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 decapsulation of the carrier. The concept behind this strategy is that strong negative signal enhancement due to the T2/T1* effects of iron oxides dominates the positive T1 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 T1 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 T2/T1* 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 small molecular 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, distribution, and ζ-potential of the resultant liposomes. Gd contents were determined by T1 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 with 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 T1 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 T2* 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.

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.

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