Perfusion, oxygenation status and growth of experimental tumors upon photodynamic therapy with Pd-bacteriopheophorbide

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Abstract. The aim of this study was to assess the anti-tumor effect of photodynamic therapy (PDT) using a novel bacteriochlorophyll derivative, palladium-bacteriopheophorbide (TOOKAD) on tumor growth, perfusion and oxygenation. Rat DS-sarcomas were treated with either TOOKAD-PDT (2 mg/kg, i.v., immediate illumination) or one of the control treatments (sham-treatment, illumination without photosensitizer, or photosensitizer without illumination). The light source was an infrared-A irradiator fitted with appropriate filters, so that the wavelengths applied (665-800 nm) included the absorption maximum of TOOKAD at 763 nm. Tumor volume was monitored for 90 days after treatment or until a target volume (3.5 ml) was reached. TOOKAD-PDT dramatically inhibited tumor growth with 92% of tumors not reaching the target volume within the observation period. In further experiments, tumor perfusion was assessed using laser Doppler flowmetry. Upon TOOKAD-PDT treatment, a rapid, pronounced decrease in perfusion was seen, down to levels corresponding to only 3% of initial values. Tumor oxygenation monitoring revealed parallel decreases, with levels corresponding to anoxia being reached. The significant anti-tumor effects presented in this report, taken together with the chemical and pharmacokinetic properties of the novel photosensitizer TOOKAD, underline the therapeutic potential of this approach in which flow stasis and induction of anoxia are key elements.

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Abbreviations: BOLD, blood oxygenation level-dependent; MABP, mean arterial blood pressure; Pd, palladium; PDT, photodynamic therapy; pO_2, oxygen partial pressure; RBC, red blood cell

Key words: photodynamic therapy, Pd-bacteriopheophorbide, perfusion, oxygenation

Introduction

Photodynamic therapy (PDT) is a cancer treatment in which an inactive photosensitizing drug undergoes photoexcitation when light of a wavelength matched to the absorption properties of the drug is applied to the tissue to be treated. The photoexcitation initiates a series of photochemical, chemical and biological reactions which culminate in cell death (1). This treatment option is becoming increasingly accepted as a therapy modality for a range of oncological, dermatological, ophthalmic and cardiovascular diseases (2). To date, the most commonly used photosensitizers have been hematoporphyrin derivatives (for oncological purposes) and a benzoporphyrin derivative (for treatment of age-related macular degeneration). The widespread use of hematoporphyrin agents in malignant disease has however been limited by a number of characteristics of these agents. Firstly, the commercial products available, despite purification, still consist of up to 60 different compounds, making the reproducibility of the drug composition difficult (1). Secondly, the extinction coefficients of the active components are generally low so that relatively high doses of drug and/or light are necessary for a satisfactory phototherapeutic response, and furthermore, the selectivity in terms of tumor drug accumulation may be poor, so that normal tissue within the light field may also become necrotic upon therapy. Thirdly, the depth of effective treatment is limited to 4-5 mm since light corresponding to the absorption maxima of the hematoporphyrin derivatives at approximately 630 nm only penetrates poorly into tissues. A further considerable problem is the prolonged cutaneous photosensitization due to slow drug clearance. When these issues are taken together, the heterogeneity in treatment response obtained both within a given tissue and between patients is hardly surprising (1). These difficulties have prompted the development of a second generation of photosensitizers, some of which are currently being evaluated in clinical trials. The bacteriochlorophyll derivatives are, in this respect, a potentially interesting group of substances exhibiting a number of photochemical and pharmacological characteristics which are superior to those of photosensitizers currently in clinical use. One compound, palladium-pheophorbide (TOOKAD®, STEBA BIOTECH; NEGMA-LERADS, France)

was developed at the Weizmann Institute of Science (A.S., Y.S.) in collaboration with STEBA BIOTECH; NEGMA-LERADS, France (3). It is a novel and pure bacteriochlorophyll derivative in which the central magnesium atom is replaced by palladium, absorbing maximally in the near-infrared (763 nm), with a high extinction coefficient (ε_o in $CHCl_3$ $\approx 10^5$ mol^{-1} cm^{-1}) and rapid clearance from the circulation (4). These features should enable a more efficient photodynamic treatment at appreciably greater tissue depth with considerably less cutaneous phototoxicity. Studies with other bacteriochlorophyll-based photosensitizers, e.g., bacteriochlorophyll-serine, suggested that the anti-tumor effects could be mainly ascribed to an anti-vascular action (5-7). The literature available to date describing studies of TOOKAD-based PDT suggest a similar mode of action with histological assessment revealing extensive vascular damage in rat C6 glioma xenografts (8), and in human prostatic small cell carcinoma xenografts (9). A study in experimental melanomas using blood oxygenation level-dependent (BOLD) contrast magnetic resonance imaging (MRI) also showed changes suggestive of a primarily anti-vascular effect (4).

