Cisplatin treatment monitoring by sodium MRI relaxometry at 4.7 T in colorectal tumors implanted on mice

C. D. Thomas[1,2], M. Lupu[1,2], C. Walczak[1,2], A. Volk[1,2], and J. Mispelter[1,2]
[1]Institut Curie, Orsay, France, [2]INSERM-U759, Orsay, France

Objectives:
Generally, tumor treatments are eventually inducing structural changes of the external side of the cellular membranes or changes in cellular density (apoptosis and necrosis). These effects may be monitored by sodium MRI and relaxometry in a completely non-invasive way, due to the quadrupolar characteristics of the nuclei. Indeed ^{23}Na relaxation mechanisms, dominated by the interactions between the quadrupolar moment with the electrical field gradients generated by the environment are targeting any structural changes appearing in the extracellular compartment. The purpose of this preliminary work using ^{23}Na-MRI was to follow the effect of an alkylant drug, cisplatin, on colorectal tumors implanted in nude mice.

Methods:
Experiments were performed on colorectal tumor xenografts implanted in six nude mice on a Bruker Biospec system (4.7T). The RF coil was a home-made system comprising a double tuned linear birdcage coil, operating at 53/200 MHz as transmitter and ^{1}H receiver and a 53 MHz surface coil as receiver. Mice received one I.P. administration of cisplatin (CPT) (0.2 mg). A monitoring over 18 days was performed.
Multi-slice, multi-echo ^{1}H images were recorded for localization purpose and growth curves determination, (respiratory trigger, FOV=6.8 cm, evenly separated echoes by TE=7 ms, NE=8, matrix 256x256, slice thickness 1 mm).
Single slice, multi-echo (32 echoes) images were recorded for sodium studies, (TR=350 ms, evenly separated echoes by TE=6.7 ms, FOV=6.8 cm, matrix 64x64, slice thickness 3 mm) using 160 averages. In order to get proper decay curves, the images were post-processed [1]. Firstly, the images were phase-corrected in order to avoid the Rician noise created by modulus reconstruction. The relaxation curves, measured in most sodium hyper intense pixels were forward linear projected in order to reduce the noise amplitude and to reach the base line, required for a proper multiexponential fitting. This was done by singular value decomposition method that needs no initial input for fitting. Error estimations were performed by Monte Carlo simulations on 500 runs.
After MRI experiments the tumor was removed for histological examination (H&E). For estimation of tumor necrosis, five adjacent sections located at the tumor center (at 600 µm intervals) were selected, in order to cover the entire sodium slice, and scanned at 4000 dpi (Nikon Super Coolscan 8000, Tokyo, Japan). The percentage of necrosis was determined by manual delimitation using Amira software (Mercury Computer Systems; TGS, Bordeaux, France).

Results and Discussion:
Sodium MRI revealed necrosis (confirmed by histological results : Fig.1e, 2e) with a high contrast (Fig.1a,c, Fig.2a,c) due to the diminished cellular density and consequently an increase of extracellular sodium. CPT didn't seem to modify the percentage of necrosis, but seemed to act on the tumor growth (Graph) : treated tumors had a slower rate of development as compared to control. Sodium T_2 relaxation curves were nonexponential for both control and treated tumors, allowing a biexponential fit. The non-exponential behaviour implies the existence of a bound sodium population in the extracellular compartment. T_{2fast} was poorly defined due to TE but necessary to fully determine T_{2slow}. Both experimental relaxation times (slow and fast T_2) are apparent resulting from an exchange process between the bound and free extracellular sodium. The control group was characterized by rather constant T_{2slow} relaxation times while the treated group showed an important increase of the slow relaxation constant after CPT treatment, followed by a decrease, close to control values (Table). Platinium was known to induce mainly the apoptotic process [2]. From ex-vivo studies [3], it was shown that the apoptotic process induces the change of the cellular membrane polarity which became negatively charged at the external side, followed by cell shrinkage. The cellular shrinkage is increasing the overall extracellular volume increasing thus the exchange rate between free and bound Na ions. Consequently, both apparent relaxation times are increasing after treatment. As the tumoral developing occurs, the initial conditions are restored and the relaxation constants tend to control values, characterizing mainly the necrotic core of the tumor.

