Vascular targeted photodynamic therapy (VTP) stops blood supply and chokes the tumor

Department of
Biological Regulation

Prof. Yoram Salomon

Catherine Tempel-Brami, Esther Shai, Eran Neumark*, Yoav Gal*, Dina Preise, Itai Glinert*, Noa Madar*, Sarit Migdal

**In collaboration with Prof. A. Scherz, , Deptartment of Plant Sciences*

☎ 972 8 934 3930
FAX 972 8 934 2813
@ yoram.salomon@weizmann.ac.il
www.weizmann.ac.il/Biological_Regulation

Introduction

Studies in our laboratory focus on development of vascular targeted photodynamic therapy (VTP) based on bacteriochlorophyll derivatives as photosensitizer drugs, elucidation of its mechanism of action and development of new applications for photodynamic therapy (PDT) such as treatment of Extrauterine Pregnancy (EUP). These studies are in collaboration with Prof. Avigdor Scherz Dept. of Plant Sciences, (see respective abstract in this book)

Vascular Targeted Photodynamic Therapy, a strategy that cuts off the tumor blood supply

VTP is an anti-cancer treatment modality that selectively targets and occludes the vasculature that supplies blood to solid tumors in cancer patients, leading to their cure. VTP consists of i.v. infusion of a non-toxic photosensitizer-drug and of local illumination of the target tumor with non hazardous light at a wavelength that matches with the maximal absorption of the photosensitizer (NIR, 755-763nm). Using interstitial optic fibers, light can be delivered to tumors practically anywhere in the body. The resulting cytotoxic reactive oxygen species (ROS)-induced injury of established tumor blood vessels leads to local thrombosis, vasoconstriction and blood stasis within minutes. Consequent downstream hypoxia and necrosis lead to tumor eradication (over several weeks) and healing with high cure rates. The clinical treatment session requires approximately one hour, including the 10-17 min illumination time and is completed in a single session. In contrast to VTP, anti-angiogenic therapy that inhibits development of tumor neovasculature requires life long chronic drug administration. VTP with the Pd-bacteriochlorophyll derivative WST09 (developed in our laboratories) is presently in phase II/III clinical trials for prostate cancer therapy in Canada, UK and the USA, while another bacteriochlorophyll derivative, WST11 also developed in our laboratories is in phase II clinical trials for ophthalmic age-related macular degeneration (Berdugo et al. 2008) in France.

Research objectives

Studies in the recent years were aimed at:

i Elucidation of the mechanistic basis of VTP.
ii Design of new photosensitizers.
iii Development of new targeting techniques.
iv Identification of molecular ROS targets.
v Improvement of treatment efficacy.
vi Online guidance and monitoring of tumor treatment by functional psMRI that detects the incident light in vivo.
vii Examination of long-term tumor immunity induced by VTP and its role in tumor ablation.
viii Development of a new photodynamic modality for potential application for treatment of Extrauterine Pregnancy.
ix Development of bioluminescence imaging based monitoring of Tumor response to VTP and application of repeated VTP (Fleshker et al. 2008).

The Hemodynamic Basis Of Vascular Targeted Photodynamic Therapy: Online Optical And Magnetic Resonance Imaging

To study the acute hemodynamic response of the tumor to VTP we used the mouse earlobe tumor model, applying two non-invasive complementary online imaging techniques: **(i)** fluorescent Intravital Microscopy (fIVM) and **(ii)** Photosensitized (ps) Blood Oxygenation Level Dependent (BOLD) contrast magnetic resonance imaging (psMRI).

The treatment protocol consisted of illumination of the entire earlobe (Light Control) of an anesthetized mouse immediately followed by intravenous administration of the sensitizer. The protocol was terminated after 10 minutes by turning off the light. Changes in blood vessel morphology and diameter in course of VTP were monitored online in mice injected with a 250kDa molecular weight dextran, a fluorescently-labeled pool marker.

