Selective MRI and MRS of PEGylated Compounds

S. D. Swanson[1]

[1]Department of Radiology, The University of Michigan, Ann Arbor, MI, United States

Introduction:

To reduce clearance time, delay breakdown time, and increase clinical efficacy, small molecule pharmaceutical agents are sometimes covalently bound to polyethylene glycol (PEG). Current PEGylated pharmaceuticals include agents for treatment of hepatitis (Pegasys), ovarian cancer (Doxil), and macular degeneration (Macugen). Pegylation has a number of desirable properties for magnetic resonance. First, polyethylene glycol is comprised of a $(–CH_2-CH_2-0-)_n$ residue. This residue repeats n times in the PEGylated pharmaceutical where n may as large as 1,000 resulting in a significant number of ethylene protons. Second, though the PEGylated molecule is large and diffuses slowly, PEG is a very flexible polymer. The internal molecular motions create a long T_2 and a narrow resonance for the ethylene protons. Third, these ethylene protons resonate at a single frequency and generate a relatively large MR signal. For instance, there may be up to 4,000 ethylene protons for each pharmaceutical molecule. Finally, the decreased diffusion constant allows use of magnetic field gradients to suppress the signal from small molecules such as water that typically dominate the MR signal. The net result is the ability to selectively detect the PEGylated pharmaceutical and an increased sensitivity of up to three orders of magnitude.

Methods:

Pegaptanib Sodium (Macugen) is an anti-VEGF RNA aptamer shown to reduce the rate of vision loss in patients with age related macular degeneration. 1.6 mg of Macugen (FW = 50 kD) was dissolved in 2.5 ml of water. This sample contains 277 mmoles of water protons, 32 nmoles of Macugen, and 115 µmoles of PEG protons. The ratio of water protons to PEG protons in this sample is 2,411.

Selective detection of Macugen was accomplished using diffusion filtered NMR spectroscopy and imaging. Crusher gradients were applied about each of the 180° pulses in a PRESS pulse sequence. The duration of each of the four gradients was 40 ms. The amplitude of the gradient pulses was varied so that the b value ranged from 0 to 2 10^6 s cm^{-2} (gradient strengths from 0 to 3.0 G cm^{-1}) (Fig. 1). A phantom was made using the Macugen sample described above and an additional vial with water only (Fig. 2).

Results and Discussion:

The self-diffusion constant of water at 19 °C was measured to be 1.9 10^{-5} cm^2 s^{-1} and the diffusion constant of Macugen was measured to be 3 10^{-7} cm^2 s^{-1} (Fig. 1). Magnetic field gradients in a spin echo experiment attenuate the signal intensity by a factor of exp{-bD}, where b is the Stejskal-Tanner gradient factor and D is the self-diffusion or diffusion constant. At b = 10^6 s cm^{-2}, the water signal is 5 10^{-9} of the initial signal while the Macugen signal is 74% of the initial signal. This large discrepancy in attenuation allows elimination of the water signal in a diffusion weighted spin-echo imaging experiment (TR/TE 1000/100) (Fig. 2).

Conclusion:

PEGylated pharmaceuticals can be selectively detected using MRI or MRS. High molecular weight, flexible polyethylene chains, and superposition of many ethylene resonances combine to create a condition favorable for MRI and NMR detection. In this example we have selectively created an image Pegaptanib Sodium with only 32 nmoles of compound.

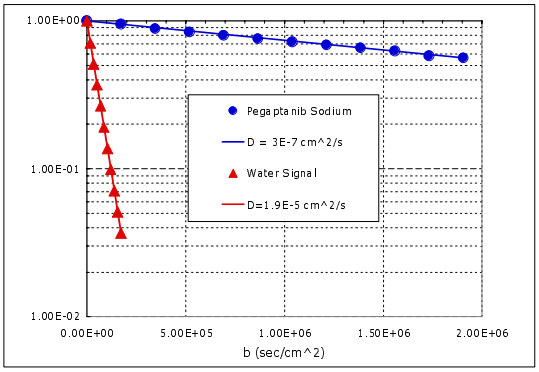

Figure 1. Signal decrease for ethylene protons on Pegaptanib Sodium (blue circles) and water proton (red triangles) as a function of increased b values in diffusion weighted press experiment.

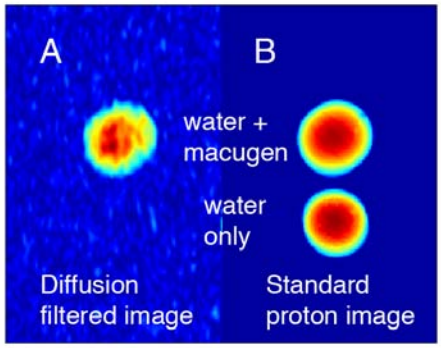

Figure 2. A phantom consisting of a vial of dilute Macugen (top) and a vial of water (bottom) was imaged. Diffusion filtered image (A) was acquired with b = 3.8 10^5 s cm^{-2}. Due to the large b factor, only the Macugen signal survives and the water signal in both vials is eliminated. A standard water proton image of the phantom is shown for comparison (B).