A NEW MT SIGNAL AT -1.6 PPM VIA NOE-MEDIATED SATURATION TRANSFER

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Target audience: Investigators interested in the development and application of MT, CEST and NOE imaging methods.

Purpose: Magnetization transfer (MT) provides a unique mechanism for producing contrast and makes MRI sensitive to the presence of metabolites, mobile macromolecules, and semisolid macromolecules through their 'magnetic coupling' effects on the water signal. The 'magnetic coupling' between water protons and the main biological tissue components has been investigated previously by using water-exchange filter spectroscopy (WEX) and two-dimensional nuclear Overhauser enhancement (NOE) spectroscopy (2D NOESY). Chemical exchange at 3.5 ppm from water between water protons and amide protons of proteins or peptides has been extensively reported. NOE effects at around -3.5 ppm from water between water protons and mobile proteins or methylene protons of membrane lipids, as well as an NOE effect at around -1.6 ppm from water between water protons and methyl protons on the choline head group of membrane phospholipids have been reported from tissue extracts and model lipids. Although CEST signal at +3.5 ppm (amide proton transfer, APT) and NOE signal at around -3.5 ppm have been widely studied, to date no *in vivo* MT effect at -1.6 ppm has been reported. In the present work, we report how to detect this new MT signal at -1.6 ppm on live rat brain and try to interpret the origin of this signal.

Methods: MT measurements were performed by applying 5 s continuous wave (CW) irradiation before single-shot SE-EPI acquisition on 9.4 T and 4.7 T Varian small animal scanners. MT Z-spectra were acquired with RF offsets from -5 ppm to 5 ppm (-2000 Hz to 2000 Hz at 9.4 T and -1000 Hz to 1000 Hz at 4.7T) and RF powers of 0.5μT and 1μT. Images were acquired with matrix size 64 × 64, field of view 32 × 32 mm and number of acquisitions = 1.

Eighteen male healthy Fischer F344 rats (three groups: 5, 10, and 20 weeks; and six in each group) were scanned at 9.4 T and 4.7 T. Twelve male Fischer F344 rats (10 weeks) were divided into 3 groups for study of the restoration of the MT(-1.6 ppm) at different time points after a 4 hour intake of isoflurane (ISO). These rats were anesthetized with a 2%/98% isoflurane/oxygen mixture for 4 hours and scanned on the first day, and then scanned again on the third and seventh days after ISO-induced anaesthesia, respectively.

Reconstituted phospholipids were used to study the molecular origin of the MT signal at -1.6 ppm. The model membrane lipids were prepared by adding Egg PtdCho (Egg PC) to a chloroform solution. Solutions were then evaporated/dried under vacuum, and re-suspended to 1: 3 Egg PC: water by weight.

Ten rats bearing 9L tumors were scanned to study the potential applications of MT(-1.6 ppm) in cancer. Four postmortem rats were made by tail vein injection of saturated KCl solution and were scanned to check the change of MT(-1.6 ppm) signal after animals were immediately euthanized.

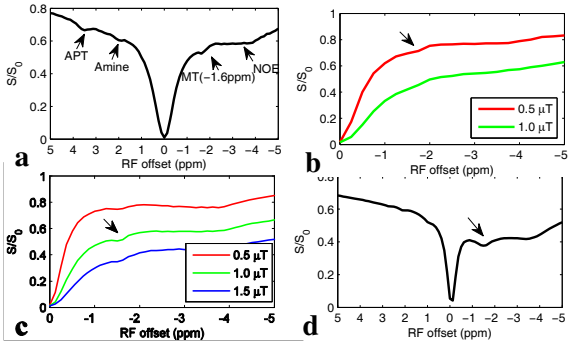

Fig. 1: MT z-spectra from GM of a representative healthy brain at a power of 1μT on 9.4T (a), at a variety of powers at 4.7T and 9.4T (b and c), from reconstituted Egg PC at a power of 1μT on 9.4T (d).

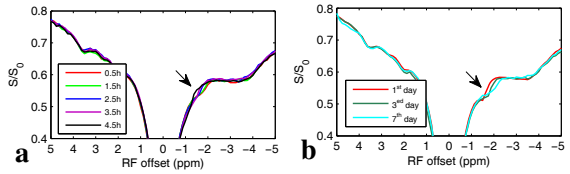

Fig. 2: MT z-spectra from GM of a representative healthy brain at different time points after onset of ISO (a) and later days after 3 hours intake of ISO (b).

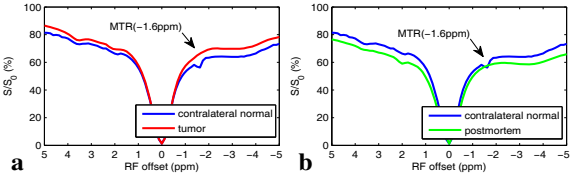

Fig. 3: Representative MT z-spectra from a rat brain bearing 9L tumor (a) and from live and postmortem of a rat brain (b).

Results: Among the forty four rats, forty two animals showed significant detectable MT(-1.6ppm) signals from normal brain if measured within 3 hours of ISO-induced anaesthesia. Fig. 1a shows the MT Z-spectra from gray matter (GM) of a representative healthy rat brain at 9.4T. It was found that in addition to MT signals from APT at 3.5ppm, amine-water exchange effects at 2 ppm, and NOE at -3.5 ppm, significant MT signal at -1.6 ppm was clearly resolved. Fig. 1b and 1c shows the MT Z-spectra acquired with a variety of RF irradiation powers at 4.7T and 9.4T, respectively, from GM of a representative healthy rat brain. It was found that MT(-1.6ppm) is optimized at 1μT at 9.4T, but at 0.5μT at 4.7T. Fig. 1d shows the MT Z-spectra from model membrane lipids. An obvious MT signal at -1.6 ppm (solid arrow) was observed. Fig. 2a shows the MT Z-spectra from GM of a healthy rat brain at different time points after intake of ISO. It was found that the magnitude of MT(-1.6ppm) becomes weaker and the resonance frequency of MT(-1.6 ppm) shifts downfield after a long-lasting intake of ISO. Fig. 2b shows the MT(-1.6 ppm) signal on the first, third, and seventh days after 3 hours' intake of ISO on the first day. It was found that the MT(-1.6 ppm) signal was influenced by the ISO even after several days. Fig. 3 shows that unlike normal brain tissue, MT(-1.6 ppm) becomes very weak in tumors (Fig. 3a) and postmortem (Fig. 3b).

Discussion: Our data from model membrane lipids show that the new MT(-1.6ppm) is consistent with magnetization transfer with the choline head groups of phospholipids in membrane. It has previously been reported that choline head groups exhibit magnetic coupling with water protons via dipole-dipole interaction and thus can cause NOE effects in MT experiments. The interaction depends on the rotational correlation time of the choline head group and depends on the membrane conformation as well. We found that the MT(-1.6ppm) signal becomes weak after a long duration of ISO which may be explained by the addition of amphiphilic ISO molecules into bilayer cell membranes which in turn affect membrane conformation and thus the NOE signal.

Conclusion: The MT signal at -1.6ppm may reflect the NOE effect between choline head groups of membrane phospholipids and water protons, and is reliably detected in healthy rat brain. Its magnitude decreases postmortem and after extended periods of ISO anesthesia.

References:

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[2] Avni, R. et al., Journal of Magnetic Resonance 199, 1 (2009)