Upon initiation of VTP a significant increase in fluorescence in the tumor vessels was observed, indicating local increase in perfusion. Off-line analysis revealed temporal vasodilatation in the tumor feeding and draining vessels as well as in the tumor vascular bed, but not in the non-tumor surrounding vasculature. Towards termination of the protocol, the tumor blood vessels appeared collapsed. Nevertheless, more distant non-tumor peripheral blood vessels, also exposed to the entire VTP protocol did not show dramatic change in morphology.

Monitoring changes in blood flow rates during the entire treatment protocol (10min) showed a decline of arterial flow rate culminating with stasis within a minute or so while venal blood flow declined with a slight delay in a hasty manner. Upon arterial stasis, reversed venal flow direction, at a moderate rate was observed, to the end of data acquisition (up to 30min). However, switching the light off at 5 min (partial treatment) led to resumption of normal arterial and venal blood flow that reached pretreatment levels within few minutes, indicating the reversibility of the hemodynamic behavior at early stages of the treatment. In agreement with the above VTP-induced changes in flow rate, BOLD-MRI contrast changes also showed a biphasic behavior in the same time windows. In the first phase (tVTP<5min) BOLD contrast increased but remained reversible upon early termination of the illumination at 5 minutes. This was followed by a second phase (tVTP >5min) where reversibility was lost i.e BOLD contrast kept rising and remained high even after termination of the protocol at

10 minutes. These results provide new mechanistic insight into the hemodynamic basis of VTP and may provide presently unavailable tools for intra-operative guidance and follow up of the procedure.

Light Images Detected By Simple Tissue Can Be Visualized By Photosensitized Magnetic Resonance Imaging (psMRI)

In this study, we show how light can be absorbed by the body of a living rat due to an injected pigment circulating in the blood stream. This process is then physiologically translated in the tissue into a chemical signature that can be perceived as an image by magnetic resonance imaging (MRI). We previously reported that illumination of an injected photosynthetic bacteriochlorophyll-derived pigment leads to generation of ROS, upon oxygen consumption in the blood stream. Consequently, paramagnetic deoxyhemoglobin accumulating in the illuminated area induces changes in image contrast, detectable by a Blood BOLD-MRI protocol, termed by us photosensitized (ps)MRI. In this study we showed that laser beam pulses synchronously trigger BOLD-contrast transients in the tissue, allowing representation of the luminous spatiotemporal profile, as a contrast map, on the MR monitor. Regions with enhanced BOLD-contrast (7-61 fold) were deduced as illuminated, and were found to overlap with the anatomical location of the incident light. Thus, we conclude that luminous information can be captured and translated by typical oxygen exchange processes in the blood of ordinary tissues, and made visible by psMRI (Fig. 1). This process represents a new channel for communicating environmental light into the body in certain analogy to light absorption by visual pigments in the retina where image perception takes place in the central nervous system. Potential applications of this finding may include: non-invasive intra-operative light guidance and follow-up of photodynamic interventions, determination of light diffusion in opaque tissues for optical imaging and possible assistance to the blind (Tempel-Brami et al. 2007).

Photodynamic Ablation Of A Selected Rat Embryo: A Model For The Treatment Of Extrauterine Pregnancy

Extrauterine pregnancy (EUP) is the abnormal implantation of an embryo outside the uterus, almost always in the Fallopian tube (~97% of EUP cases). Left untreated, this highly prevalent (1-2% of all pregnancies) condition may cause a life threatening rupture of the Fallopian tube. Therefore, EUP is the leading cause of maternal death in the first trimester in the western hemisphere. Current treatment options include expectancy, chemotherapeutic treatment with methotrexate or surgery. Both medical and surgical therapies entail high morbidity and severe adverse effects, mainly impaired fertility, as does failed expectancy treatment. The aim of our study was to assess the feasibility of applying Pd-bacteriochlorophyll-based PDT for the ablation of a specific embryo within a rat litter as a prospective new treatment modality for EUP. As such, the protocol should spare the rest of the litter, allow normal parturition and preserve fertility. A positively charged Pd-bacteriochlorophyll-based photosensitizer (PMRDA) was used. A treatment protocol was developed allowing successful ablation of nearly 80% of treated embryos in single session, without damaging remaining embryos or future fertility (Fig. 2). We conclude that Pd-bacteriochlorophyll-based PDT shows potential for EUP-treatment allowing high success rates with minimal adverse effects (Glinert et al. 2008).