The aim of the present study was to assess this anti-vascular effect in a rat tumor model. *In vivo* tumor growth was monitored subsequent to TOOKAD-based PDT and the consequences of this treatment on the tumor microenvironment in terms of perfusion and oxygenation were also evaluated. Additional monitoring of oxygenation in skeletal muscle during PDT allowed an evaluation of differences between tumor and normal tissue in the response to this treatment modality.

Materials and methods

Animals, tumors and surgical procedures. The DS-sarcoma was the tumor model used in this study. Tumors were implanted subcutaneously (0.4 ml of a cell suspension containing approximately 10^4 cells/μl) on the hind foot dorsum of male Sprague-Dawley rats (Charles River Wiga, Germany; body weight at time of tumor implantation: 140-200 g), which were housed in our animal care facility and allowed unlimited access to standard food and water. The tumors reached a volume of between 0.6 and 0.8 ml approximately one week after implantation, when treatment took place. In experiments examining the effect of treatment on normal skeletal muscle tissue, animals of the same weight class were used, without implantation of tumors. During treatment, animals were anesthetized with sodium pentobarbital (40 mg/kg, i.p., Narcoren®, Merial, Germany), laid supine on a thermostatically controlled heating pad and the rectal temperature maintained at 37.5-38.5˚C. Tumor temperature was also monitored using a thermocouple (ø250 μm; type 2ABAc, Philips, Germany). Animals breathed room air spontaneously. For tumor growth monitoring, animals were allowed to recover from the anesthetic. In the case of acute experiments (laser Doppler flux and oxygenation status), cannulation of the thoracic aorta (via the left common carotid artery) using a polyethylene catheter allowed for continuous monitoring of the mean arterial blood pressure (MABP) following connection to a pressure transducer (PD23 ID, Gould, USA). Likewise, cannulation of the left external jugular vein allowed administration of additional anesthetic, as necessary. Following these surgical procedures, animals were allowed to stabilize with data collection commencing only after constant baseline readings had been obtained for at least 20 min.

Photosensitizer. Palladium-bacteriopheophorbide (TOOKAD) was prepared at a concentration of 5 mg/ml in a Cremophor® EL (Sigma, USA)-based vehicle by the manufacturer. The sensitizer concentration was determined spectroscopically at 763 nm after dilution in chloroform, assuming an extinction coefficient of 1.086×10^5 mol^{-1} cm^{-1}. Following preparation, the drug was protected from light and stored at 4˚C prior to administration as a bolus via a tail vein at a dosage of 2 mg/kg. Drug administration was immediately followed by illumination. Control animals received an equivalent amount of the vehicle.

Light source. The light source used has been described in detail previously (10-12). It encompasses a halogen lamp emitting over the spectral range 420-1400 nm. Following insertion of a long-wave pass (665 nm) and a short-wave pass filter (800 nm) into the light path, radiation could be applied over the range 665-800 nm. In this way, the infrared bands which would otherwise result in tissue heating were removed. Illumination was applied continuously over the treatment period. A radiometer/photometer (IL1400A, International Light, USA) with a calibration traceable to the National Physical Laboratory (USA) was used for light dosimetry purposes. Irradiation was applied solely to the tumor or exposed skeletal muscle tissue, the remainder of the animal being shielded by aluminum foil.

Treatment groups. Animals were allocated randomly to the following treatment groups: i) Control: animals underwent sham-treatment, receiving the drug vehicle, but no drug or light. ii) Light only: animals received drug vehicle, followed immediately by illumination (17 min; 665-800 nm) with a fluence rate of 200 mW/cm^2 and a total energy dose of 200 J/cm^2. iii) TOOKAD-dark: animals received TOOKAD (2 mg/kg, i.v.), but no illumination. iv) TOOKAD-PDT: animals received TOOKAD (2 mg/kg, i.v.) and illumination (17 min; 665-800 nm) with a fluence rate of 200 mW/cm^2 and a total energy dose of 200 J/cm^2.

