BOLD & DYNAMIC ENHANCED MRI CONTRASTS: ONLINE FOLLOWUP OF TUMOR PHOTODYNAMIC THERAPY WITH Pd-BACTERIOCHLOROPHYL DERIVATIVES

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Introduction: The objective of this study was to use online BOLD- and GdDTPA-contrast enhanced MRI for online follow up of photodynamic therapy (PDT) of solid tumors. We previously demonstrated that photogeneration of circulating deoxyhemoglobin (DeoxyHb) during PDT of tumors can be imaged by functional MRI (1). Antivascular PDT of tumors relies on in situ photosensitization of the i.v injected photosensitizer (PS) drug and local generation of cytotoxic reactive oxygen species (ROS). Photogeneration of ROS in the blood is coupled with consumption of O_2 and buildup of paramagnetic DeoxyHb that affects the T2* contrast. The consequent photodamage induces vascular stasis within minutes culminating in ischemic necrosis and tumor eradication (1, 2, 3).

Animals, tumor model and PDT: Wistar female rats were s.c grafted (flank) with rat B33 glioma cells, and tumors were grown to a size of ~1 cm. The rats were anesthetized [Ketamine 100 ml/kg /Diazepam 7.5mg/kg) and positioned supine/lateral above the tumor. The photosensitizer (Pd- 3^1-oxo-15-methoxycarbonylmethyl-rodobacteriochlorin 13^1 -(2-sulfo-ethyl) amide dipotassium salt (WST11) or Pd-bacteriopheophorbide (TOOKAD) 10mg/kg, Steba Biotech] was i.v injected via catheter (tail vein) while the tumor was illuminated (light spot, 1 cm^2) with a 1W diode laser (CeramOptec, Germany), emitting at 765 nm, 60 J/cm2/10 min. A 5mm test tube with water was used as external S/N reference. T2*w images were acquired continuously before PDT (3.5 min) (control), during illumination without PS (3.5 min) (light control) and upon PS injection (PDT, for 10 min) and for 10 min after the light was turned off. **MRI parameters:** MR images were acquired on a horizontal 4.7 T Bruker-Biospec spectrometer using a volume coil. On line guidance of PDT: sequential axial Gradient echo images for BOLD contrast were acquired with spatial resolution 35s, TE/TR/α 10/138/30°, ns=2, in plane resolution 430 µm, slice thickness 2 mm, 128x128. Assessing tumor enhancement: Gd-DTPA (0.1 mmol/kg) was injected twice, via catheter, once before and once 20 min after PDT. Spin echo (axial, TR/TE 200/14.5 ms, ns 2, 256x256, FOV 5.5cm). The pre and post Gd-DTPA images were subtracted and enhanced pixels are shown in red (Fig1 A, B).

Results and Discussion: T2* activation maps as percent of control was calculated: [Activation= (image/average of control - 1) x100]. PDT of tumors treated with the novel water soluble WST11 (n=6) (Fig.1) or the lipophilic TOOKAD (n=8) showed abrupt changes in T2*w within the illuminated zone (yellow arrow, Fig.1 D) as reflected in the calculated maps (Fig.1 C-E circles). During PDT a high variation in % activation was observed in the tumor with a large coefficient of variance (COV) = 26 (Fig. 1 G) compared to untreated, light control and unilluminated regions (black arrow, Fig.1 D) where the dispersion in T2*w values was much smaller COV= 0.4 (Fig.1 H). Positive changes in the maps were related to increase in DeoxyHb (BOLD contrast). Negative pixels may possibly be associated with increased blood flow. When illumination was terminated there was no re-equilibration to pretreatment values of T2*w (Fig.1 E) indicating vascular stasis and no perfusion as confirmed by Gd-DTPA injection (Fig 1B) as soon as 20 minutes post PDT. The illuminated region responds non homogeneously to PDT. Those variations could be associated with changes in BOLD contrast due to heterogeneity of blood vessel distribution, blood flow/volume, uneven illumination and variations in tissue optical properties. Changes in BOLD contrast observed in the unilluminated abdominal cavity above the tumor are associated with bowel motions.

Conclusion: The combination of functional MRI and Gd-enhanced contrast enables online monitoring of the progress of photodamage within the tumor to the endpoint of blood stasis. *Supported by STEBA BIOTECH and NEGMA LERADS, France.*

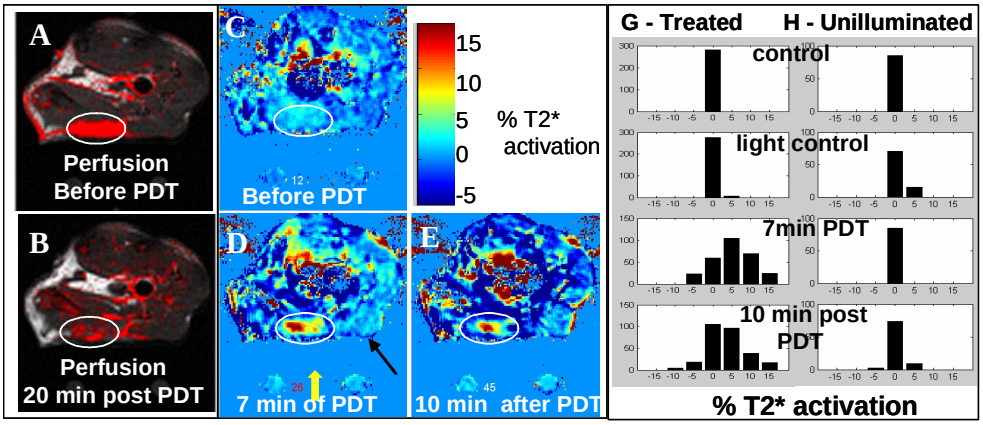

Fig 1: Changes in BOLD contrast and enhancement during WST11-based PDT. (A,B) in red : enhanced pixels of difference images after Gd-DTPA injection. (C-E) maps of the percent change in T2* and dispersion of their values upon PDT in the tumor (G) and unilluminated region (H). *Shown are: white circle: the illuminated tumor region, yellow arrow: light beam direction, black arrow: unilluminated ROI.*

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