MR Apoptosis Imaging and Evidence of Apoptotic Nanoprobe Passing Across the Blood Brain Barrier

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Introduction

Over the past a few years, iron oxide based nanoparticles have been utilized as MR targeting contrast agents for in vivo tumor studies. This is because spin-spin relaxation times T_2 and T_2* are very sensitive to local magnetic environments that can be regulated by the iron concentration of the superparamagnetic nanoparticles. Therefore, the presence of nanoparticles conjugating to targeting agent(s) can be a powerful source of detecting tumor in vivo. Intravenous injection of such nanoprobes into animals induces particle depositions in internal organs but not in the brain. The main reason for the nonappearance of the nanoprobes in the brain is due to its own protecting mechanism of the blood brain barrier (BBB). In order to have nanoparticles passed across the BBB, researchers have induced a temporary BBB disruption at the intravenous injection of nanoprobes[1, 2].

In the present study we developed the nanoprobe to achieve the MR apoptosis imaging for early stage of brain tumor (medulloblastoma D283) using annexin V which has a high binding affinity to phospatidylserine (PS) that is externalized during the apoptosis. Synthesized nanoparticles were coated with PEG (polyethylene glycol) and followed by the conjugation to annexin V. Linear relationship was obtained between the degree of apoptosis and iron concentration of nanoprobe. We also found the evidence that the synthesized nanoprobe passed across the BBB. The initial uptake of np-PEG-annexin V particles by the brain maintained for 4-5 hours and the particles cleared out approximately 9 hours after the intravenous administration of the nanoprobe.

Method

Nanoparticulated contrast agents were synthesized with their core materials of iron oxide, PEG coating and annexin V conjugation. Base material of the PEG coated nanoparticles was smaller than 15 nm. Pre-contrast images were obtained prior to the administration of the nanoprobe (np-PEG-annexin V) while longitudinal imaging was performed for 24 hours to monitor longitudinal behavior of the nanoprobe within a mouse in vivo. Iron concentration of 571.8 μg Fe/mL for the nanoprobe was administered with the injection volume of 0.1 mL. T_2 weighted MR images were acquired with four different TE (echo time) values then their corresponding spin-spin relaxation rate R_2 values were calculated to investigate the nanoparticle uptake by a normal brain. MR images were acquired on a 4.7 T magnet equipped with Varian INOVA spectrometer and a home-built Alderman-grant volume coil.

Results

Spin-spin relaxation rate R_2 values were calculated by acquired T_2 weighted MR images. Figure 1 displays R_2 variation prior to (time = 0) and after the administration of the nanoprobe. Significant uptakes were observed up-to ~5 hours after the administration and the recovery of R_2 was detected at ~9 hours after the administration. Image slice in the medulla area closed to brain stem was selected among 10 image slices and two ROIs (regions of interest) were chosen.

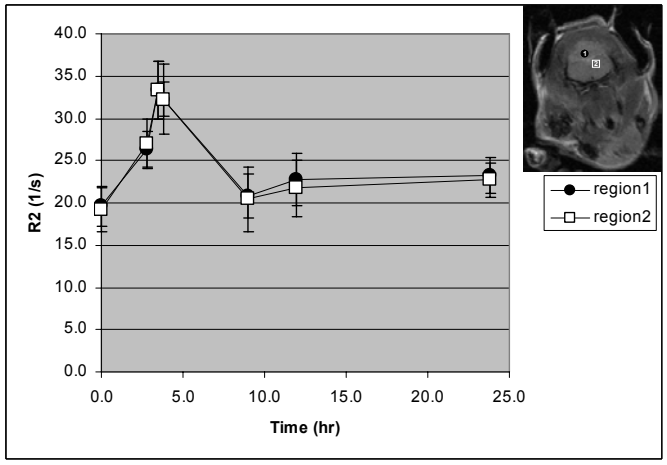

Figure 1. R_2 values were serially measured after the administration of nanoprobe on two different locations of normal mouse brain in vivo as depicted in the image insert on the right upper corner of the graph. Data points marked at time 0 were acquired from pre-contrast images that were obtained prior to the administration of the nanoprobe.

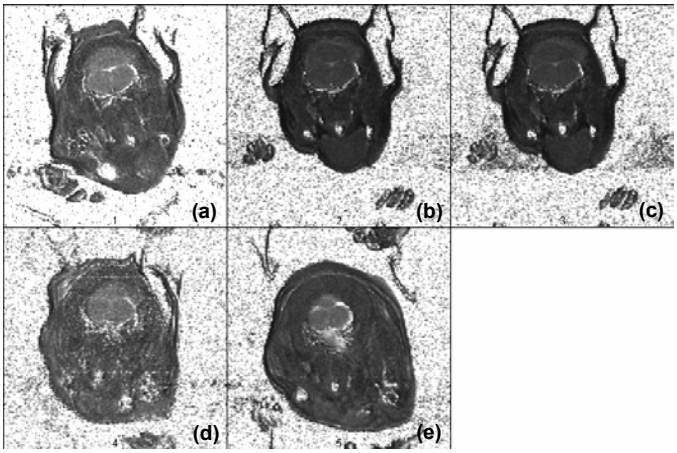

Figure 4 Longitudinal T_2 maps generated from multi-spin echo images that were acquired before administration (a), and at 3.2 hrs (b), 4 hrs (c), 13.7 hrs (d), and 24 hrs (e) after the administration of annexin V-conjugated nanoparticles.

Discussion

Significant degree of nanoprobe uptake was detected in a normal mouse brain administered with np-PEG-annexin V nanoprobe. The particle uptake by brain tissues was observed up-to the ~ 5 hr time point then decreased to the initial condition approximately 9 hours after the administration. This indicates a strong evidence that the small particle size (< 15 nm) of the nanoprobe synthesized for the present study helped passing across the endothelial lining of the blood brain barrier. We are in progress that the nanoprobe of np-PEG-annexin V will be utilized for the apoptotic imaging of the early stage of brain tumor. Both of the linearity of the apoptosis to T2 variation observed for in vitro cells and the presence of the nanoprobe within brain may indicate the high feasibility of our synthesized nanoprobe in the study of targeting apoptosis using MRI, which may eventually enable to examine the treatment response of the early stage of intracranial tumors in their small sizes subsequent to the diagnosis and targeted treatments.

References

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