Assessment of the in vivo tumor response. Subsequent to treatment, animals were allowed to recover from the anesthetic and kept under subdued lighting conditions for 24 h, before returning to our animal housing facilities. Daily measurement of the three orthogonal diameters (d) of the tumors was performed so that the tumor volume (V) could be determined using the ellipsoid approximation $V = \pi/6 \times d_1 \times d_2 \times d_3$. In this study, the end point of the tumor growth assessment was the attainment of a tumor volume of 3.5 ml rather than survival. This target volume was selected to ensure that the tumor burden at the end of the study never exceeded 1% of the body weight. In animals where tumors did not reach the target volume, monitoring was carried out for 90 days subsequent to treatment.

Laser Doppler flowmetry. A laser Doppler flowmeter (Periflux 2B, Perimed, Sweden) was used to determine the red blood cell

(RBC) flux through tumor tissue. This technique assesses the frequency change undergone by light when reflected by moving objects such as red blood cells, and has been described as a valid technique for measurement of microcirculatory function in small tissue areas (13). RBC flux signals were obtained using a needle probe (PF 302, o.d.: 0.45 mm), which was inserted into the centre of the tumor, following a small incision into the skin covering the tumor made with a 24-gauge needle. Additionally, the total backscattered light was monitored to ensure an optimum and constant probe positioning and minimum tissue compression. At the end of each experiment, the laser Doppler probe was left in place and the animal given an overdose of anesthetic so that the biological zero RBC flux signal could be established and subtracted from all previous values for each animal (14). Data were collected for 10 min prior to TOOKAD/vehicle injection and for 60 min thereafter, and were subsequently expressed relative to the value obtained immediately prior to commencement of treatment.

Oxygen partial pressure (pO_2) measurements. Assessment of tissue oxygenation was carried out polarographically using a flexible O_2 sensitive catheter electrode (ø350 µm, LICOX pO_2, GMS, Germany). Calibration of the pO_2 electrode was carried out using room air in a constant temperature chamber, taking into account the prevalent barometric pressure. For tumor measurements, the electrode was inserted into the center of the tumor. In animals where skeletal muscle oxygenation was assessed, the hind leg adductor muscles were exposed following a skin incision, and the catheter electrode inserted so that it lay parallel to the muscle fibers at a depth of approximately 5 mm. In both tumor and skeletal muscle, pO_2 values were monitored continuously, with data being recorded 10 min prior to TOOKAD/vehicle injection, and for 60 min thereafter. Data were subsequently expressed relative to the value obtained immediately prior to commencement of treatment.

Experimental guidelines and statistical methods. All procedures had been reviewed by the responsible regional Ethics Committee, and were carried out in strict accordance with the UKCCCR guidelines (15) and the German Law for Animal Protection of 1987.

In order to describe *in vivo* tumor growth characteristics, Kaplan-Meier statistics were applied and a log-rank analysis was used to assess the significance of differences between the various treatment groups. The significance level was set at α=5%.

Results

In experiments in which tumor growth was monitored for up to 90 days, no phototoxic effects on normal skin were observed, and animals in all groups recovered rapidly after treatment. A Kaplan-Meier analysis showing the probability of the tumor volume being <3.5 ml during the observation period was performed, the results of which are shown in Fig. 1. In sham-treated animals (control), no spontaneous tumor cure was seen, with all tumors reaching the target volume by day 7 after treatment. Similar observations were made for the light only and TOOKAD-dark groups, where all tumors reached the target volume by days 9 and 8 respectively. Log-rank analysis showed no significant differences between the control, light only and TOOKAD-dark groups. Tumors treated with TOOKAD-PDT showed signs of edema one day after treatment. Thereafter, the edema subsided and the tumor became pale. TOOKAD-PDT treatment increased the probability that a tumor would not reach the target volume within the 90 day observation period (92% on day 90). This effect was found to be significant when compared with all other groups (control, light only or TOOKAD-dark; $p<0.001$ in all cases). No residual tumor was evident (as assessed by macroscopic examination) in those animals still in the study at day 90 after treatment.

