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Correlation of Photodynamic Activity and Fluorescence Signaling for Free and Pegylated mTHPC in Mesothelioma Xenografts

THESE

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Introduction

Les premières applications cliniques de la thérapie photodynamique (PDT) remontent à plus d'une vingtaine d'années. Basée sur l'activation d'un médicament photosensibilisateur par une source lumineuse à une longueur d'onde spécifique, la PDT permet la destruction sélective de tissus contenant le produit actif.

Ce procédé a été expérimenté dans le traitement de cancers en raison de la propriété du médicament à se concentrer dans les tumeurs tout en épargnant les structures normales contigües. Cependant, les photosensibilisateurs utilisés jusqu'à ce jour n'ont pas démontré une accumulation exclusive dans les tissus néoplasiques mais également dans les structures saines avoisinantes induisant une destruction tissulaire non sélective. Notamment, d'importantes complications ont été rapportées suite à l'utilisation de la PDT dans la cavité thoracique après la résection de mésothéliomes pleuraux, et ce malgré l'arrivée de photosensibilisateurs de secondes générations.

De ce fait, plusieurs études expérimentales ont été menées afin d'améliorer la sélectivité tumorale du médicament en modulant différentes conditions de traitement et en modifiant la structure du photo-sensibilisateur par pégylation.

Le but de cette étude expérimentale est de corréler l'activité photodynamique, la phototoxicité et la distribution du m-tetrahydroxyphenylchlorin (mTHPC) et de sa forme pégylée, le PEG-mTHPC. De ce fait, un modèle de souris nues porteur de xenogreffes de mésothéliome humain a été utilisé pour étudier les deux photosensibilisateurs.

De récents travaux avec ce modèle ont montré que la mesure de la concentration tissulaire du mTHPC et de sa forme pégylée par HPLC restait limitée afin de prédire l'activité photodynamique. De ce fait, nous pensons que les mesures de fluorescence peuvent être plus appropriée. Le signalement fluorescent est mesuré dans le tissu tumoral et dans une région contrôle de la peau afin d'étudier la distribution et l'intensité des deux sensibilisateurs.

Méthode

Des souris nues (cd1nu/nu mice) de 8 semaines ont été transplantées avec des fragments de mésothéliome malin humain (H-meso-1). Ces derniers ont été obtenus à partir d'une suspension cellulaire. Au moins trois passages ont été faits dans les animaux, avant que le traitement soit initié.

Deux groupes de 6 souris chacun ont été utilisés pour l'injection intraveineuse par la queue du mTHPC à 0.15 mg/kg et du PEG-mTHPC à dose équimolaire. Après trois jour, la tumeur ainsi qu'une région contrôle de la cuisse ont été illuminées sur une surface d'un diamètre de 1.2 cm et pendant 133 secondes avec un laser à une longueur d'onde à 652 nm (fluence 20 J/cm^2, fluence rate 150 mW/cm^2). Les animaux ont été ensuite sacrifiés 72 heures après l'illumination. L'étendue de la nécrose tumorale et de la région contrôle ont été déterminées en aveugle par histomorphometrie par un pathologue (HJA).

La fluorescence microscopique a été évaluée dans 12 souris à une concentration de 0.15 et 0.5 mg/kg pour le mTHPC, et à doses équimolaires pour le PEG-mTHPC. Trois animaux ont été injectés avec le mTHPC à 0.15 mg/kg, 3 autres à dose équimolaire avec la forme pégylée et 6 souris avec le mTHPC à 0.5 mg/kg et à dose équimolaire.

Les animaux ont été sacrifiés 72 heures après injection. L'intensité fluorescente des sensibilisateurs a été mesurée dans la tumeur et la région contrôle.

Suite à cela, les coupes ont été fixées par H&E et superposées aux images fluorescentes, afin de localiser la distribution des deux photosensibilisateurs dans les différents compartiments tissulaires. Six souris transplantées n'ayant ni été injectées avec les sensibilisateurs ou illuminées ont servi de groupe contrôle.

