Magnetic Resonance Spectroscopic Imaging of Choline Diffusion in Experimental Gliomas During the Programmed Cell Death

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Introduction: During the recent years BT4C/HSV-tk rat glioma gene therapy model with ganciclovir (GCV) -induced cell death has been studied by numerous MR indices such as; conventional MR imaging contrasts and T1rho [1-2]; MR spectroscopy in vivo, in vitro and ex vivo – low molecular weight metabolites, lipids (especially polyunsaturated fatty acids) [3-5]; diffusion weighted MRI and MRS of water [7-8] and diffusion weighted single voxel MRS of choline [8]. It has been shown using TUNEL-stained histology that the GCV-induced programmed cell death occurs largely via the apoptotic pathway in this model. The specificity of the above mentioned imaging findings can potentially come into question with necrotic cell death, however. On the other hand, apoptotic volume decrease (AVD) is connected with the intracellular viscosity and diffusion characteristics for intracellular molecules providing means to approach apoptotic cell death by MR diffusion. In the present study we have quantified choline-moiety (CHO) apparent diffusion coefficient (ADC) maps (diffusion MRSI [9]) in BT4C-gliomas during the gene therapy to evaluate their use as a strictly apoptotic imaging marker.

Materials and Methods: BT4C gliomas, transfected with viral HSV-tk gene, were induced by implanting 10^4 HSV-tk+ cells in 5μL of Optimem to a depth 2.5 mm into the corpus callosum of female BDIX rats weighing 180-250 g. Rats in the treatment group (N=4) were injected with ganciclovir (GCV) (25 mg/kg i.p. twice daily) for the duration of the study. Non-treated animals (N=3) served as a control group. For the detection of tumor volume changes and MRSI slice selection, T2-weighted multi slice images were acquired using double spin-echo sequence with adiabatic refocusing pulses; TR 3 s, TE 80 ms, matrix size of 256x128, FOV 35 mm and slice thickness of 1 mm. For the detection of water diffusion changes slice corresponding the MRSI slice was acquired using the above mentioned sequence with TR 1.5 s, TE 45 ms, matrix size 256x128, FOV 35 mm, slice thickness of 3 mm. Diffusion weighting (b-value; 0, 500, 1000 s/mm2) was achieved by using 4 bipolar gradient pairs in all three orientations yielding trace of the diffusion tensor in single acquisition. STEAM sequence (TR 2.5 s, TE 19 ms, SW 2 kHz, NA 1) [10] with diffusion gradients (b-value; 0, 500, 1300, 2000 s/mm2) and phase encoding gradients (matrix size 16x16, FOV 20x20 mm^2, slice thickness of 3 mm) for MRSI was used for the acquisition of CHO ADC maps. MRSI data was analyzed using jMRUI software, with hamming k-space apodization and zero filling yielding 32x32 matrix. ADC values for both water and CHO were analyzed regionally by dividing the tumor radius in plane by 2 leading to center and border sections as interpreted in results.

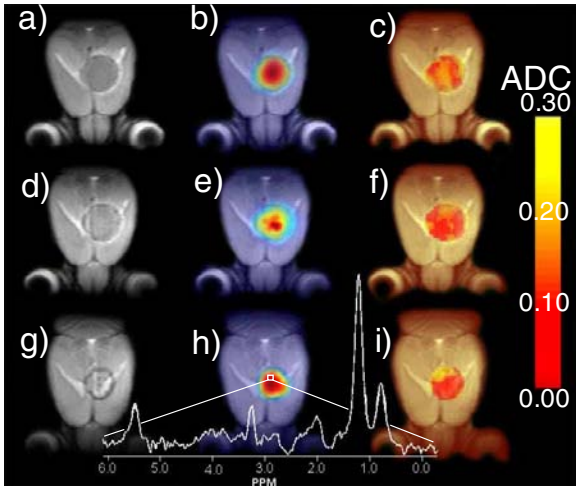

Figure 1. *a, d, g) Anatomical images at 0, 4 and 8 days after the start of treatment. b, e, h) Regional Cho signal intensity maps at 0, 4 and 8 days and typical spectrum from single voxel. c, f, i) Cho ADC maps at 0, 4 and 8 days, respectively. Colorbar denotes ADC values (*10^{-3} mm^2/s) for Cho diffusion in c, f and i.*

