Vit. A-MLs) are MRI contrast agents, which were designed to specifically be taken up by HSCs. They consist of an iron oxide core coated with an anionic lipid bilayer, functionalized with Vitamin A (Vit. A) residues.

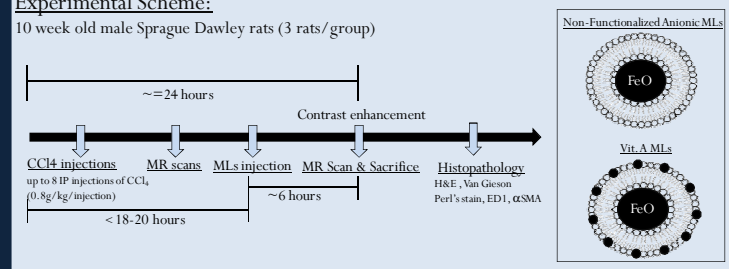
HSCs are resident perisinusoidal cells, which take up Vit. A (retinol) from the circulation by receptor-mediated endocytosis (retinol binding protein receptor), and store it. Stimulation of HSCs results in activation and transformation to proliferative, fibrogenic and contractile myofibroblasts. Upon activation of HSCs both Vit. A and lipids are depleted form the HSCs. However, little is known about the uptake of Vit. A-MLs after HSCs activation, although one publication mentioned that Vit. A uptake in activated HSCs was as effective as in resting HSCs. (ref. Sato *et al*).

Our main objective was to visualize the early onset of liver fibrosis in the rat CCl4 model with Vit. A-MLs as a biomarker for HSC activation and to correlate with the histopathological findings.

METHODS

Experimental Scheme:

10 week old male Sprague Dawley rats (3 rats/group)

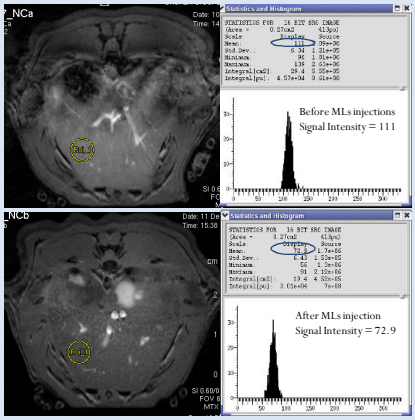


MR image processing: Animals were analyzed for their change in the signal intensity post-contrast enhancement. Three regions of interests (ROIs) were chosen at different areas of the liver (excluding areas of vasculature). Signal intensities (SI) before and after contrast agent administration were compared.

RESULTS

Decrease in the SI post MLs injections

In all rats, the liver signal intensities decreased after contrast agent injection (resulting in darker livers), due to the uptake of functionalized/non-functionalized MLs. This decrease in signal intensity (post MLs) was more pronounced in CCl_4 dosed rats compared to the vehicle rats, and animals which received functionalized MLs (Vit. A-MLs) clearly showed enhanced contrast (lower T2 values, hypointensity in T2-weighted MRI) compared to non-functionalized MLs.



Detecting centrilobular changes

The presence of a ´cobbled stone´ (or granular structures) appearance on the liver tissue, post-contrast administration in CCl_4 dosed rats, was considered to be related to the centrilobular-oriented changes caused by CCl4 injections, and most likely due to the uptake of MLs by activated centrilobular macrophages (ED1 stain).

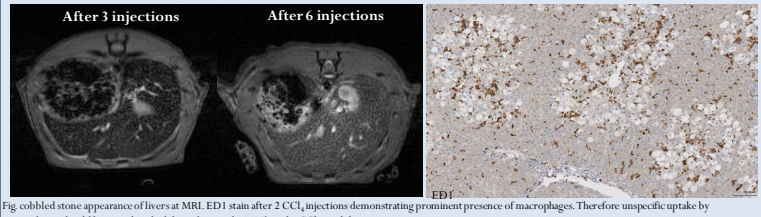


Fig cobbled stone appearance of livers at MRI. ED1 stain after 2 CCl_4 injections demonstrating prominent presence of macrophages. Therefore unspecific uptake by macrophages should be considered while evaluating the MRI's in the CCl_4 model.