Résultats

Trois jours après l'illumination, la PDT provoque une nécrose tumorale de 10 ± 5.4 mm^2 pour le mTHPC à 0.15mg/kg et 5.2 ± 4.6 mm^2 pour sa forme pégylée à dose équimolaire. Cependant, la nécrose tumorale induite par les deux formulations du sensibilisateur est significativement plus élevée que dans le groupe contrôle (0.33 ± 0.58 mm^2) (P=0.02). Toutefois, le mTHPC pégylé provoque une photosensibilité cutanée moins importante que la forme non-pegylée. Dans les deux groupes, aucune nécrose n'a été observé dans la cuisse des animaux.

Trois jours après l'injection du mTHPC et de la forme pégylée à 0.15 mg/kg, aucune activité fluorescente n'a été détectée. Cependant, à 0.5 mg/kg, la fluorescence microscopique révèle une distribution hétérogène des deux photo-sensibilisateurs dans le tissu tumoral avec une accumulation prédominante dans les régions peri-vasculaires. Les deux médicaments montrent une distribution intra-cellulaire homogène dans le cytoplasme et une absence de signalement dans le nucleus.

La mesure de l'intensité fluorescente du mTHPC à 0.5mg/kg ne montre pas de différence significative entre le tissu tumoral et la région contrôle. Par contre, le PEG-mTHPC montre une intensité fluorescente supérieure dans le tissu tumoral que dans la peau (ratio tumeur- peau 0.94 pour le mTHPC et 1.73 pour le PEG-mTHPC).

Conclusion

L'utilisation du mTHPC à 0.15mg/kg induit une nécrose tumorale similaire à celle du PEG-mTHPC à dose équimolaire. Cependant, ce dernier démontre une photo-toxicité plus atténuée de la peau. La fluorescence microscopique permet de localiser les deux sensibilisateurs dans les différents compartiments tissulaires à partir d'une dose de 0.5 mg/kg.
Le PEG-mTHPC induit un signalement fluorescent supérieur dans le tissu tumoral par rapport à la peau.
La mesure du signalement fluorescent a le potentiel de prédire l'activité photodynamique du mTHPC et de sa forme pégylée dans les xénogreffes de mésothéliome humain dans un modèle de souris nue.

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Correlation of Photodynamic Activity and Fluorescence Signaling for Free and Pegylated mTHPC in Mesothelioma Xenografts

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Background/Objectives: Correlation of photodynamic activity (PDT) and fluorescence signaling for free and pegylated meta-tetrahydroxyphenylchlorin (mTHPC) in nude mice with mesothelioma xenografts.

Study Design/Materials and Methods: Twelve animals received light delivery (20 J/cm^2, 150 mW/cm^2, spot size 1.2 cm) on the tumor and the hind leg 3 days after sensitization with 0.15 mg/kg free mTHPC ($n=6$) or equimolar-dosed pegylated mTHPC ($n=6$). Groups of three animals each were sensitized with 0.15 and 0.5 mg/kg free mTHPC or equimolar dosed pegylated mTHPC followed after 3 days by fluorescence microscopy measurements.

Results: Pegylated mTHPC resulted in a similar extent of PDT-related tumor necrosis but in lower skin phototoxicity than free mTHPC. Both mTHPC formulations were heterogeneously distributed in the tumor and were mainly localized in perivascular areas. Pegylated mTHPC revealed a higher tumor to skin fluorescence intensity ratio than free mTHPC ($P<0.001$).

Conclusions: Fluorescence signaling measurement has the potential to predict the photodynamic activity for both mTHPC formulations in mesothelioma xenografts. Lasers Surg. Med. 39:237–244, 2007. © 2007 Wiley-Liss, Inc.