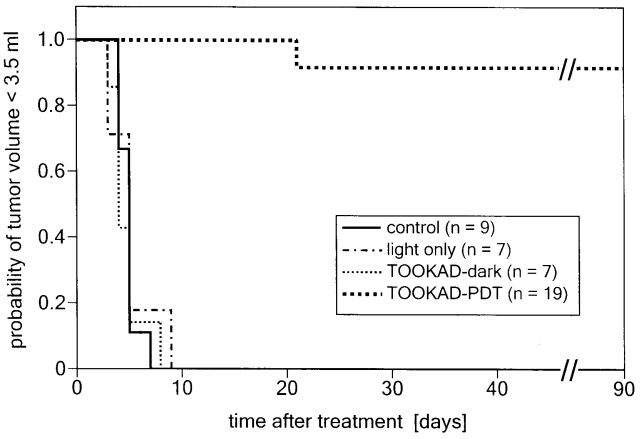

Figure 1. Probability of tumor volume being <3.5 ml as a function of time following treatment as assessed using a Kaplan-Meier analysis. Rats bearing DS-sarcomas underwent either sham-treatment (control), injection of vehicle followed immediately by illumination (light only; 200 mW/cm^2, 200 J/cm^2), TOOKAD injection (2 mg/kg, i.v.) without illumination (TOOKAD-dark) or TOOKAD injection (2 mg/kg, i.v.) followed immediately by illumination (TOOKAD-PDT; 200 mW/cm^2, 200 J/cm^2). n = number of tumors treated.

The time course of changes in MABP and RBC flux in tumor microvessels, relative to values at t=0 min are shown in Fig. 2. MABP at t=0 min was 122±2 mmHg (mean ± SEM of values from 26 animals). No pronounced changes in this parameter were seen over the treatment period, with values varying by only up to 10% of the initial values. This finding indicates that the changes in tumor RBC flux and oxygenation described below are not related to changes in perfusion pressure.

During sham-treatment, RBC flux remained relatively constant. In comparison, mean RBC flux values in the light only group tended to be slightly higher (up to 10% higher than at t=0 min). This may reflect a physiological response to the approximately 2˚C increase in tumor temperature seen during illumination. In contrast, RBC flux decreased (up to 15%) following drug administration in the TOOKAD-dark group, an effect which prevailed for the whole of the observation period. Treatment with TOOKAD-PDT resulted in a characteristic biphasic change in RBC flux. This encompassed an initial rapid decrease starting approximately 5 min after treatment commencement, in which the RBC flux fell to 70% of the initial value within the following 3 min of

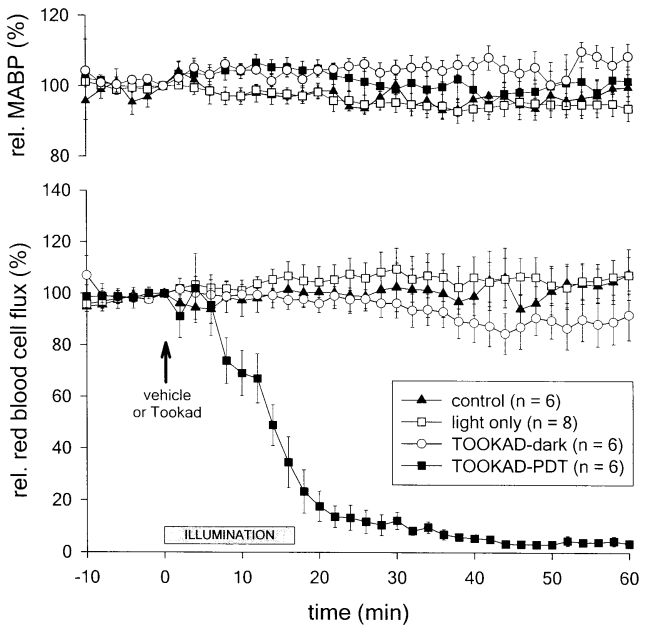

Figure 2. Relative mean arterial blood pressure (rel. MABP) and relative red blood cell flux in tumor microvessels as a function of time during sham-treatment (control), injection of vehicle followed immediately by illumination (light only; 200 mW/cm^2, 200 J/cm^2), TOOKAD injection (2 mg/kg, i.v.) without illumination (TOOKAD-dark) or TOOKAD injection (2 mg/kg, i.v.) followed immediately by illumination (TOOKAD-PDT; 200 mW/cm^2, 200 J/cm^2). The period of illumination is indicated by the shaded bar, and the time of drug or vehicle injection by the arrow. Each data point indicates mean values (± SEM) for the number of tumors treated (n).

