Vascular Targeting Photodynamic Therapy (VTP): Correlations between hemodynamic and photosensitized fMRI response parameters in the mouse ear tumor model

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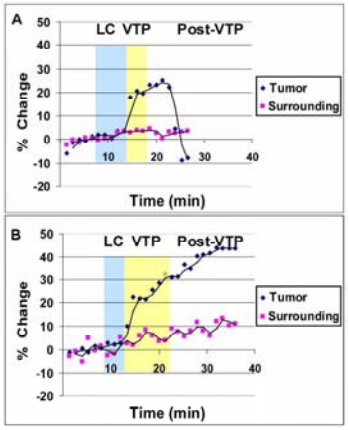
Introduction: Vascular Targeting Photodynamic Therapy (VTP) of solid tumors with Pd-Bacteriochlorophyll derivatives relies on local photosensitization of the circulating photosensitizer (PS) and intravascular generation of cytotoxic Reactive Oxygen Species (ROS), which lead to blood stasis, tumor necrosis and eradication. Local photogeneration of ROS is coupled to consumption of oxygen and temporal rise in deoxyhemoglobin (DeoxyHb) levels in the illuminated zone. It was previously demonstrated by us that photogeneration of DeoxyHb can be visualized by photosensitized BOLD contrast MRI (1). In this study, differential photodynamic response parameters of the tumor vasculature during VTP were examined online by two independent imaging methods, fluorescent IntraVital microscopy (fIVM) and fMRI using the mouse ear model.

Methods: Mice and tumor model: MADB106 rat mammary carcinoma tumors were s.c. grafted to the CD1 nude mouse ear. VTP was performed on tumors 2-4mm in diameter**.** Photosensitizers and VTP protocol: Pd-Bacteriopheophorbide (WST09,10mg/kg), Steba Biotech) was i.v. administrated via tail-vein catheter. During VTP, the tumor and its surrounding were illuminated using a 1W diode laser (CeramOptec, Germany), emitting at 763 nm, 57/114[J/cm2] for 5/10 min respectively. MRI studies: Anesthetized mice were subjected to 5/10 min VTP inside a horizontal 4.7T Bruker-Biospec magnet. The mouse ear was gently attached to a 1.5cm surface coil and was covered with water-based gel for air isolation. T_2*w images were continuously acquired before VTP (Control), during illumination (Light Control, LC), during VTP (PS+light) and after VTP. MRI parameters: Gradient echo images, TE/TR/α 10/100/30°, ns=6, time resolution=90sec, slice thickness 0.7-1 mm, 128x128, FOV=2cm. Gd-DTPA (0.1 mmol/kg) was administered via catheter ~20 min after VTP to monitor vascular tumor perfusion. Images were acquired by Spin echo sequences (TR/TE 250/9 ms, ns=1, 128x128, FOV=2cm). fIVM studies: Anesthetized mice were subjected to 5/10 min VTP under a zoom-upright macroscope (Olympus SZX-RFL2). Online monitoring of blood flow was performed with a video camera (Mintron 12V1-EX CCD, Taiwan). Red Blood Cells from a donor mouse were stained ex-vivo (4-(4-(didecylamino)styryl)-N-methylpyridinium iodide, 4-Di-10-ASP, Molecular Probes) and i.v. injected (1% V:V) to the tumor-bearing treated mouse. VTP protocol was identical to the one described for MRI above. VTP-induced changes in blood flow were monitored before, during LC, during VTP and after VTP. Changes in blood flow velocity were quantified offline (Fig.2).