Key words: mTHPC Foscan; nude mice; animal model; tumor; skin phototoxicity; fluorescence microscopy; fluorescence intensity

INTRODUCTION

Following its advent more than two decades ago, photodynamic therapy (PDT) has emerged as a promising treatment modality for superficially growing tumors. The mechanism of action of PDT is based on the activation of a photosensitive drug by laser light of a specific wave length and selective destruction of tissues containing the sensitizer. Since the sensitizer is expected to accumulate preferentially in malignant tumors, PDT may lead to selective tumor destruction while sparing normal non-affected tissues. However, the photosensitizers studied to date do not exclusively accumulate in malignancies as normal tissue also present some uptake. This has led to relevant morbidity after PDT of the chest cavity after surgery for pleural mesothelioma [1–6], even in the presence of new second generation sensitizers [7–13]. Several experimental studies focusing on PDT for mesothelioma have shown that enhanced tumor selectivity may be obtained by modulating treatment conditions [14–18] and by pegylation of the sensitizer [19,20].

The current study compares the photodynamic activity, phototoxicity, and sensitizer distribution in tumors and skin for free and pegylated meta-tetrahydroxyphenylchlorin (mTHPC) in nude mice with mesothelioma xenografts. Since previous work has shown that tissue concentration measurements of free and pegylated mTHPC performed by HPLC were of limited use for predicting the photodynamic activity in this model [15,19], we hypothesized that fluorescence signaling measurements by fluorescence microscopy might be well suited in this respect. Fluorescence signaling of free and pegylated mTHPC was analyzed in tumors and skin by use of fluorescence microscopy in order to assess the photosensitizer distribution in tumors and to measure the tumor to skin fluorescence intensity ratio for both mTHPC formulations.

MATERIALS AND METHODS

Study Design

The study design is summarized in Table 1. Free mTHPC and equimolar dosed pegylated mTHPC were compared with respect to photodynamic activity, phototoxicity and drug distribution within tumors and skin of nude mice bearing mesothelioma xenografts 3 days after

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TABLE 1. Study Design

	Assessment of tumor necrosis and normal tissue damage		Assessment by fluorescence microscopy	
	Drug dose	#Animals	Drug dose	#Animals
Free mTHPC	0.15 mg/kg	6	0.15 mg/kg	3
			0.5 mg/kg	3
Pegylated mTHPC	0.15 mg/kg	6	0.15 mg/kg	3
			0.5 mg/kg	3
Non-sensitized, non irradiated		3		3

i.v. photosensitizer administration. Six tumor-bearing animals were given 0.15 mg/kg of free mTHPC and six were given an equimolar dose of pegylated mTHPC. Three days later, all 12 animals received laser light on the tumor and an equally sized area on the hind leg at a drug-light interval (i.e., time interval between sensitizer administration and light delivery) of 3 days, followed by photodocumentation and histological assessment of the treated areas 72 hours after photoirradiation. Another 12 tumor-bearing animals were given photosensitizer. Of these 12 animals, 3 were given 0.15 mg/kg free mTHPC, 3 were given an equimolar dose of pegylated mTHPC, 3 were given 0.5 mg/kg free mTHPC, and 3 were given an equimolar dose of pegylated mTHPC. This was followed by fluorescence microscopic assessment of the photosensitizer distribution within tumors and skin 3 days after drug administration. Six tumor-bearing animals that did not receive drug and were not photo-irradiated served as controls.

Animals and Housing

Specific Pathogen-free female nude mice (cd1nu/nu mice, Charles River Wiga, Sulzfeld, Germany), 6 to 8 weeks old at tumor implantation, were used. They were kept in autoclaved cages in a laminar-air-flow bench at $25 \pm 2°C$ and $55 \pm 5\%$ relative humidity under artificial light in a 12-hour rhythm. The animals were cared for in accordance with the established guidelines of the Local Ethical Committee on Animal Care of the University of Lausanne.

