Gas-filled Microbubbles as Intravascular Susceptibility Contrast Agent for Brain MRI

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Introduction

Gas-filled microbubbles have the potential to become a unique MR contrast agent due to their magnetic susceptibility effect, biocompatibility and localized manipulation via ultrasound cavitation. In this study, two types of microbubbles, custom-made albumin-coated microbubble (A-MB) and a commercially available lipid-based clinical ultrasound microbubble contrast agent (SonoVue®), were examined as intravascular MR brain contrast agents using dynamic susceptibility MRI in Sprague-Dawley (SD) rats at 7 Tesla.

Methods

Microbubble Preparation and Animal Procedures: SonoVue® microbubbles (Bracco) consist of sulphur hexafluoride gas stabilized in aqueous dispersion by phospholipid monolayer. Air-filled A-MBs were produced by sonication as previously described [1]. Briefly, 5% bovine serum albumin (USB) solution was preheated to about 70 ºC and sonicated using 20 kHz ultrasound. Five normal SD rats (~250-350 g) were used. Each rat was anesthetized with IP injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). Femoral vein catheterization was performed with a 1-m long tube (Braintree) connected to a 27-gauge needle. The dead space in the catheter was about 0.2 mL. During imaging, animals were anesthetized with isoflurane/air using 1.0-1.5% via a nose cone with respiratory monitoring.

Microbubble Administration: Microbubbles were first warmed slowly to room temperature. A-MB suspension was resuspended by inversion and rotation for 2 min until a homogenous milky-white suspension formed. For SonoVue®, 1.2 mL sodium chloride 0.9% w/v solution was used for dispersion of powder in the vial. Resuspension was performed by vortexing for ~20 s until a homogenous suspension formed. For each imaging session, 0.2 mL of microbubble suspension (of ~4% volume fraction for A-MB and ~3.5% volume fraction for SonoVue®) was slowly injected (over ~10 s) into femoral vein at a rate of 1.2 mL/min to avoid possible microbubble destruction due to high pressure and shear stress.

MRI: All MRI experiments were performed on a 7 T Bruker MRI scanner using a 38-mm quadrature RF resonator. Anatomical images were acquired with 2D FLASH sequence with resolution = 0.20 × 0.20 × 2.0 mm^3. Dynamic susceptibility weighted MRI was performed with single-shot GE-EPI sequence using TR = 1000 ms, TE = 30 ms, FA = 90º, FOV = 29 mm × 29 mm, slice thickness = 0.7 mm, acquisition matrix = 96 × 96, BW = 221 kHz and NEX = 1. Microbubble suspension was injected about 5 min after the start of dynamic imaging. A minimum lapse of 10 min was used to ensure sufficient clearance of the microbubbles before the next injection. The susceptibility effect of microbubbles was compared with that of a well-established intravascular contrast agent, monocrystalline iron oxide nanoparticles (MION; MGH), in brain by single dose of 0.6 mg Fe/kg injection using identical injection protocol and imaging sequence.

Image Analysis: GE-EPI images were first co-registered using AIR5.2.5 [2]. ΔR_2^* maps were computed on a pixel-by-pixel basis as $\Delta R_2^* = \ln (S_{pre}/S_{avg\text{-}post})/TE$, where S_{pre} is the average intensity in 100 preinjection images and $S_{avg\text{-}post}$ is the average intensity in 40 postinjection images with maximum susceptibility contrast for microbubbles (or 100 postinjection images at steady-state contrast for MION). To quantify the ΔR_2^* values, ROIs were manually drawn in cortex (Ctx), cauduate putamen (CPu) and blood vessel (BV) regions based on the high resolution FLASH images. Assuming that ΔR_2^* is proportional to microbubble concentration C(t) at time t, C(t) can be estimated as $C(t) = k \ln \{S_{pre}/S(t)\}/TE + C_B$, where S(t) is the image intensity at time t, k a proportionality constant, and C_B a constant residue to account for any postinjection baseline [3]. Given the relatively long injection time and the limited lifetime of microbubbles in vivo, C(t) were approximately modeled with a gamma-variate function by curve fitting [4]. Full width at half maximum (FWHM) and time-to-peak were then measured from the fitted C(t) time courses.

Results and Discussion

Fig. 1 and 2 illustrate the rat brain images typically observed during A-MB injection (0.2 mL of ~4% volume fraction) and SonoVue® injection (0.2 mL of ~3.5% volume fraction) respectively. Note that the microbubbles induced ΔR_2^* maps were observed to be similar to those caused by intravascular MION. Table 1 shows the in vivo measurements of the ΔR_2^*, FWHM and time-to-peak of A-MB and SonoVue® as well as the ΔR_2^* and time-to-peak of MION in cortex area among all rats studied. With identical injection protocol, time-to-peak was found to be shorter for MION. This is largely expected as microbubbles, with size comparable to that of red blood cells, flow slower than blood plasma while MION nanoparticles flow together with plasma. ΔR_2^* was 2.49 ± 1.00 s^{-1}, 2.41 ± 1.18 s^{-1} and 1.98 ± 0.36 s^{-1} for A-MB, SonoVue® and MION, respectively. This indicates that the in vivo susceptibility effects of A-MB and SonoVue® at the dosage used are comparable to that of 0.6 mg Fe/kg MION in brain tissue at 7 T.

