Activated MR Contrast Agent by A Dual Contrast Technique And Their Application

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Introduction: Gadolinium (Gd)-based contrast agents represent positive contrast agents while superparamagnetic iron oxide (SPIO) nanoparticles are categorized as negative contrast agents. Both types of agents have been used extensively as a single agent, and concomitant use has also been tried pre-clinically (1) and clinically (2) to improve the contrast in diagnostic MR images. The strategy of the activated MR contrast agent developed here is based on the encapsulation of both a positive and a negative contrast agent within the same carrier and subsequent decomposition of the carrier. The concept behind this strategy is that strong negative signal enhancement due to the T_2/T_2* effects of iron oxides dominates the positive T_1 contrast generated by a Gd-based contrast agent when these agents are in close proximity, such as within an intact nanocarrier encapsulating GdDTPA/SPIO, and positive T_1 contrast becomes evident upon release of Gd-based contrast agent from the carrier once the distance between Gd-based contrast agents and SPIO molecules is beyond the T_2/T_2* enhancement range, as illustrated in Fig. 1. One of the suitable applications for the activated contrast agent is noninvasive release monitoring of small molecular weight cargo molecules, GdDTPA, from the carrier and subsequent intratumoral distribution of GdDTPA. Activation of MR contrast enhancement also involves the diffusion of a low molecular weight Gd-based contrast agent from the areas of negative signal enhancement generated by massive SPIO nanoparticles upon degradation of the carrier. This phenomenon is based on a significant restriction of the free diffusion of massive SPIO nanoparticles due to their large sizes (40-70 nm), which results in a significantly shorter diffusion range. Therefore, the low molecular weight Gd-based MR contrast agent can be used as a surrogate marker for other small molecule anticancer agents, assuming their diffusion rates are similar, on the grounds that intratumoral delivery of larger molecules is restricted due to short diffusion distance from vascular surface compared to smaller molecules (3). In this study, we sought the feasibility of the activated MR contrast agent using a dual contrast technique *in vitro* phantom study and *in vivo* mice xenografted tumor models.

Methods: Liposomes were used for a model carrier system. Omniscan® (GdDTPA-BMA) and Feridex® (SPIO)-loaded liposomes (Lip-Gd/Fe) were prepared by the sonication method, followed by extrusion through polycarbonate membrane (pore size: 200 nm first, then 100 nm). Empty liposomes (Lip) and single contrast agent-loaded liposomes (Lip-Gd and Lip-Fe) were also prepared for controls. A dynamic laser-light scattering (DLS) was used for the measurement of particle size, size distribution, and ζ-potential of the resultant liposomes. Gd contents were determined by T_1 relaxation time measurement using MRI, after destruction of liposomes. The encapsulation of SPIO nanoparticles in liposomes was confirmed using atomic force microscope (AFM). *In vitro* visualization study was performed using 2% agarose gel as illustrated in Fig. 1. A multislice-multiecho pulse sequence wi th an echo time (TE) of 15 ms, and six different repetition times (TR) were used on a Bruker 9.4T spectrometer. *In vivo* release monitoring of GdDTPA-BMA from liposomes and subsequent intratumoral distribution were investigated using two xenografted cancer models, a rat prostate cancer MatLyLu and a human breast carcinoma MCF-7 in mice. To obtain both released GdDTPA-BMA and SPIO distribution images, a 3D fast spin echo (RARE: Rapid Acquisition with Relaxation Enhancement) sequence with an effective TE of 50 ms and a TR of 1000 ms was acquired before and after intratumoral or intravenous administration of Lip-Gd/Fe. For the quantitative T_1 map, a 3D RARE pulse sequence with a TE of 50 ms, and five different TRs was acquired with a spatial resolution of 0.125×0.250×0.250 mm. For T_2* acquisition, a 3D fast low-angle shot (FLASH) pulse sequence was used with the following parameters: TE/TR = 7/100 ms; NA = 4. The distribution of the SPIO relative to the tumor vasculature was also visualized *ex vivo* 48 hrs post-i.v. administration of Lip-Gd/Fe in MatLyLu tumor xenograft.