Generation of Human Mesothelioma Xenografts

Tumor transplantation was performed in animals under general anesthesia with Narketan (100 mg/kg i.p) and Xylapan (5 mg/kg i.p.) (Chassot AG, Bern, Switzerland). Using the trocar technique, fragments of human malignant mesothelioma (H-meso-1, Mason, Worchester, MA) [21], based on a human mesothelioma cell suspension, were implanted subcutaneously behind the left omoplate of the nude mice as previously described [15]. At least three passages on nude mice were performed by the trocar technique after thawing from liquid nitrogen before treatment was initiated [20]. Experiments were performed on 30 animals at a tumor size of 8 mm diameter.

Photosensitizer Administration

Free mTHPC (Foscan®) and pegylated mTHPC were kindly provided by Scotia Pharmaceuticals (Guildford, UK). mTHPC was dissolved in a pharmaceutical-grade solution of 40% ethanol and 60% propylene glycol for administration [15]. Pegylated mTHPC is a tetrakis-(m-methoxypolyethylene glycol) derivative of 7,8-dihydro-5,10,15,20-tetrakis(3-hydroxyphenyl)-21-23-[H]-porphyrin with a molecular weight of approximately 6,300 Da and results from the addition of four polyethylene glycol chains with a length of 2000 by use of triazine moieties to native mTHPC [19]. Pegylated mTHPC was dissolved in sterile 0.9% NaCl for administration [19,20].

Nine animals received 0.15 mg/kg free mTHPC and another nine animals received an equimolar dose of pegylated mTHPC. Three animals received 0.5 mg/kg and three an equimolar dose of pegylated mTHPC. Both sensitizer formulations were injected intravenously into the tail vein of the animals under general anesthesia and a volume of 0.1 ml/10 g body weight of drug solution was used for injection.

Photoirradiation

Illumination was performed under general anesthesia with Narketan (100 mg/kg i.p) and Xylapan (5 mg/kg i.p.) (Chassot AG). The mice were positioned on a warm pad during light application. A diode laser emitting at 652 nm (Applied Optronics, South Plainfield, NJ) was connected by an SMA 905 connector to an optical quartz fiber (600 nm) containing a lens (Medlight, Ecublens, Switzerland). In each animal, non-contact surface irradiation was performed on the tumor (through the intact skin overlying the tumor) and on an equal size area of the hind leg [15]. The irradiated spots were 1.2 cm in diameter and the treated surfaces were situated perpendicular to the incident laser beam. The power at the end of the fiber was measured by a power meter calibrated for 652 nm. The fluence rate and fluence on the treated surfaces was 150 mW/cm^2 and 20 J/cm^2, respectively. The exposure time of 133 seconds was controlled by a time shutter.

Assessment of Photodynamic Activity on Tumors and Normal Tissue

The treated areas were inspected and photo-documented on day 1, day 2, and day 3 after illumination. Seventy-two hours after illumination, the animals were sacrificed by an overdose of ether and were fixed in buffered formalin (10%). The irradiated areas of the tumor and the hind leg were cut at right angles to the surface from the center to the

periphery and were H&E-stained. The extent of tumor necrosis was determined by planimetry to better assess the inhomogeneous necrosis in a nodular tumor as previously described [15]. A transparent grid with 1 mm spacing was placed over the histological section taken through the largest diameter of the tumor and the number of grid intersections falling within the necrotic or non-necrotic tumor area was counted with the aid of a dissecting microscope. This procedure was repeated three times at different angles and the median value was used for subsequent statistical analysis. The extent of normal tissue damage (skin and underlying muscle of hind leg) was measured in mm with a dissecting microscope fitted with an eyepiece graticule. The pathologist (HJA) was blinded to the treatment conditions.