Histology of the livers, after one to four CCl4 injections, showed centrilobular congestion with single cell necrosis and chronic inflammation, surrounded by ballooning degeneration and vacuolization of hepatocytes.

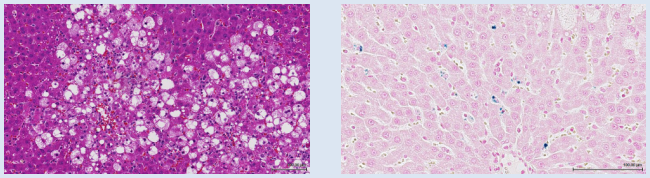


Fig. HE stain showing centrilobular lesions after 2 CCl_4 injections, Perl's stain showing iron uptake in the liver of rat (3 CCl_4injections) with vit. A-MLs

MR images, image processing and histology findings

Changes in the SI were represented in the form of a histogram. From three injections onwards, a shift in SI was noted post-contrast administration. This increase in SI correlated histologically with minimal increase in αSMA staining, indicative for HSC activation/proliferation, after three injections and with slight to moderate HSC activation/proliferation from four injections onwards.

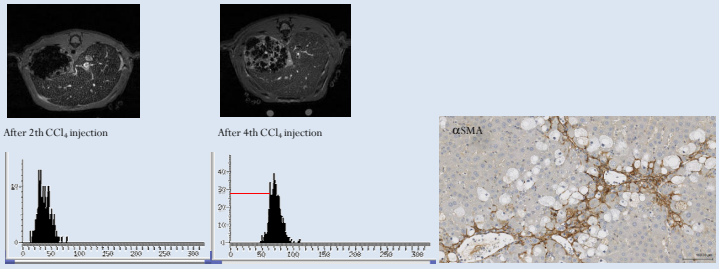


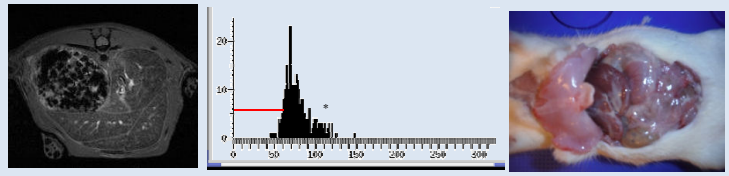
Fig histograms showing the shift in signal intensity. α-SMA stain after 6 CCl_4 injections: showing activation and proliferation of hepatic stellate cells.

The SI distribution varied with multiple injections, this could be indicative for loss of anatomical structure, probably related to the CCl_4-induced lesions, and at later time points the progression of liver fibrosis.

MR images and liver fibrosis

Histologically the first signs of liver fibrosis could be observed after five CCl4 injections.

After 8 CCl_4 injections: white stripes in the MRI indicated the presence of liver fibrosis, at necropsy a white and irregular liver surface was noted. In the histogram an area with SI above 100 was present*, this was comparable to normal livers of control (non Vit. A-MLs injected) rats, which indicated absence of magnetoliposome uptake in these areas.



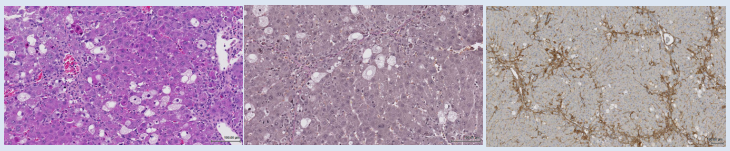


Fig. HE, Van Gieson and α-SMA stain: showing chronic inflammation, fibrotic strands and activation and proliferation of HSCs after 8 CCl_4 injections

CONCLUSION

These preliminary results indicate the possibility to detect the onset (and progression) of liver fibrosis *in vivo*, using MRI imaging with Vit. A functionalized magnetoliposomes. This technique might therefore be valuable in longitudinal studies, to follow the onset, progression and recovery of liver fibrosis in individual animals, in liver disease research and potentially also as a biomarker for clinical use. However due to the limited number of rats used in this study and the confounding morphological changes caused by the CCl4 injections, future work is needed to confirm these results.

REFERENCE

Sato et al. Nature Biotechnology Volume 26 number 4, April 2008, p 431-442