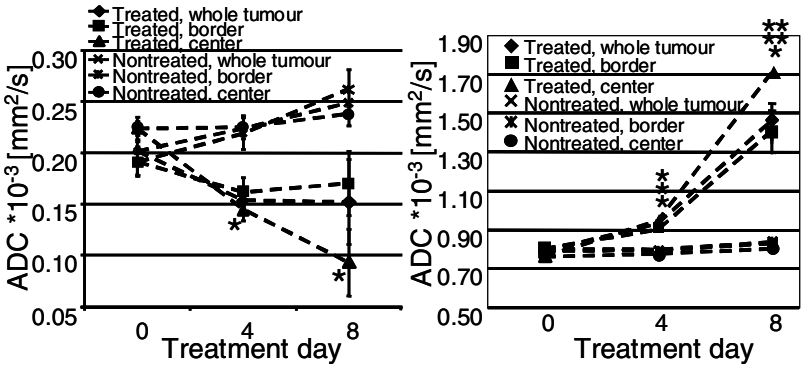

Figure 2. *ADC values for CHO signals measured by diffusion MRSI (left) and ADC values for water (right) as measured by diffusion MRI. Regional values are calculated as described in methods section. Student's t-test used in statistical analysis: * p<0.05, ** p<0.01.*

Results: As control parameters, we measured tumor volumes and ADC of water. Relative tumor volumes have their peak value at day 4 after the start of the treatment, and similarly to previous reports ~50% decline in tumor volumes is detected from peak values by day 8. The relative volume in untreated tumors went up to 250-300% (data not shown). We also analyzed water ADC maps regionally by dividing the tumor into center and border sections. ADC changes (Figure 2 right) over the treatment period were (0.75 ± 0.01 – 1.71 ± 0.06), (0.91 ± 0.01 – 1.41 ± 0.10) and (0.79 ± 0.01 – 1.46 ± 0.08) x10^{-3} [mm^2/s]. CHO diffusion maps (Figure 1c) at the start of the treatment revealed that the ADC in the border section is systematically lower (10-30%) than in the center section in single animals – this however, is not significant over the whole treatment group (figure 2 left) because of the variation in starting values. Most importantly however, the ADC values of CHO at the center section are significantly reduced already by day 4 from the start of the treatment. During the treatment period CHO ADC in the center section went down from (0.224 ± 0.006) to (0.093 ± 0.032) x10^{-3} [mm^2/s].

Discussion: This study is – to our knowledge – the first report utilizing MRSI technique with diffusion weighting *in vivo*. Values for the overall choline ADC decline are well in line with previous single voxel measurement in tumors (0.14-0.08 x 10^{-3} mm^2/s)[8], which could be expected with apoptotic cell shrinkage. With DW MRSI we show here that the diffusion of CHO is region dependent, diffusion being slower at center sections of BT4C-gliomas at the end of successful therapy. It has previously been shown that the regional apoptotic index (% of TUNEL-positive cells) is highest in the center section at day 8 after the start of treatment [7]. Interestingly, water ADCs at 8th day in the center sections are more than twice the values at the start of the treatment. If necrosis were a major contributor to the cell death process, we would expect to observe a release of choline moieties into the extracellular space and ADC behavior similar to water. We therefore expect DW-MRSI to be the least sensitive to potential necrotic processes during anticancer therapy. Histological analysis of cell properties and larger animal group studies to verify this fact are undergoing.

References: [1] Poptani H, *et al.* (1998), Cancer Gene Ther, 5;101-109, [2] Hakumäki JM, *et al.* (2002), Cancer Gene Ther, 9;338-345, [3] Hakumäki JM, *et al.* (1999), Nature Med, 5; 1323-1327, [4] Griffin JL, *et al.* (2003), Cancer Res, 63;3195-3201, [5] Lehtimäki KK, *et al.* (2004), J Biol Chem, 278;45915-45923, [7] Valonen PK, *et al.* (2004), J Magn Reson Imag, 19;389-396, [8] Hakumäki JM, *et al.* (1998), Cancer Res, 58;3791-3799, [9] Bito Y, *et al.* (1995), MRM 33;69-73, [10] Tkac I, *et al.* (1999), MRM, 41;649-656.