Targeting Inflammation in MRI with a Superparamagnetic Contrast Agent

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INTRODUCTION

The non-invasive diagnosis of inflammation has a particular significance due to its involvement in a broad spectrum of pathologies. Sialyl-Lewis^x (sLe^x) is one of the ligands (Figure 1) expressed by leukocytes to interact with endothelium during inflammation (Figure 2).
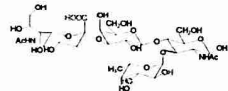

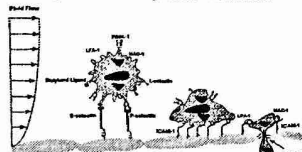**Figure 1.** The sialyl-Lewis^x molecule.

Figure 2. Leukocytes adhesion and transmigration into tissues. The interaction between endothelial selectins and sialyl-Lewis^x is very important for this event.

A molecule mimicking sLe^x (1) has been synthesized and coupled to Gd-DTPA (2) (Figure 3).
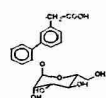

Figure 3. The sialyl-Lewis^x mimetic.

Its potential to image inflammation by interaction with endothelium has been confirmed (3).

In the present study, a new contrast agent was synthesized by coupling the sLe^x mimetic to ultra small particles of iron oxide (USPIO-g-sLe^x). USPIO-g-sLe^x has been tested on a mouse model of hepatitis induced by the injection of Concanavalin A (ConA) (4).

USPIO have long circulation times but are nevertheless captured by the RES cells. Such accumulation of particles in Kupffer cells induces a signal decrease of the liver parenchyma on MR images. The retention of USPIO-g-sLe^x on the vascular endothelium should however limit their uptake by the RES cells.

SUBJECTS AND METHODS

Relaxometry

The NMRD relaxation profile was recorded from 20 kHz to 10 MHz on a STELAR field cycling relaxometer (Mede, Italy). Additional measurements at 20, 40, 60 and 300 MHz were performed on Bruker Minispec systems and on an AMX spectrometer (Bruker, Karlsruhe, Germany)

Animal studies

Hepatitis was induced on NMRI mice after i.v. injection of ConA (20 mg/kg). USPIO-g-sLe^x was injected i.v. at a dose of 30 µmol Fe/kg to ConA-treated mice and to healthy mice as controls. USPIO was also injected at the same dose to healthy and ConA-treated mice as a non-specific controls.

MR imaging and data analysis

The images were obtained on a Bruker AVANCE-200 system (4.7 T). One pre-contrast and several post-contrast axial images of the liver were acquired at different delays (2 min to 120 min) after the injection of the particles with a 2D GE sequence (TR/TE: 58.8/5.2 ms, FOV: 5.5 cm, matrix: 256x256,flip angle: 50°). ConA-treated mice were analysed 5 hours after the induction of hepatitis. PARAVISION software was used to measure signal intensities (SI) of ROIs chosen in the liver on MR images. Signal to noise ratio (SNR) was calculated and relative signal enhancement was obtained with the following formula:

$$(SNR)_{relative\ enhancement} = 100 \times \frac{(SNR)_{post-contrast} - (SNR)_{pre-contrast}}{(SNR)_{pre-contrast}}$$

RESULTS

Relaxivity

The r_1 and r_2 values of USPIO-g-sLe^x are respectively 18.7 s^{-1} mM^{-1} and 56.0 s^{-1} mM^{-1} at 20 MHz and 37°C (Figure 4).
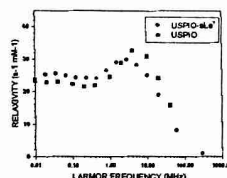

Figure 4 : NMRD profile of USPIO-g-sLe^x and of USPIO.

As compared to the original particles, the branching of the synthetic mimetic does not induce a major change of the relaxometric properties.