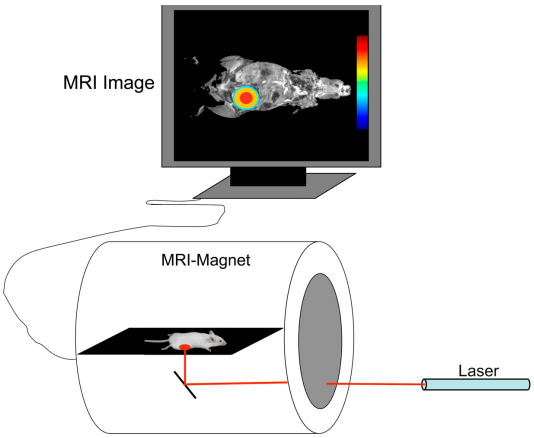

Fig. 1. *Schematic view of the experimental set up for in-vivo translation of light into an MR-image. The laser pulses are projected onto the pigment-treated animal placed inside the MRI magnet. The illuminated display is physiologically translated in the tissue into a chemical signature (paramagnetic deoxyhemoglobin) and reproduced as an image on the MRI monitor.*

VTP-Mediated Induction Of Systemic And Long-lasting Antitumor Immunity: *In Situ* Antitumor Vaccination

VTP initiates massive tumor tissue death accompanied by acute local inflammation inside the illuminated area, which is manifested by rapid infiltration of various immune cells (neutrophils, macrophages, T cells ect.). Consequently, we addressed the possibility of adaptive immune response induction by the treatment that may be considered as an integral part of the mechanism of VTP-mediated tumor eradication. The effect of VTP on the host immune system was investigated using WST11 and some of the results were also confirmed with WST09 (TOOKAD®). We found that mice lacking adaptive immune system (BALB/Nude and NOD/SCID strains) responded poorly to WST11-VTP with tumor recurrences developing shortly after treatment, while immunocompetent BALB/c mice demonstrated high cure rates (~80%), implying that a functional immune system is essential for successful VTP-mediated tumor ablation. Moreover, VTP survivors were protected against second tumor challenge (systemic injection of live tumor cells followed by assessment of tumor growth in the lungs) for a long time period up to one year after initial VTP. This antitumor protection was found not to be strictly

tumor specific as cured mice also rejected tumors from origin different than the tumor initially subjected to VTP. We propose that this interesting phenomenon can be attributed to involvement of overlapping antigens possibly originating from tumor blood vessels that are host-derived and thus shared by different tumors. Adoptive transfer experiments with different T cell populations and sera from VTP-cured or naïve mice revealed that antitumor immunity induced by VTP involves both cellular and humoral components. Using Diphtheria toxin (DTx) receptor (DTR) transgenic mice (FVB-Tg Itgax-DTR/GFP 57Lan/J) kindly provided by S. Jung from the Dept. of Chemical Immunology demonstrated that dendritic cells (DCs) are essential for VTP as their absence during the VTP protocol and shortly after significantly decreased treatment success. Additionally, we found that tumor cells exposed to photodynamic action *in vitro* can be used as effective vaccines to induce protective immunity against subsequent tumor challenge in the vaccinated animals. Based on our findings we suggest that local VTP might be utilized in combination with other anticancer therapies (e.g. immunotherapy) for further enhancement of host antitumor immunity. It might enable development of novel treatment protocols expanding the application of this modality for the treatment of disseminated disease (Preise et al. 2008).