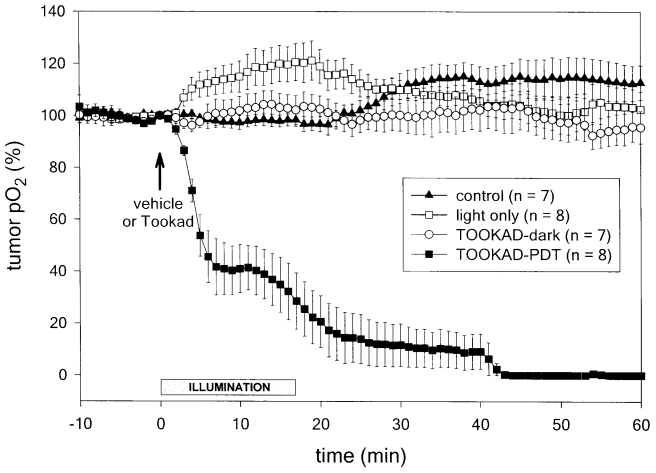

Figure 3. Relative tumor O_2 tension (pO_2) as a function of time during sham-treatment (control), injection of vehicle followed immediately by illumination (light only; 200 mW/cm^2, 200 J/cm^2), TOOKAD injection (2 mg/kg, i.v.) without illumination (TOOKAD-dark) or TOOKAD injection (2 mg/kg, i.v.) followed immediately by illumination (TOOKAD-PDT; 200 mW/cm^2, 200 J/cm^2). The period of illumination is indicated by the shaded bar, and the time of drug or vehicle injection by the arrow. n = number of tumors treated. Each data point indicates mean values (± SEM) for the number of tumors treated (n).

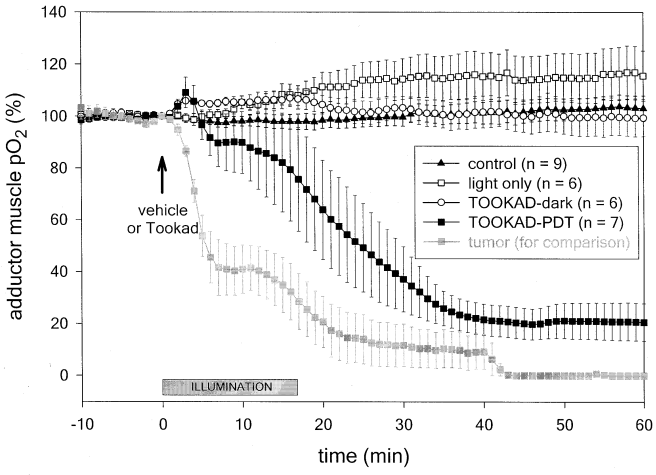

Figure 4. Relative O_2 tension (pO_2) in adductor muscle as a function of time during sham-treatment (control), injection of vehicle followed immediately by illumination (light only; 200 mW/cm^2, 200 J/cm^2), TOOKAD injection (2 mg/kg, i.v.) without illumination (TOOKAD-dark) or TOOKAD injection (2 mg/kg, i.v.) followed immediately by illumination (TOOKAD-PDT; 200 mW/cm^2, 200 J/cm^2). The period of illumination is indicated by the shaded bar, and the time of drug or vehicle injection by the arrow. n = number of adductor muscles treated. Each data point indicates mean values (± SEM) for the number of muscles treated (n). For comparison, values from Fig. 3 for TOOKAD-PDT in tumors have been added in gray.

treatment. This was succeeded by a transient plateau phase lasting approximately 5 min and a further steady decline in RBC flux down to 3% of the initial flux. No recovery was evident during the remainder of the observation period.

