In-vivo Investigations on Dye-Enhanced Photothermal Tumor Therapy with a Naphthalocyanine Derivative

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Abstract:

Chromophore-enhanced photothermal therapy involves the application of an exogenous chromophore in combination with irradiation, using an appropriate wavelength, exposure duration and sufficient irradiances. The chromophore palladium(II) octabutoxynaphthalocyanine ($PdNc(OBu)_8$) accumulates at satisfactory concentrations and with good selectivity between both tumor and muscle and tumor and skin in tumor-bearing mice. In an attempt to thermally damage tumor tissue with concurrent sparing of adjacent normal tissue, the potential of $PdNc(OBu)_8$ for photothermal therapy was investigated. Using a Balb/c mouse model with subcutaneously implanted EMT6 adenocarcinoma, 90-100 hours after intraperitoneal application of $PdNc(OBu)_8$, the tumor and surrounding tissue were irradiated with a 826nm continuous-wave diode laser. The thermal effects on tumor and normal tissue were evaluated histologically. Our results indicate that after $PdNc(OBu)_8$ administration and tumor irradiation using $5W/cm^2$ for 100 seconds, pronounced selective heating of the tumor was achieved in mice, while in control animals merely an unspecific and marginal overall increase in temperature over the entire irradiation area was observed. Histological evaluation of treated areas indicated that the $PdNc(OBu)_8$ -targeted tumor tissue showed severe thermal damage while peripheral tissue like skin and muscle remained largely unaffected. This study shows the potential of creating localized thermal effects by using $PdNc(OBu)_8$ and continuous-wave light for chromophore-enhanced photothermal therapy.

Introduction:

As was shown by Anderson and Parrish [1], localized thermal tissue damage can be obtained by an increased target tissue absorbance compared to surrounding normal tissue in combination with radiation using appropriate wavelengths, irradiances, and irradiation times. Using the approach of selective photothermolysis, endogenous absorbers like (oxy)hemoglobin and melanin are now widely exploited for local destruction of vascular lesions (e.g. port-wine stains), for laser hair removal [2], or, as shown by Roider and coworkers, for precise targeting of a single layer of pigmented cells like the retinal pigment epithelium [3]. As an exogenous absorber indocyanine green (ICG) was used in experimental studies. ICG binds to plasma-proteins and therefore stays in the vasculature. The target for ICG-mediated phototherapy are blood vessels [4-6]. With ICG,

depending on the irradiances used, photochemical and/or photothermal mechanisms are discussed [7].

In selective photothermolysis local confinement of thermal effect can be achieved by matching the irradiation time to the thermal relaxation time of the absorbing structure [1]. Using appropriate irradiances and wavelengths, macromolecules and organelles may be targeted with picosecond and nanosecond pulses [8], cells and vessels can be selectively damaged with pulses in the microsecond and millisecond regime [2,3].

In this study we investigated the potential of an exogenous absorber, which is preferentially accumulated in tumor tissue, for localized thermal damage of our target. We use infrared light provided by a diode laser for deep tissue penetration.

Material and Methods:

Palladium(II)octabutoxynaphthalocyanine ($PdNc(OBu)_8$) was kindly provided by Malcolm Kenney, Dept. of Chemistry, Case Western Reserve University, Cleveland, OH, USA [9].

The animals used were Balb/c female mice, 20-25 g. The mice carried an EMT-6 mammary adenocarcinoma tumour line (NCI Frederick Cancer Research and Development Center CDT Tumor Repository) that was maintained in the mouse by subdermal injection of homogenized tumor tissue in the right hind leg [10].

Mice were injected intraperitoneally with 0.5 mg $PdNc(OBu)_8$/kg body weight in a Cremophor-based delivery vehicle as described by Bucking et al. [10]. Irradiation was performed with a 5 W diode laser emitting at 826nm 90-100 hours post application of the absorber. An air flow was used during irradiation to avoid unspecific damage of the skin covering the tumor.

Results and Discussion:

In this study, palladium(II)octabutoxynaphthalocyanine ($PdNc(OBu)_8$) was used as an absorber for in-vivo photothermal therapy. $PdNc(OBu)_8$ shows strong absorption in the near infrared wavelength range (828 nm, with an extinction coefficient of $2.8x10^5$), permitting a good penetration depth of the exciting light. With $PdNc(OBu)_8$ excitation energy is predominantly released as vibrational energy [9]. As described by Bucking et al. [10], a pharmacokinetic study performed in a Balb/c-EMT6 tumor model revealed a good accumulation of the $PdNc(OBu)_8$ in tumor compared to muscle and skin tissue.

