MRI monitoring of the influence of US contrast agent destruction for local delivery of a MRI blood pool contrast agent in the rat liver

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Introduction

Local drug delivery by ultrasound is a promising strategy for increasing the drug concentration at the target location [1], and decrease systemic toxicity effects. The presence of microbubbles during sonication at the target location increases the likelihood for cavitation. For local drug delivery the most important bio-effect of cavitation is the increased capillary permeability [2], which causes an increased extravasation of co-administered macromolecules that normally do not spontaneously diffuse outside the vessels [3,4]. The objective of our work was to show the ability of MRI to visualize and measure the time course of a MRI blood pool contrast agent deposition by ultrasonic destruction of microbubbles, in vivo on the liver of rats. In addition, the potential cell damage induced by microbubbles destruction was evaluated by histology.

Material and methods

Experimental protocol: Male Wistar rats aged from 3 to 5 months (350 to 550g) were anesthetized with Isoflurane (2%) in air and positioned supine in a 1.5Tesla MRI scanner (Philips Achieva) with a 4.7 cm in diameter surface coil taped on the abdomen. Four transverse slices centered on the liver were repeated before and during 1 hour after injection of contrast agent to measure the potential changes of the T1 in the liver. Imaging was performed with a fast inversion-recovery sequence (look-locker: LL) with the following parameters: TR=23ms, TE=11ms, EPI factor=11, flip angle=30°, FOV=96x96mm^2 matrix=96x96, NSA=4 [5]. About 30 acquisitions at different recovery times were performed to measure the recovery of the longitudinal magnetization, with respiratory triggering, leading to an acquisition time of 2 to 3 minutes, depending on the breathing period. After acquisition of reference images with the LL sequence, a macromolecular MRI contrast agent (Vistarem®, Guerbet-France) was injected in the tail vein (slow bolus of 20-30 sec) at a dose of 40 µmol/kg [6], followed by a second injection (0,05mL/100g) of a US contrast agent (Sonovue®, Bracco-Italy). In one group of rats (n=5), these microbubbles were immediately visualized with a clinical echograph (Acuson Sequoia 512, Siemens) located in the MR room, and were then destroyed with the same apparatus, using a pulsed mode imaging with a high mechanical index (MI>1.6) and a low frequency (2 MHz) for 2 minutes. The total duration of injections and sonications was less than 6 minutes. Then, the animals were rapidly repositioned at the isocenter of the magnet without displacing them from their holder, in order to measure the temporal evolution of T1 values. The second group of animals (control group, n=7) received identical injections without application of ultrasound. Body temperature was continuously monitored with a rectal optical probe (Luxtron), since variations of temperature may influence T1 values. Image processing: The images obtained from each look-locker measurement were automatically fitted with an in-house developed software written in IDL language (ITT corporation): for each pixel, the temporal evolution of the magnitude of the longitudinal magnetization was fitted (marquardt-levenberg routine) with the following equation $Mz(t) = M0.|a-b\,e^{-t/T1}|$ to obtain a parametric map of the T1 values (see Figure 1). This automatic process was repeated for each dynamic acquisition. Then, three regions of interest (ROI) were manually selected in the left, center and right parts of the liver to analyze the evolution of T1 (mean+/-sdev) as a function of time. Histology: The animals were sacrificed 24 hours after the injection and routine histological analysis (Hematoxylin-Eosin staining) was performed on each liver to detect potential tissue alteration.

Results

Figure 1 shows typical results of the evolution of the signal obtained with the look-locker sequence in a single pixel located within the liver (dots) and the corresponding fit (green curve). The resulting parametric map of M0 and T1 are also displayed. In most of the liver, the precision of the mean T1 value was less or equal to 15 ms. Figure 2 shows the temporal evolution of T1 values (normalized to the initial value) determined with the proposed method at 7, 15 minutes and 60 minutes (t=0 corresponded to the beginning of the injection of Vistarem®) in the ROI located in the center of the liver for the two groups of animals. A clear difference was observed with a systematic lower value of T1 for the group with destruction of microbubbles, indicative of a change of the interaction of the MRI contrast agent with the tissue. This difference was higher immediately after application of ultrasound (T1 at 7 minutes after injection of Vistarem® represented 63% (+/-6%) in the group with microbubbles destruction versus 81% (+/-4%) in the control group) and progressively decreased in time. However, a difference remained one hour after injections. As expected, a similar behavior was observed for the ROIs located on the left and right parts of the liver since the ultrasonic field induced by the probe (4C1) used for destruction of the Sonovue® covered the complete liver. Body temperature remained stable during the experiment (mean variation of 1.1+/-0.7 °C). The microscopic analysis of the liver did not reveal the presence of tissue damages for the two groups (data not shown).

Conclusion and perspective

Destruction of US contrast agent can be used to influence the interaction between a MRI vascular contrast agent and the surrounding tissue. The proposed method allows for a non invasive measurement of T1 change in time after injection of a blood pool MRI contrast agent and destruction of US contrast agents. This approach can be very useful to determine the time course and reversibility/irreversibility of this process, depending on the administered doses and ultrasonic parameters (duration, duty cycle, mechanical index). Since Sonovue® is used in clinical diagnostic imaging, such an approach could be applied to increase the release outside the blood of large molecules having a therapeutic effect, without inducing tissue damage.

Figure 1: Typical results a look-locker measurement. The signal intensity was measured for 29 successive inversion times (black dots). The green curve is the result of the fit (Eq [1]). The parametric maps of the M0 and T1 values are displayed on the right side of the graph to illustrate the quality of the results. The selected ROI for temporal analysis of the T1 is displayed on the M0 map.

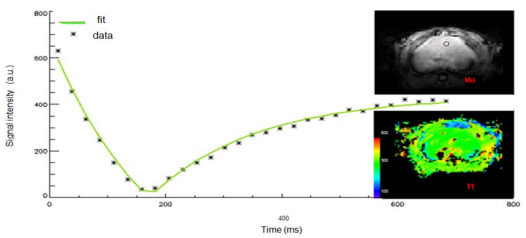
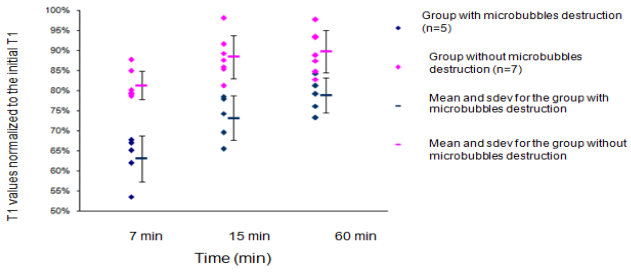


Figure 2: Temporal evolution of T1 values. The graph represents T1 values normalized to the initial T1 value at 7, 15, 60 min in the ROI located in the center of the liver (see Figure 1) for all the rats of the 2 groups, with mean and standard deviations (sdev).



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