Results: VTP was associated with a sharp increase in BOLD contrast [20-30% (calculated as % change = (image/average of control - 1) x100] that declined after a short lag time to pretreated values when illumination was terminated at 5 min (Fig.1A). When illumination lasted for 10 min, further increase of BOLD contrast up to 50% was observed in the illuminated tumor zone. However, termination of the light did not lead to reduction of the BOLD contrast. Changes in BOLD contrast in the normal surrounding tissue (Fig.1B-D), were much smaller (a few %), possibly due to lower blood content. Post-VTP Gd-DTPA enhanced imaging indicated rehabilitation of blood flow or blood stasis following termination of VTP at 5 or 10 min respectively (data not shown), in agreement with an earlier report in B33 tumors (2). In order to better understand the differential BOLD contrast responses associated with VTP, changes in blood flow during the treatment were monitored by fIVM in the tumor vascular bed and its main feeding artery and draining vein (Fig.2A). Blood flow in the tumor cortex stopped shortly after ~1-2min illumination and did not resume up to 30min post-VTP, irrespective of the duration of illumination (5 or 10 min). Collapses of the tumor main artery, accompanied with random pauses and reversal venous blood flow were observed regardless of treatment duration (5 or 10 min VTP, Fig.2B-C). While blood flow in the main vessels was fully restored after 5 min illumination (Fig.2B) VTP for 10 min induced irreversible arterial blood stasis. Interestingly, reversed venous blood flow (Fig.2C) continued to the end of data acquisition (15-30 min post VTP, data not shown).

Discussion: The increase in BOLD contrast associated with VTP was comprised of two phases, an early one (t_{VTP}<5min) that was light-dependent and reversible in the dark, followed by a second phase (5min>t_{VTP} >10min) that was light-independent and BOLD contrast remained high even after the light was switched off. These results were well correlated with the hemodynamic changes monitored by fIVM, which were also characterized by the same two phases: an early phase, in which blood flow velocity decreased temporarily but resumed to pre-treatment values when the light was switched off; and a later phase, characterized by arterial collapse and reversal of venous flow direction. Vascular stasis and reversed venous blood flow contributed to saturation of DeoxyHb and sustained levels of BOLD contrast. Consequently, we suggest that in the first phase of VTP the BOLD contrast is comprised of a photochemically-induced reversible hemodynamic effect that gradually converges into irreversible blood stasis that is light-independent. These results newly provide local hemodynamic insight of VTP that substantiate our understanding of the photochemical and hemodynamic basis of photosensitized MRI.

Conclusion: Parallel studies in the tumor- mouse-ear model with fMRI and fIVM differentiate between light dependent and independent trends in BOLD contrast which are highly correlated with changes in blood flow velocity in the tumor during VTP.



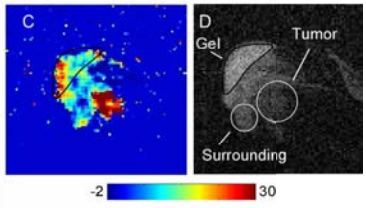
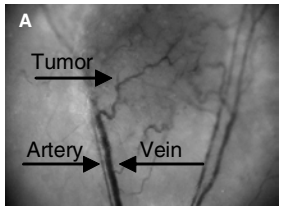


Figure1: fMRI studies: Changes in BOLD contrast during VTP
Changes in BOLD contrast during 5 min (**A**) and 10 min (**B**) VTP. **C-** BOLD activation map of 10 min VTP **D-** T_2W image of the ear. Tumor, normal surrounding tissues and gel are marked. Note- contrast in gel due to dryness.



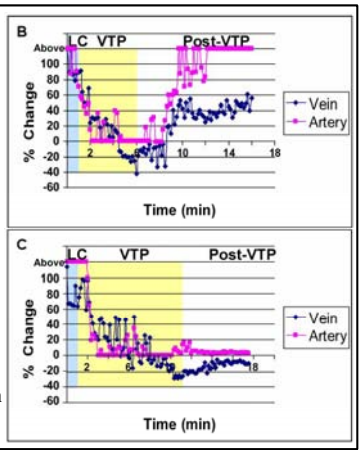


Figure2: fIVM studies: Changes in blood flow during VTP
A- The mouse ear model: MADB106 Tumor, main artery and vein are marked by arrows. **B,C:** Changes in blood flow in the main vessels during 5 min (**B**) and 10 min (**C**) VTP. Above=above detection range. Sub-Zero values=reversed flow

References: **1.** Gross S, Gilead A, Scherz A., Neeman M., Salomon Y. Nature Med 2003 9:1327-31. **2**. Brami C., Neeman M., Scherz A., Salomon Y. ISMRM 2005 Miami.