The treatment parameters were based on theoretical calculations on the temporal and spatial temperature distributions in tumor and surrounding tissue. We used an analytical solution of the equation for heat diffusion, including the absorption coefficient and the pharmacokinetic behaviour of the absorber, published values for optical properties of tissue in the near infrared wavelength range, and published data on the kinetics of thermal tissue damage according to the Arrhenius formalism. Our calculations indicated, that the drug-mediated increase in target tissue absorbance is sufficient for localized thermal damage of our model tumor.

During irradiation of the tumor and surrounding tissue the skin surface was protected from thermal damage by cooling with an air flow. The thermally induced effects on tumor and normal tissue were evaluated histologically 48 h after treatment.

A thermography camera was used for monitoring the rise in temperature on the mouse skin during irradiation. Fig. 1 shows an example for the rise in temperature at the end of irradiation for 100s with 5W/cm². A pronounced selective heating of the skin covering the absorber-stained tumor can be observed (Fig 1a). On the other hand, in control mice, who did not receive the absorber but were irradiated with the same irradiation parameters, merely an unspecific and marginal overall increase in temperature over the entire irradiation area was observed (Fig 1b).

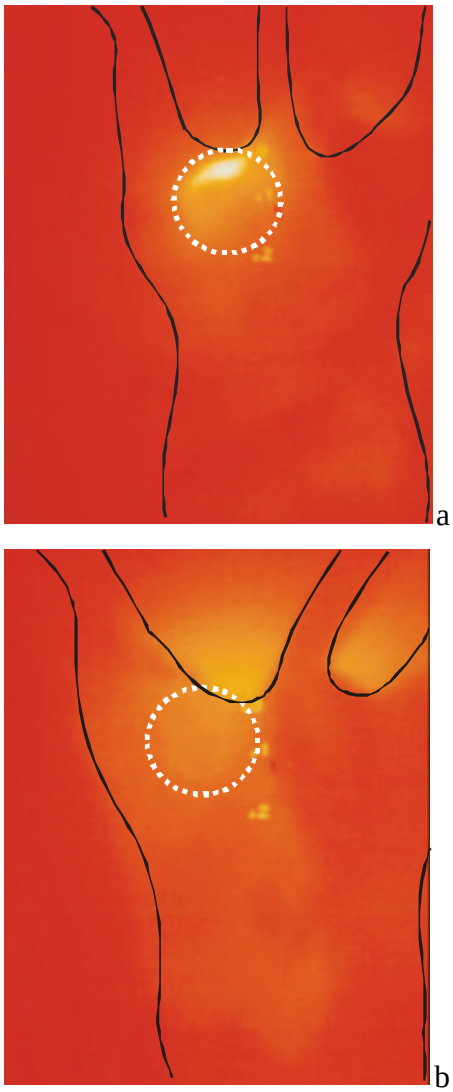

Fig. 1: *Irradiation-induced selective rise in temperature in skin overlaying the PdNc(OBu)$_8$–stained tumor (a) compared to control mice (b) monitored with a thermography camera.*

Histological evaluation showed severe thermal damage of the PdNc(OBu)$_8$–targeted tumor while surrounding tissue like overlying skin and adjacent muscle remained largely unaffected [11]. In controls no thermal damage was observed. In some cases, a zone of thermally damaged muscle cells of about 0.5 mm below and adjacent to the damaged tumor was noticed [12]. This observation was not unexpected and can be explained by the rather long exposure times of 100s which were used in these experiments. The maximum power of diode laser was limited to 5W. In

order to achieve a temperature-time regime sufficient for thermal damage of the tumors, irradiation times of 100 seconds were applied which exceeded the thermal relaxation times of the tumors (e.g. 10 seconds for a tumor with a diameter of 5 mm). Consequently, the spatial confinement of the thermal effect was lost due to heat diffusion. With a more powerful diode in future experiments higher irradiances and shorter irradiation times will be used.

In summary: This pilot study shows the potential of creating localized thermal damage of a target tissue by using $PdNc(OBu)_8$ and continuous-wave light for chromophore-enhanced photothermal therapy.

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