	Control		Treated	
T_2 Na (ms)	T_{2slow}	T_{2fast}	T_{2slow}	T_{2fast}
Before CPT	33.6±1.3	3.6±1.6	30.6±2.8	8.3±2.3
4 days after CPT	35.6±2.5	10.6±1.3	44.6±3.0	14.6±1.5
14 days after CPT	32.6±2.0	10.6±1.6	40.0±3.0	12.0±2.0
18 days after CPT	34.6±2.4	13.6±2.0	26.6±2.3	7.6±2.0

Table: Relaxation results on two colorectal tumors

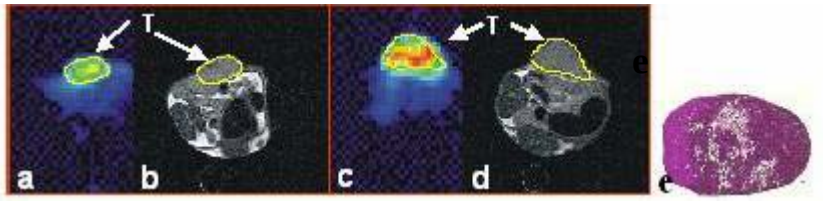
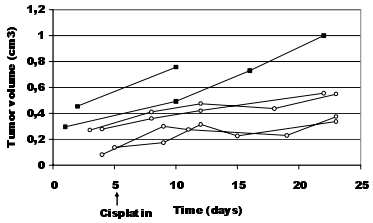


Fig.1. Control tumor (T: tumor)
a) ^{23}Na slice, first echo, b) corresponding ^{1}H slice TE= 28ms, c) ^{23}Na slice 18 days after saline, d) corresponding ^{1}H slice TE= 28ms, e) histology (H&E)



Graph: Tumor growth
(Black squares: control, white circles: treated)

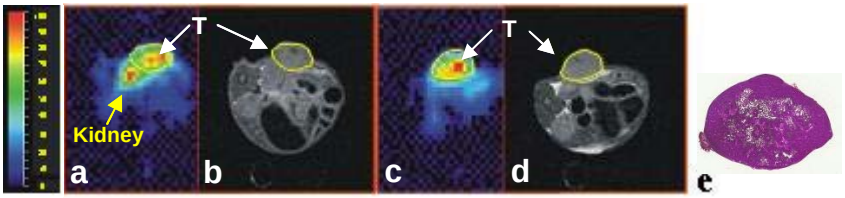


Fig.2. Treated tumor (T: tumor)
a) ^{23}Na slice (first echo) before CPT, b) corresponding ^{1}H slice TE= 28ms, c) ^{23}Na slice 18 days after CPT, d) corresponding ^{1}H slice TE= 28ms, e) histology (H&E)

Conclusion:
To our knowledge it's the first ^{23}Na relaxometry follow-up of treatment performed on mice bearing colon carcinoma. ^{23}Na-MRI was a good method to monitor non-invasively the effect of CPT on colorectal tumor on nude mice. The authors suggest that the relaxation changes on treated mice were due to the cellular shrinkage induced by the apoptotic process. Further investigations are needed to confirm the great potential of sodium relaxometry to become an endogenous marker for therapy response.

Acknowledgments: The authors acknowledge "Canceropôle Ile de France" for supporting this work.
References: [1]: M. Lupu, C.D. Thomas, J. Mispelter,. Retrieving accurate relaxometric information from low signal to noise ratio ^{23}Na MRI performed in vivo. C.R. Chimie, 2007, in press.
[2]: Kelland L. The resurgence of platinium-based cancer chemotherapy, Nature Reviews, 2007, 7, 573-584.
[3]: Lupu M., Ojcius D., Perfettini J.L., Patry J., Dimicoli J.L., and Mispelter J. Evidences on sodium ions compartmentalization in biological systems due to pathological states. A non-invasive NMR study. Biochimie, 2003, 85, 849-861.