Initial mean tumor pO_2 was 27±2.3 mmHg (mean of values from 30 tumors), as measured polarographically. Upon treatment, tumor oxygenation showed similar changes to those seen for RBC flux (Fig. 3). In the TOOKAD-PDT group, tumor pO_2 started to fall dramatically already 2 min after TOOKAD administration and illumination commencement, reaching 40% of initial values within the first 6 min. This was followed by a short plateau phase. Thereafter, a further - albeit slower - decrease in pO_2 was observed which continued even after light delivery ended. By 45 min after commencement of light and TOOKAD administration, the tumor pO_2 was 0 mmHg (= anoxia) in all tumors, with no recovery evident during the remainder of the 60 min observation period. In order to assess the selectivity of these effects on tumor tissue, oxygenation measurements were also made in normal adductor muscle tissue (Fig. 4). Immediately prior to treatment, mean pO_2 in this skeletal muscle was 44±2.4 mmHg (mean of values from 28 muscles). In the control and TOOKAD-dark groups, relatively constant muscle pO_2 values were seen. In the light only group, pO_2 values increased by approximately 15% upon illumination, which may again reflect an increase in muscle blood flow as a physiological response to the ≈2˚C increase in muscle temperature seen during illumination. Within 4 min after commencement of TOOKAD-PDT treatment, muscle pO_2 rose by approximately 10%. Thereafter, the muscle pO_2 fell continuously, reaching 20% of initial values approximately 40 min after TOOKAD administration and commencement of illumination. No recovery was evident during the remainder of the 60 min observation period. When

changes in tumor and muscle pO_2 are directly compared (Fig. 4), it is evident that the decreases in pO_2 in muscle tissue occur more slowly than those found in tumor tissue and are considerably smaller (down to 20% of initial values, as opposed to down to 0% in tumor).

Discussion

The objective of this study was to determine the effects of a single treatment with TOOKAD-PDT on the growth of an experimental tumor in the rat. In order to gain insight into the mechanisms underlying the anti-tumor effect of this therapy, measurements of tumor perfusion and oxygenation were also performed. In the tumor model used, this treatment approach proved to be highly effective, resulting in a high probability (92%) for tumors not reaching the target volume. The therapeutic potential is additionally underlined by the fact that there was no evidence of residual tumor presence in animals still undergoing observation on day 90. A number of studies have examined the anti-tumor effects of photodynamic treatment using bacteriochlorophyll derivatives (6-8,12,16,17). One compound, bacteriochlorophyll-serine showed phototoxicity under *in vitro* conditions approximately 200 times greater than that found with hematoporphyrin derivatives (17), and *in vivo* was found to be effective in inhibiting tumor growth in a melanoma model in mice where a cure rate of 81% was achieved (6). In the tumor model also used in the present study, PDT using bacteriochlorophyll-serine (20 mg/kg, i.v.) led to a 36% probability of tumors not reaching the target volume within the 90 day observation period (7). When this is compared with the probability of 92% obtained in the present study with TOOKAD-PDT, using a photosensitizer dose of only 2 mg/kg, then it would appear that TOOKAD is the more effective of these two bacteriochlorophyll derivatives. In this context, a further study with the same rat model should be noted where 5-amino-levulinic acid-based PDT resulted in a probability of tumor control of only 15% (18). In further studies with TOOKAD-PDT a cure rate of 64% was obtained for rat C6 glioma xenografts (8) and of 69% in human prostatic small cell carcinoma xenografts (9). In light of the effectiveness of TOOKAD-PDT in tumor models, this compound was selected for use in clinical trials on the treatment of prostate cancer which commenced in July 2002 (Gertner *et al*, CapCure Scientific Retreat, Abst, Washington, DC, USA).

The investigation of tumor perfusion in the present study using laser Doppler flowmetry showed a rapidly induced vascular stasis, suggesting that PDT with TOOKAD primarily induces vascular damage, as was the case with bacteriochlorophyll-serine (6). The protocol for PDT in the present study involved immediate tumor illumination following drug administration (i.e., a drug-light interval of 0 min). In preliminary experiments in which a variety of drug-light intervals were assessed (0, 15, 30 min, 24, 48, 78 h), the greatest anti-tumor effect was found when illumination commenced immediately after TOOKAD administration, with the effect diminishing with increasing drug-light intervals (data not shown). Similarly, Borle *et al* found the greatest PDT response at the shortest drug-light interval with TOOKAD (19). These findings suggest that a high photosensitizer concentration in blood is an important factor in the success of TOOKAD-based PDT, in line with the anti-vascular effects seen. Further strong evidence for a primarily anti-vascular action of TOOKAD-PDT has recently also been presented by Preise *et al* who showed this treatment to be equally effective in xenografts derived from either isogenic wild-type HT29 or MDR-HT29 cells, despite the fact that the latter cell line was resistant to TOOKAD-PDT *in vitro* (20).