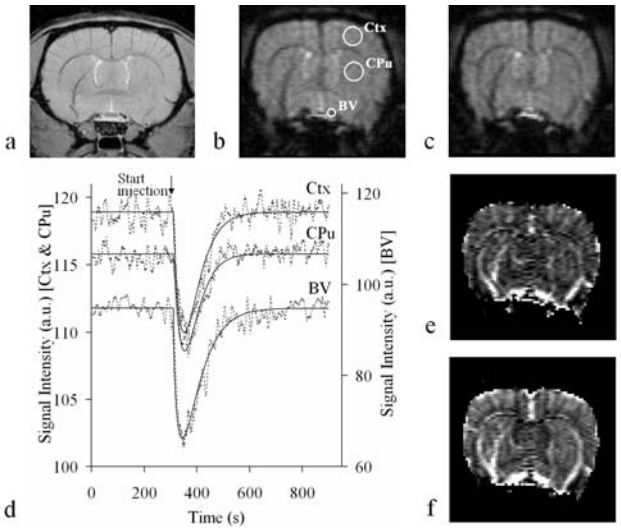

Fig. 1. Typical images from a rat brain during A-MB injection (0.2 mL of ~4% volume fraction over ~10s): (**a**) anatomical image; (**b**) preinjection GE-EPI T_2^*-weighted image; (**c**) postinjection GE-EPI T_2^*-weighted image with the maximum susceptibility contrast; (**d**) T_2^*-weighted signal time courses in different ROIs during A-MB injection; (**e**) ΔR_2^* map computed from A-MB data; and (**f**) ΔR_2^* map obtained using intravascular contrast agent MION at 0.6 mg Fe/kg. Three ROIs used for time course measurements are shown in (b) and gamma-variate fitted data shown in sold lines.

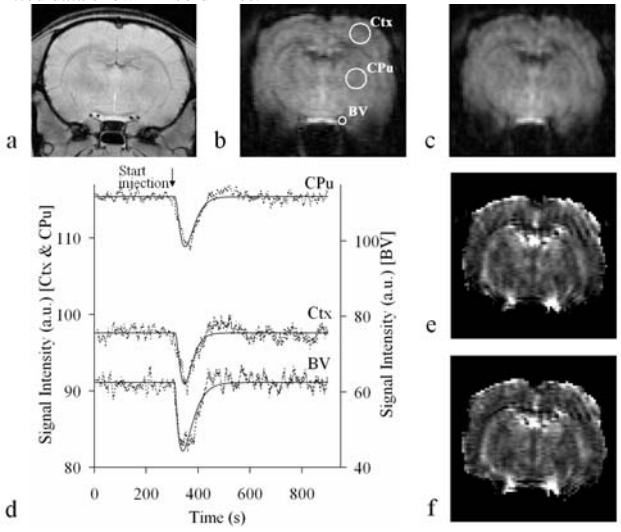

Fig. 2. Corresponding images from a rat brain during SonoVue® injection (0.2 mL of ~3.5% volume fraction over ~10s).

Table 1. Measurements of ΔR_2^*, FWHM and time-to-peak of the concentration time courses for A-MB (0.2 mL of ~4% volume fraction), SonoVue® (0.2 mL of ~3.5% volume fraction) and MION (0.6 mg Fe/kg) in rat brain cortex (mean ± standard deviation, $N = 5$).

	ΔR_2^* (s^{-1})	FWHM (s)	Time-to-peak (s)
A-MB	2.49 ± 1.00	114 ± 39	57 ± 20
SonoVue®	2.41 ± 1.18	86 ± 16	48 ± 12
MION	1.98 ± 0.36	N.A.	24 ± 2

Conclusion

We demonstrated, for the first time, the feasibility of gas-filled microbubbles as an intravascular susceptibility contrast agent for brain MRI at 7 T. Considerable susceptibility induced changes were observed and characterized in rat brains using custom-made albumin-coated microbubbles and a commercially available clinical ultrasound microbubble contrast agent. The results indicate that microbubbles can serve as a unique intravascular MR contrast agent at high field. As microbubble fabrication technology is advancing, substantially increased in vivo lifetime by using surfactant molecules [5] and molecular targeting capability by means of ligand incorporation [6] have been demonstrated. All together, these technologies may enable microbubble MRI to provide effective real-time imaging guidance in various microbubble-based drug delivery and therapeutic applications.

References [**1**] D Cerny et al, United States patent 4957656 1990. [**2**] RP Woods et al, J Comput Assist Tomogr 1998;22:139-152. [**3**] KK Wong et al, Magn Reson Med 2004;52:445-452. [**4**] DR Pickens et al, Invest Radiol 1992;27:S12-17. [**5**] E Dressaire et al, Science 2008;320:1198-1201. [**6**] KW Ferrara et al, Adv Drug Deliv Rev 2008;60:1097-102.

Acknowledgements: This work was supported by GRF7642/06M.