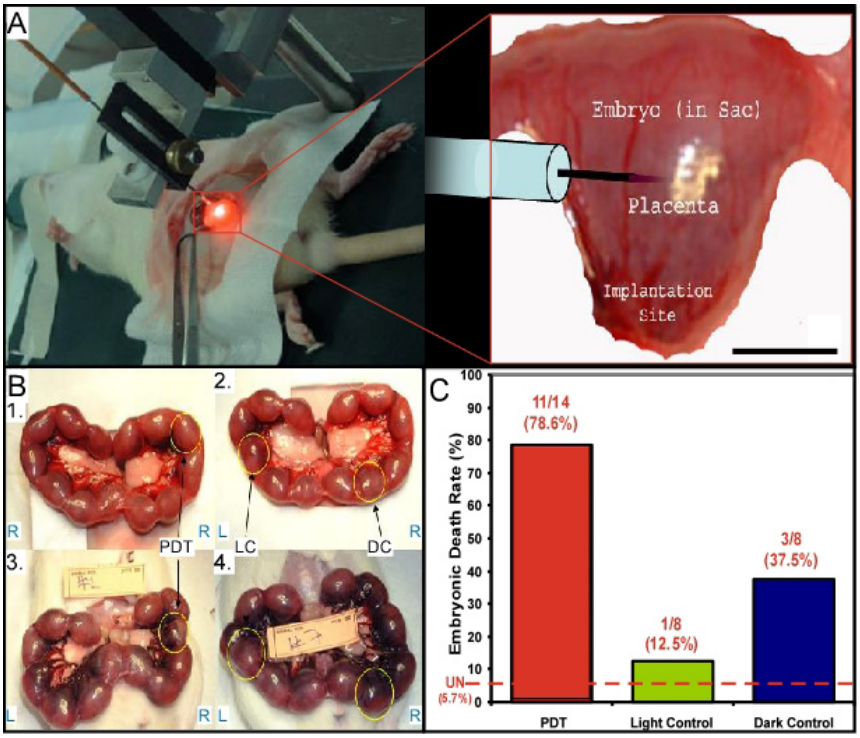

Fig. 2. *Uterine PMRDA-PDT experimental layout and results: A. Layout of the procedure. Enlarged picture: a single embryo and injection approach (Scale bar 1cm). B. Exposed rat uteri on embryonic day 14 (E14) with embryos selected for treatment (marked by yellow circles, B1), or 48h after PDT (E16, B3), light/dark controls (LC/DC) before (E14, B2) or 48h after treatment (E16, B4). Note the damage to treated embryo (shrinkage and discoloration, B3) compared to unharmed control embryos (normal size and color, B4). C. Summary of results: embryo death rates following PDT (red), LC (green) and DC (Violet). Dashed line: experimental background embryonic mortality rate (UN).*

Selected publications

Tempel-Brami, C., Pinkas I., Scherz A. and Salomon, Y. (2007). Detection Of Light Images By Simple Tissues As Visualized By Photosensitized Magnetic Resonance Imaging. PLoS ONE. 2007 Nov 21;2(11):e1191.

Glinert, I.S., Geva, E., Tempel-Brami, C., Brandis, A. Scherz, A., and Salomon, Y., (2008) Photodynamic Ablation of a Selected Rat Embryo: A Model for the Treatment of Extrauterine Pregnancy, Biology of Reproduction (in press).

Fleshker, S., Preise, D., Kalchenko A., Scherz, A., and Salomon, Y., (2008). Prompt Assessment Of Wst11-Vtp Outcome Using Luciferase Transfected Tumors Enables Second Treatment And Increase In Overall Therapeutic Rate. Photochem. Photobiol. (in press).

Berdugo, M., Bejjani R., Valamanesh F., Savoldelli, M., Jeanny JC., Scherz A., Salomon y., BenEzra D., and Behar-Cohen, F. (2008). Evaluation of the new photosensitizer STAKEL (WST-11) for photodynamic choroidal vessel occlusion in the rabbit and rat eye. Investigative Ophthalmology & Visual Sci. 49:1633-44.

Preise, D., Oren, R., Glinert, I., Kalchenko, V., Jung, S., Scherz, A., and Salomon, Y. (2008). Systemic antitumor protection by vascular-targeted photodynamic therapy (VTP) involves cellular and humoral immunity. Cancer Immunology and Immunotherapy (in press).

Acknowledgements

Y.S. is the incumbent of the Tillie and Charles Lubin Professorial Chair in Biochemical Endocrinology. Study supported by STEBA-BIOTECH France.

INTERNAL support

Keren Yeda