Aggregation-Induced Reduction in T_2 Relaxation Time of MR Visible Lipids Observed in Ischemic Rat Brain

L. Wei[1], H. Lei[1]

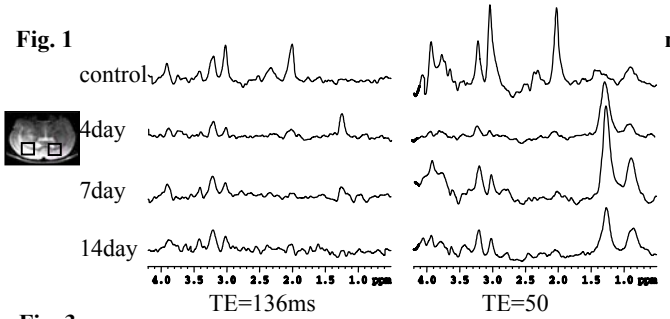
[1]State Key Laboratory of Magnetic Resonance and Atomic and Molecular Physics, Wuhan Institute of Physics & Mathematics, Chinese Academy of Sciences, Wuhan, Hubei, China, People's Republic of

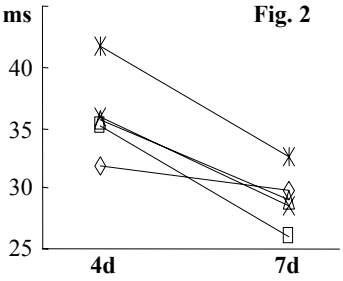
Introduction Magnetic resonance visible lipids (MRVL) (i.e., CH_2 signal at1.26 ppm and CH_3 signal at 0.9 ppm) increase in responses to pathological conditions[1,2]. It has been proposed that MRVL can potentially be used as indicators for monitoring inflammatory responses after stroke[3], and that CH_2/CH_3 intensity ratio could be used as a quantitative marker of apoptosis in studying tumor treatment[4]. Most of the previous studies measured MRVL using short-echo time MRS[3]. It is shown recently that T_2 of certain species of MRVL can be very long, enabling their detection by long-echo time MRS[5]. The facts that different MRVL species could have different T_2 and that T_2 of MRVL could potentially change with disease evolution complicate MRVL quantification. In this study, localized *in vivo* ^{1}H MRS with different echo-times was used to monitor the temporal changes of MRVL and their apparent T_2 relaxation times in a rat model of transient focal ischemia.

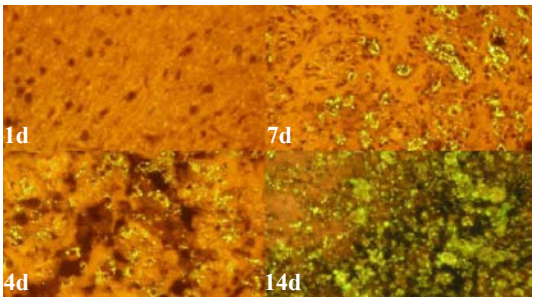
Materials and Methods Ninety-minute transient middle cerebral artery occlusion (MCAO) was induced in 15 male Wistar rats (180~200 g) by suture insertion from the common carotid artery. Rectal temperature of the rats was maintained at 37±1 ^{o}C during ischemia. MR experiments were carried out on a 4.7 T/30 cm Bruker Biospec scanner at 1, 4, 7 and 14 days after ischemia. T_2-weighted MRI was used to locate ischemic lesions (TE of 120 ms, TR of 2.5 s and FOV of 3 cm×3 cm). Localized ^{1}H spectra were acquired from the ischemic lesions and the corresponding locations in the contralateral hemisphere (Fig. 1) using a PRESS sequence with TE of 136 and 50 ms, TR of 1.5 ms, and 512 averages. The brain of the rats was removed after the MR experiments to prepare frozen sections (20 μm) for Nile-red staining, which gives a yellow fluorescence when neutral lipids (NLs), such as cholesterylester (CE) and tricylflyceride (TAG), are present[6]. Statistical analysis was performed by one-way ANOVA and paired student's *t*-test.

Results Figure 1 shows the MR results obtained from a representative rat. Compared to control (top row), the signal intensities of NAA, Cr and Cho in the ischemic lesion decreased significantly at 4, 7 and 14 days after ischemia. Comparing the spectra obtained from the ischemic lesion, it was found that the intensity of the lipid signal at 1.26 ppm was the highest at 4d when TE= 136 ms, but peaked at 7d when TE=50 ms. Apparent T_2 relaxation time of the lipid signal at 1.26 ppm, calculated by comparing the peak areas obtained with different TE, was significantly ($p< 0.05$, paired *t*-test) shorter at 7d than at 4d (Fig. 2). The number of Nile-red positive lipid droplets found in the ischemic lesion increased evidently from 1d to 14d (Fig. 3). Being in a circular shape at 4d and granular aggregates at 14d (Fig. 3), the average size of the Nile-red positive lipid droplets also increased with time (Fig. 4).

Discussion The main finding in this study is that apparent T_2 of the MRVL observed in the ischemic lesion decreases with time as the lesion matures. The time course of the T_2 decrease coincides with that of the increases of amount as well as size of the Nile-red positive lipid droplets. It is therefore most likely that shortening of apparent T_2 for MRVL is caused by aggregation of lipid droplets which have been shown to reside mainly in the infiltrating macrophages and/or reactive microglia that can uptake oxidized lipoprotein and stores NLs in the cytoplasm without limit[7].







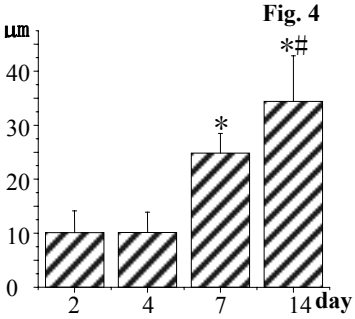


Fig. 1 ^{1}H spectra obtained from the contralateral (1d, top row) and ipsilateral cortex at 4, 7 and 14 days after ischemia with TE= 50 ms and TE=136 ms, respectively.

Fig. 2 Changes of apparent T_2 of MRVL with time.

Fig. 3 Changes of Nile-red staining in the ischemic area with time.

Fig. 4 Changes of the average size of Nile-red positive lipids droplets with time (*p<0.001 compared to 2d and #p<0.001 compared to 7d by ANOVA and post hoc Tukey's tests).

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References: 1) Hakumki JM et al, *Trends Biochem. Sci.* 2000; 25:357–362; 2) Zoula S et al, *NMR Biomed.* 2003; 16:199–212; 3) Graham GD et al, *Stroke* 2001; 32:2797-2802; 4) Blankenberg FG et al, *Blood* 1997; 89:3778–3785; 5) Wei L et al, *Proc. Intl. Soc. Mag. Reson. Med.* 2004; 2433; 6) Fowler SD et al, *J. Histochem. Cytochem.* 1985; 33(8):833-839; 7) Chait A et al, *Curr. Opin. Lipidol.* 1994; 5:365-370.