Proton and sodium MRI follow-up of human colorectal tumors implanted in mice. Comparison between two photodynamic therapy protocols.

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Introduction: Photodynamic therapy is an established cancer treatment in which a non-mutagen photosensitizing (PS) agent is activated by exposure to visible light. Absorption of light initiates the photochemical reactions leading to the generation of cytotoxic products (reactive oxygen species - ROS) responsible for the therapeutic effects. Vasculature damage and necrosis or apoptosis decrease cell density and increase the local sodium concentration [1]. Since sodium magnetic resonance imaging (^{23}Na MRI) directly monitors variations of sodium concentrations in a non-invasive way, it can be used to follow-up the tumor response to therapy from the very beginning and throughout the treatment [2].

Methods *MRI*: ^{1}H and ^{23}Na MRI were performed at 4.7 T using a Bruker Biospec small animal MRI scanner. The MRI probehead consists of a double tuned volume resonator (birdcage) used for transmitting / receiving ^{1}H signals and transmitter ^{23}Na r.f. channel, and a surface coil nested inside the birdcage for receiving ^{23}Na signals. Multi-slice, multi-echo ^{1}H images were recorded for localization purposes, (respiratory trigger, FOV=6.8cm, TE=12ms, NE=10, matrix 256x256, slice thickness 1mm) and tumor volume determination. Single-slice, multi-echo (NE=28) ^{23}Na images were recorded for sodium studies, (respiratory trigger, TE=6.7ms, FOV=6.8cm, matrix 64x64, slice thickness 3mm) using 160 averages. The sodium resolution was (1x1x3) mm^3 and the acquisition time was 75 min. This sequence allowed us to image mainly the extracellular tumor compartment. ***Treatment protocol***: Flank implanted nude mice with human colorectal tumors were used. Since the damage induced by PDT is strictly related to PS localization during illumination, two regimens were devised for this study. Mice treated with a single-dose, anti-vascular regimen received one i.v. injection of PS (0.6 mg/kg polyethylene glycol 400/ethanol/physiological serum: 3/2/5 per volume) followed 10 min later by exposure to red light (650 nm). Since the majority of PS was still in the vascular system, the overall effect of the PDT was anti-vascular. The second protocol targeted both cancer cells and blood vessels. One i.v dose of PS was followed by a second dose, separated by a 3h interval. This time lapse allowed the first dose of PS to penetrate inside tumor cells. 10 minutes after the administration of the second dose, tumors were exposed to light. Consequently, both cancer cells and blood vessels were targeted. 4 mice for each treatment protocol were used and 4 controls.

Results: 11 days after the antivascular PDT, the anatomical ^{1}H-images indicated an increase in tumor volume (Fig.1) and the ^{23}Na images suggested that vessel damage was not permanent and/or new vessels have been formed (Fig.2 A,B,C). Anyhow the blood vessels positioned beyond the red light penetration depth were not damaged by the treatment and continued the nutritional supply. Two hours after the PS activation in the second treatment protocol a moon-crescent shaped region with high sodium signal appeared on an average depth compatible with the red light penetration into the tissue (4-5 mm at 650 nm) (Fig.2E). 24 h after the PS activation the ^{1}H image showed an increase of the tumor volume possibly due to inflammatory edema that often accompanies PDT treatment. The ^{23}Na image (Fig.2F) showed widespread high sodium concentration in the tumor slice indicating an increase of the extracellular space. This change in sodium signal suggests that a massive process of necrosis and/or apoptosis was taking place in a much larger area than the moon-crescent shaped region where ROS damage was initially localized. Histological analysis confirmed the presence of necrosis.

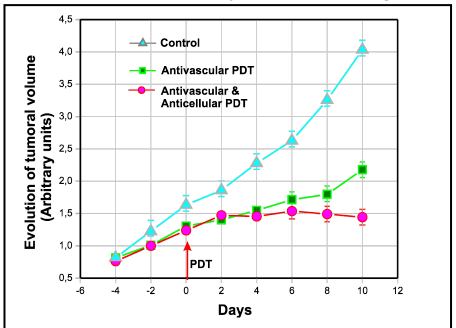

Fig.1 Tumoral evolution over 11 days.

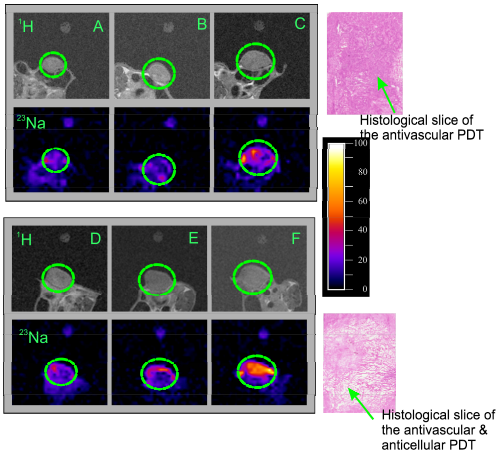

Fig.2 ^{1}H & ^{23}Na MRI of tumors evolution (A-before, B-2 h, C-11 days after antivascular PDT, D-before, E-2 h, F-11 days after anticellular & antivascular PDT).

Conclusions: A single-dose antivascular-PDT does not prevent the tumoral growth. Conversely, the second PDT protocol, which targets both blood vessel and tumor cells, led to important damage of the tumor architecture. Our work indicates that *in vivo* dual ^{1}H and ^{23}Na MRI is a non-invasive technique well suited for both longitudinal follow up and early treatment assessment. We could easily follow the transition from low sodium signal before treatment to high sodium signal characteristic of damaged areas without adding any exogenous contrast agent.

References: [1] Thomas CD et al., Proc.Intl.Soc.Mag.Reson.Med., 2008, 16, 2795. [2] Lupu M et al., PDPDT, 2009, 6, 214

Acknowledgements: This work was supported by INCa-DHOS and Canceropôle Ile-de-France.