The primary target for PDT was traditionally considered to be the tumor cell itself. As with ionizing radiation and some chemotherapeutic agents, the PDT effect is O_2-dependent (21) and it was considered that O_2 deprivation due to vascular damage may result in a 'self-limitation' of PDT treatment (22). More recently however, attention has been focused on the prospect of treating solid tumors by targeting the tumor vasculature, not only with PDT (23,24) but also by using other agents which have a direct anti-vascular effect, e.g., combretastatin (25). In PDT treatment of age-related macular degeneration, the blood vessels are the exclusive target (2). The changes seen in this study in tumor perfusion and oxygenation occur rapidly and concur with the recent findings of Gross *et al* (4) who monitored the effects of TOOKAD-PDT in melanomas in mice using blood oxygenation level-dependent (BOLD) contrast magnetic resonance imaging. They proposed that two processes occurring during TOOKAD-PDT are responsible for the changes seen in BOLD contrast: a rapid increase in oxygen 'consumption' due to the photochemical reaction which results in a deoxygenation of hemoglobin, independent of blood flow changes, together with a local vascular occlusion and stasis.

In our study, significant changes in blood pressure during TOOKAD-PDT (or TOOKAD-dark) were not recorded and therefore appear not to play a role in the tumor perfusion or oxygenation changes. In a study of TOOKAD-PDT in canine normal tissue, a marked decrease in blood pressure was observed upon TOOKAD administration (26). This effect could be controlled by premedication and was attributed to the presence of Cremophor in the drug vehicle which can trigger an anaphylactoid reaction in the species studied. In order to ascertain the tumor specificity of the TOOKAD-PDT effects in this study, oxygenation measurements were carried out during treatment in both tumor and skeletal muscle tissue. The results presented show that TOOKAD-PDT can drastically reduce oxygenation in both tissues. However, closer comparison of the O_2 changes in these tissues suggests that the effects may differ between tumor and normal tissues. Firstly, the reduction in oxygenation in tumor tissue takes place more rapidly than in muscle tissue. Secondly, the extent of the reduction seen at the end of the observation period was greater in tumor tissue, with levels of anoxia (pO_2=0 mmHg) being reached in all tumors examined, whereas in muscle tissue, the oxygenation, though greatly reduced (down to an average of 20% of initial values), never reached anoxic levels. Since TOOKAD-PDT appears to provide a primarily anti-vascular approach to tumor treatment, it is not surprising that effects are also seen in normal tissue upon illumination and TOOKAD application. In light of the rapidity of the effects, a selective uptake into the tumor cells is unlikely to be involved in the differences found in oxygenation changes.

Rather, the differences most probably reflect a functional selectivity attributable to a differential response of tumor and normal vasculature to TOOKAD-PDT, which in turn is based on a range of structural and functional abnormalities found in tumor microvessels (27,28).

TOOKAD-PDT therefore provides a highly effective anti-vascular approach to tumor therapy. The photosensitizer TOOKAD also presents a number of features which may enhance its efficacy in comparison to many other photosensitizers. Due to the fast clearance, tissue illumination must take place shortly after, or even during drug administration. While this aspect requires a stringent clinical protocol of patient and light source positioning before drug application, it provides the significant advantage of allowing drug and light administration in a relatively short, single treatment session. The rapid drug clearance means that phototoxicity, in particular skin sensitization (a major side effect of hematoporphyrin derivative-mediated PDT), should be minimal, thus reducing the need for post-treatment management. Indeed, in this and other animal studies performed to date, skin phototoxicity was not recorded as a notable side effect with this photosensitizer (26,29). Lastly, since the maximum absorption spectrum of TOOKAD is at a much longer wavelength (around 760 nm) than the absorption maxima of the first generation photosensitizers such as Photofrin® (630 nm), the light can penetrate more deeply (>1 cm), allowing treatment of bulkier tumors. At this wavelength, appreciable light absorption by most other endogenous tissue pigments can also be avoided (19).

In conclusion, the significant anti-tumor effects presented in this report, taken together with the chemical and pharmacokinetic properties of the novel photosensitizer TOOKAD, underline the therapeutic potential of this antivascular approach, which will be assessed in the ongoing clinical trials.

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