Direct *in vivo* evidence of penetration of the Blood Brain Barrier with Nano-liposomes

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Introduction

Gadopentetate dimeglumine (Gd-DTPA; Magnevist, BayerHealthcare) is widely used as an MRI contrast agent for detection of brain tumors but is limited to the cases where the blood brain barrier (BBB) is significantly compromised by infiltration of tumors because Gd-DTPA itself is not able to cross the BBB. The tumor boundary produced by Gd-DTPA-enhanced MRI reflects only the extent where BBB is compromised instead of a true tumor-normal tissue boundary. It is desirable to develop a new contrast agent that is capable of crossing the intact BBB to detect the tumor at its earlier stage when treatment could be more effective. Previous work in our laboratory has shown that IL-13Rα2 receptors are over-expressed in most types of glioma cells [1]. We further demonstrated that IL-13, as a targeting agent, when conjugated with liposomes containing doxorubicin (anticancer drug) inhibited the glioma tumor growth more effectively in a tumor mouse model [2]. To determine the ability of our liposome to penetrate uncompromised BBB compared to free Gd-DTPA, in this work we replaced the therapeutic drug with a contrast agent, Gd-DTPA, in the liposomes and examined the dynamic contrast change in normal animal brain. Our data provided the first and direct in-vivo evidence that liposomes are capable of crossing the intact BBB in normal animals, which creates a great potential application in treating glioma.

Methods

Preparation of liposomes: Liposomes were formulated as described in our earlier work [1]. Briefly, the lipids 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), Cholesterol, 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[carboxy(polyethylene glycol)-2000] (ammonium salt) (DSPE-PEG2000), 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[maleimide(polyethylene glycol)-2000] (ammonium salt) (DSPE-PEG-Mal) in a molar ratio of 10:5:5:0.5 were dissolved in a mixture of methanol/chloroform in a ratio of 1:1 (v/v). The organic solvents were evaporated to produce a solid film. Subsequently, the film was reconstituted in a saturated solution of Gd-DTPA with poly-L-lysine to form multilamellar vesicles and covalently conjugated to human IL-13 protein. The Gd-liposomes were exhaustively dialyzed against PBS to remove the free Gd-DTPA. The average particle size was 100-150 nm as measured by Malvern Particle size Analyzer (Zetasizer Nano S). The Gd concentration in the liposomes was in the range of 4.0-10.0 mg/L quantified using ICP-AES. The relaxitivity of Liposome-Gd was 0.7mmol/L when measured at 7T.

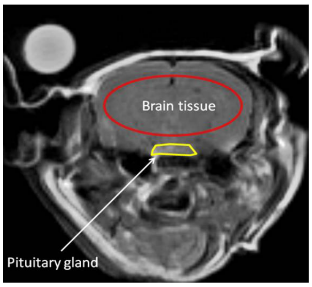

Figure 1: The two ROIs used for SNR measurement.

Imaging: Adult mice were anesthetized with 2% isoflurane/oxygen. Axial T1-weighted images were acquired on a 7T MRI system (Bruker Biospin 7/20a, Ettlingen, Germany) with 540/11ms TR/TE, 8 NEX, 192x192, 3.2cm FOV, 0.5mm thickness with 0.5mm gap at following time-points: a pre-dose and 5 post-dose scans at 10, 30, 60, 120-minutes and 24-hours following a tail vein (IV) injection of either Gd-DTPA or the IL-13 Liposome-gadolinium.

Image Data Analysis: The signal-to-noise ratios (SNR; Figure 1) of pre- and post-injections images were evaluated from two ROIs: 1) from the brain parenchyma and 2) from posterior pituitary gland on the same slice. The reason posterior pituitary gland was selected is that it does not have BBB. Its signal can be used as an internal indicator of contrast agent status in the brain tissue.

Results and Discussion

As shown in Figure 2a, SNR from pituitary gland increased up to 25% post-injection of Gd-DTPA, while SNR from brain parenchyma remained unchanged, indicating that no Gd reached the brain parenchyma, because of the blockage of BBB. In contrast, after injection of liposome-Gd, the SNR in the brain tissue followed the same trend as that from the pituitary gland as shown in Figure 2b, indicating the presence of Gd in brain tissue. This result clearly demonstrated that our liposomes are capable of delivering Gd across the intact BBB in normal animals. Previous *in vivo* studies by our team and others demonstrated that drugs delivered by liposome could more effectively reduce the cancer progression in an animal tumor model [2]. Since it is known that the BBB is significantly compromised in these tumor models, it is not possible to draw a conclusion on whether liposome could deliver the drug more effectively through BBB using animal tumor models. Thus, normal animals were used for this study and our data provided conclusive evidence that liposome mediated delivery of Gd-DTPA resulted in a SNR increase in the brain parenchymal region compared to free Gd-DTPA in the pituitary gland. Notice that the SNR increase generated by the liposome-Gd is lower (<10%) compared to free Gd-DTPA. The low relaxitivity is likely due to the "quenching effect" of encapsulation of liposome. We do not anticipate that this would limit its application as targeted liposome would increase the local concentration in the tumor and the quenching effect would disappear as the liposomes rupture upon internalization into the tumor cell.

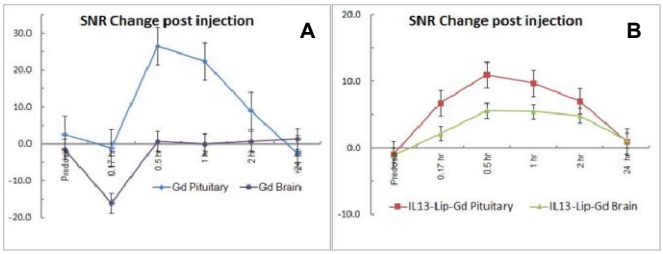

Figure 2: SNR Change after Gd-DTPA injection (**A**), and Liposome-Gd injection (**B**).

[1] Madhankumar, A.B., et al., Mol Cancer Ther, 2006. 5(12): p. 3162-9 [2] Madhankumar, A.B., et al., Mol Cancer Ther, 2009. 8(3): p. 648-54.