Results/Discussion: *In vitro* release of MR contrast agent from liposomes with or without Triton® X-100 was successfully monitored using 2% agarose gel system. Intratumoral release and distribution of GdDTPA-BMA was noninvasively monitored *in vivo* following both intratumoral and intravenous administration of Lip-Gd/Fe at a dose of 14.2 nmol eq. GdDTPA-BMA, and 0.43 μmol eq. GdDTPA-BMA, respectively. A restricted SPIO diffusion was also proved by *ex vivo* fluorescent iron staining.

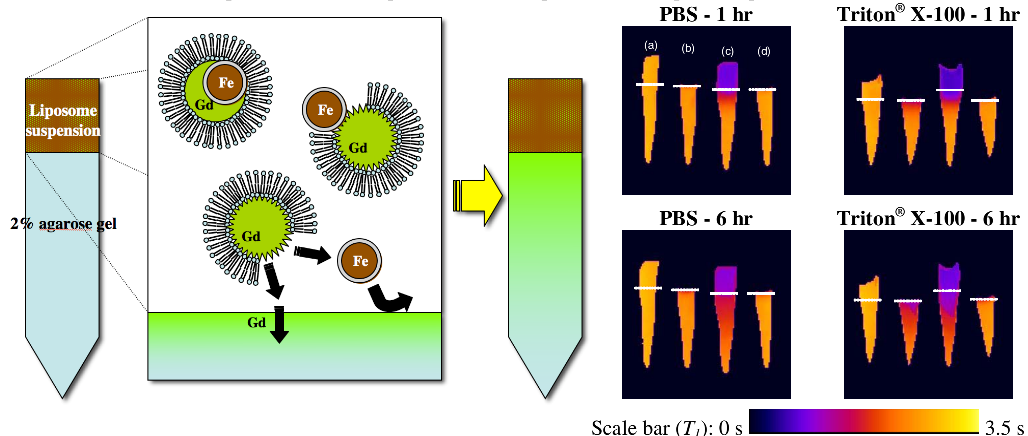

Figure 1. Schematics of *in vitro* release visualization of liposomal dual contrast agents (left panel). (a) Lip, (b) Lip-Gd/Fe, (c) Lip-Gd, (d) Lip-Fe. Quantitative T_1 maps of liposome phantoms are shown in the right panels. White broken lines represent the interface of the suspension/agarose gel layer. Parameters: 3 sagittal slices (st = 1 mm); FOV = 40 × 22 mm; matrix size = 128 × 80; TE = 15 ms; NA = 2.

Conclusion: A dual MR contrast technique could be a promising strategy for activated MR contrast agent. Non-invasive release monitoring and subsequent intratumoral distribution of cargo molecules is one of the potential applications for an activated MR contrast agent using a dual contrast technique.

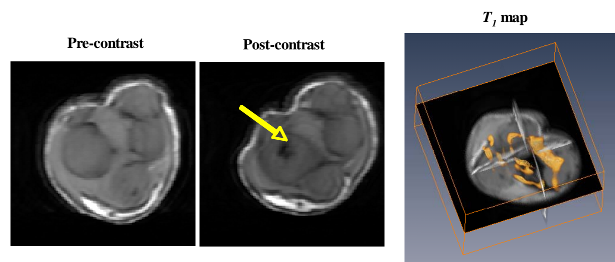

Figure 2. MR images of MatLyLu prostate cancer xenograft before and 2 hr post-intratumoral administration of Lip-Gd/Fe suspension. Post-contrast images were co-registered to the pre-contrast images for comparison. Negative contrast in the tumor is generated by extravasated liposomes (yellow arrow). Orange areas on the right image represent leaking of low molecular weight GdDTPA-BMA released from spontaneously disrupted liposomes.

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