MR imaging

Figure 5 shows the axial MR images resulting from USPIO-g-sLe^x or USPIO injection to healthy and ConA-treated mice. Signal decrease caused by the USPIO-g-sLe^x or USPIO uptake in the Kupffer cells can be observed in the liver. However, with USPIO-g-sLe^x, the liver of the healthy mice becomes darker than that of the ConA-treated mice. These results suggest that USPIO-g-sLe^x is taken up by the Kupffer cells of diseased livers to a lesser extent, probably as a result of the interaction with E-selectin on the vascular endothelium.

MR Images

A. Pre-contrast

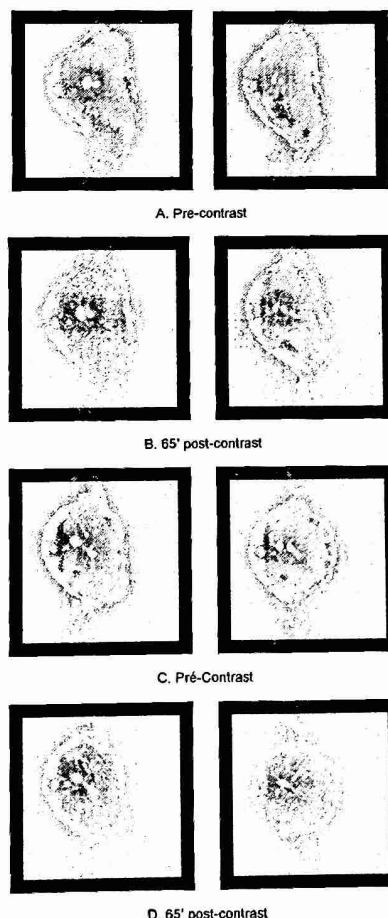B. 65' post-contrast

C. Pré-Contrast

D. 65' post-contrast

Figure 5. Axial GE MR images of healthy (left) and ConA-treated (right) mice before and 65 minutes after the injection of 30 µmol Fe/kg of USPIO-g-sLe^x (A, B) and of USPIO (C, D). Colour mapping of MR images was performed with the Osiris program.

MR image analysis

Analysis of the MR images (Figure 6) shows the reproducibility of the phenomenon one hour after the injection of the contrast agents. Relative enhancement of the S/N ratio measured in the liver of ConA-treated mice injected with USPIO-g-sLe^x is significantly higher than for the other groups.
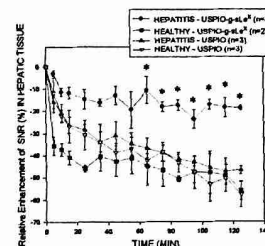

Figure 6. Relative enhancement of SNR in the liver of healthy and ConA-treated mice after the injection of USPIO or USPIO-g-sLe^x. Mean values and standard error bars are represented on the graph. (*: statistically significative (p<0.05) regarding all the control groups).

These results suggest that an interaction between USPIO-g-sLe^x and endothelial E-selectin occurs in hepatitis, preventing the uptake of USPIO-g-sLe^x by Kupffer cells.

In healthy conditions, it also seems that the USPIO-g-sLe^x is more rapidly taken up by the liver than the USPIO. This could be explained by an interaction between the C-type surface lectin of the Kupffer cells (the Mannose Receptor) and the mannose residue present in the sLe^x mimetic molecule (5).

DISCUSSION

A reduced SPIO-mediated hepatic uptake in patients with cirrhosis, as been observed by imaging (6). Kupffer cells dysfunction was invoked to explain this difference. In our experimental conditions however, the uptake of the USPIO is not significantly different between healthy and ConA-treated mice, which suggests that the function of Kupffer cells is not altered by ConA. Contrarily, in diseased liver, USPIO-g-sLe^x are taken up by Kupffer cells to a lesser extent, probably because of their interaction with E-selectin expressed on liver endothelial cells during inflammation. This observation is supported by the SNR evolution in liver parenchyma.

CONCLUSION

Our results show that USPIO-g-sLe^x has an interesting but paradoxical potential for the in vivo diagnosis of inflammatory diseases. Besides, USPIO-g-sLe^x could find application for the in vitro evaluation of sLe^x interactions with endothelial cells.

ACKNOWLEDGEMENTS

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