In vivo MRI follow-up of murine tumors treated by electrochemotherapy with bleomycin.

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INTRODUCTION: Electrochemotherapy (ECT) using electroporation (EP) to deliver an anticancer drug (usually bleomycin) to tumor cells, it makes possible to treat animal and human superficial tumors without the adverse effects of the anti-cancer drugs. Several MR indexes are known to reflect the efficiency of chemotherapies at a short time interval after drug delivery to tumor. This study aims to detect tumor modifications linked to ECT, done with normal and high dose of bleomycin known to produce different cellular effects (mitotic cells death or rapid pseudoapoptosis respectively).

MATERIALS & METHODS: Sixteen C57 Bl/6 mice, that had undergone a graft of LPB fibrosarcoma cells and developed a tumor of 4 to 7 mm diameter, were divided in 4 groups. The electrical pulses were delivered with flat electrodes laid at each side of the tumor, as described in [1]. In the EP group (E) 5 mice underwent EP twenty minutes after an IP Dotarem injection to test the extent of efficient electric field in the tumor [2] and two mice received only electrical pulses. In the treated group (B) 6 mice underwent EP 4 minutes after bleomycin injection at a dose of 4 µg/kg in 100 µL injected in the retroorbital sinus. In the group treated with a high dose of bleomycin and EP (HB) 3 mice were treated identically at a dose 800 µg/kg. Mice were anesthetized with 1.5 % isoflurane in 1 L/min oxygen during electric pulse delivery and imaging. For groups E and B, MRI examinations were performed before and 24 and 48 hrs after the treatment, and for group HB, before and at 3, 6, 10 and 24 hrs after the treatment. ADC measurements were done also at 3 hrs for 2 mice of group E. MRI studies were performed on an in-house developed 4.7 T scanner. The mice were laid prone inside a 42 mm diameter bird cage. Tumor was surrounded with alginate in order to increase the magnetic field homogeneity.
T_1-weighted SE axial images for volume measurements were obtained with TR/TE = 600/5 ms, spatial resolution 195 µm x 195 µm x 2 mm, Nex = 4. T_2 and diffusion measurements were done in a single 3 mm slice at the center of the tumor. DWI experiments were performed with a single shot EPI spin echo sequence: TR/TE = 4.2 s / 40 ms, FOV = 50 x 25 mm^2, matrix = 128 x 48, BW = 286 kHz. A saturation slab was applied to suppress fat signal and ghosting of mouse abdomen. Seven b values from 0 to 1000 s/mm^2 were applied with gradient pulse durations of 4.1 ms and interval of 7.15 ms. To correct the effect of cross term between imaging and diffusion gradient, four images at each value of b were acquired, cycling the polarity of read and phase encoding gradient [3]. Diffusion coefficients were determined from the product of these 4 images at each b value. The CPMG sequence was acquired with TR = 3 s, $TE_1/\Delta TE$ = 10/10.6 ms, 4 echoes. T_2 and ADC maps were calculated with MatLab (MathWorks, Natick, MA). Data from control and treated tumors were compared by Student's t-test analysis.

RESULTS: In the 5 animals that received EP and Dotarem, the tumor signal enhancement was homogeneous with mean value 38 ± 10 % at 48 hrs, confirming the homogeneity of the electric field applied to the tumor under our treatment conditions (Fig. 1a. and b.). Mean values and standard deviations of all parameters related to tumor evolution are given in Table 1. All the animals of group E could be pooled altogether since no differences in parameters were found between the mice that received Dotarem or not, resulting in a larger control group.

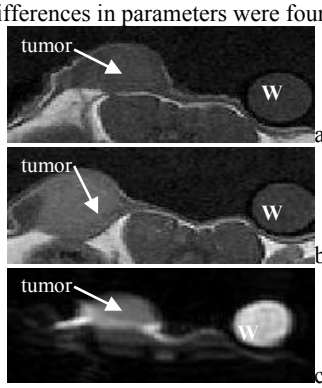

Fig. 1: central T1-weighted slice through tumor and reference tube (w) for one E group mouse at 0 (a.) and 48 hrs (b.) after treatment and corresponding slice in EPI at 0 hr (c.).

	E (N = 7)	B (N = 6)	HB (N = 3)
Volume at 0 hr (mm^3)	202 ± 92	233 ± 131	289 ± 142
Volume at 24 hrs (mm^3)			181 ± 88 **
Volume at 48 hrs (mm^3)	314 ± 123 **	240 ± 124 *	
ADC at 0 hr (10^{-3} mm^2/s)	0.647 ± 0.067	0.646 ± 0.1001	0.645 ± 0.029
ADC at 3 hrs (10^{-3} mm^2/s)	0.698 ± 0.027 #	0.684 ± 0.112	0.703 ± 0.09
ADC at 10 hrs (10^{-3} mm^2/s)			0.749 ± 0.151 **
ADC at 24 hrs (10^{-3} mm^2/s)	0.678 ± 0.0001 # *	0.716 ± 0.06 **	0.661 ± 0.053
ADC at 48 hrs (10^{-3} mm^2/s)	0.675 ± 0.058 *	0.703 ± 0.101 **	
T_2 at 0 hr (ms)	56 ± 5	49.2 ± 13.9	50 ± 1.3
T_2 at 6 hrs (ms)			61 ± 7 **
T_2 at 24 hrs (ms)	58 ± 2 *	66 ± 14 **	56 ± 2
T_2 at 48 hrs (ms)	61 ± 4	53 ± 8	

*Tab. 1: Average and standard deviation of tumor volume, tumor ADC and tumor T_2 for each group of mice in the study. At 3 and 24 hrs the ADC data were obtained from 2 mice only (#) in group E. ** correspond to a significant variation ($p<0.05$), and * correspond to a no-significant variation of a parameter compared to its initial value at time 0 hr.*

At 48 hrs the mean tumor growth was 112 ± 58 mm^3 in the control group E, whereas in group B the tumor volume decreased in three mice and stayed stable in two as observed in [1]. In the group HB the tumor volume decreased in all mice at 24 hrs.
Initial ADC values were identical in all groups. Tumor ADC increased more strongly at 24 and 48 hrs in group B than in group E. In the HB group the peak value of ADC was observed circa 10 hrs instead of 24 hrs. This tends to indicate that cell death starts 24 hrs after normal dose treatment (known to cause a mitotic cell death) and sooner after high dose treatment (known to cause a rapid pseudoapoptosis) [4].
The average T_2 value for group B (66 ± 14 ms) was significantly higher at 24 hrs than before the treatment (49 ± 13 ms), whereas in the control group the difference was not significant. At 3 hrs, T_2 values measured for the two mice in the E group showed a temporary increase that is probably linked to osmotic flux during EP, while ADC presented similar variation. For the HB group, T_2 values were also higher at 3 and 6 hrs, sooner than the ADC increase. However at 48 hrs (E) and 24 hrs (HB), the corresponding T_2 values had decreased.

CONCLUSION: Though a more detailed study during the time after ECT is needed, our results suggest that T_2 values are very sensitive to osmotic edema, and that ADC values rather reflect apoptosis and tumor death, and the accompanying loss of structural integrity that would lead to longer T_2 values.

REFERENCES: [1] S. Corovic et al. *Technol Cancer Res Treat*. 2008 Oct; 7(5): 393-400. [2]M. Paturneau-Jouas, et al. *Radiology*. 2003 Sep; 228(3):768-75. [3] J. Mattiello et al. *Magn Reson Med*. 1997 Feb; 37(2): 292-300. [4] H. Mekid et al. *Br J Cancer*. 2003 Jun 2; 88(11):1763-71