Fluorescence Microscopic Assessment of Sensitizer Distribution in Tumors and Skin

Twelve sensitized (free mTHPC = 6, pegylated mTHPC = 6) but non-irradiated animals were sacrificed by an overdose of ether 72 hours after i.v. administration. The tumors together with the overlying skin were harvested, frozen in liquid nitrogen with isopentane slush and stored at −70°C. Preparation of tissue slices and fluorescence microscopy measurements was performed as previously described [22]. Tissue sections were prepared in the dark to avoid photobleaching. The frozen tissue blocks were mounted in OCT medium (Tissue Tek II embedding compound, BDH Ltd, Poole, UK) and a series of sections was cut with a cryostat (Frigocut Model 2700, Reichert Ltd, Vienna, Austria). Six consecutive, non-stained, 5 μm thick tissue sections mounted on clean glass slides were prepared for each sample. From each frozen section, three images were recorded over three different parts of the slice to avoid photobleaching. We used a Zeiss AxioPlan-2 microscope equipped with a Zeiss Axiophot image analysis system and a filtered 100 W mercury lamp as an excitation light source. Images were recorded with a gray-scale camera with a 12-bits dynamic range and 2×2 binning, resulting in 694×520 pixel images with 4,095 grey levels. A 05 cube for epifluorescence (excitation BP395-440, Carl Zeiss, Jena, Germany) and a barrier filter (LP470 nm) were used. The photosensitizer's fluorescence was recorded using a second barrier filter (LP630 nm). The localization and specific intensity of the sensitizer's fluorescence were determined by subtracting the autofluorescence from the fluorescence image. For this purpose, a band pass D560/40 m filter was used (Chroma Tech, Brattleboro, VT). After recording the fluorescence images, the same slices were stained with H&E and compared with the fluorescence image from the same site in order to localize the sensitizer within the tissues. The relative fluorescence intensity emitted by the sensitizer was analyzed in the different areas of tumors and skin using the public domain program, NIH ImageJ 1.31 m (Bethesda, MD). The autofluorescence background subtraction procedure was performed using similar tissue samples from three non-sensitized control animals.

Statistical Analysis

A non-parametric Kruskal–Wallis test was applied where appropriate. A bidirectional hypothesis was used and significance was accepted at a $P<0.05$.

RESULTS

PDT-Related Tumor Necrosis

The extent of spontaneous tumor necrosis in non-sensitized, non-irradiated xenografts was 0.33 ± 0.58 mm^2. PDT with 0.15 mg/kg free mTHPC resulted in a tumor necrosis of 10 ± 5.4 mm^2 and PDT with equimolar dosed pegylated mTHPC in 5.2 ± 4.6 mm^2. For both mTHPC formulations, the extent of tumor necrosis was significantly larger compared to control animals ($P = 0.02$). There was no significant difference in the extent of tumor necrosis between free mTHPC and pegylated mTHPC.

PDT-Related Phototoxicity

Inspection and photo-documentation of the treated areas on day 1, day 2, and day 3 after light delivery revealed a higher degree of skin alterations with a 0.15 mg/kg dose of free mTHPC than with an equimolar dose of pegylated mTHPC. This holds true for both treatment sites in all animals assessed (Figs 1 and 2). Histological assessment of the treated areas of the hind leg 3 days after light delivery revealed the absence of necrosis of the skin and underlying musculature for both mTHPC formulations on all animals assessed.

Fluorescence Signaling Assessment by Fluorescence Microscopy in Tumors and Skin

No fluorescence signaling was detected 3 days after administration of 0.15 mg/kg free and an equimolar dose of pegylated mTHPC. Fluorescence microscopic assessment after administration of 0.5 mg/kg free and equimolar dose of pegylated mTHPC revealed a heterogeneous distribution for both mTHPC formulations in the tumors confined to perivascular areas (Figs 3 and 4). Centrally localized and non-vascularized tumor areas did not reveal fluorescence signaling for both mTHPC formulations. Both mTHPC formulations showed a homogenous intracellular distribution in the cytoplasm and absent signaling from the nuclei in areas with photosensitizer captation (Figs 3 and 4).

Fluorescence intensity measurements revealed a mean autofluorescence background signaling of 57 ± 3 units for all tissues assessed. The recorded autofluorescence background was lower than the photosensitizer emission and comparable between different animals with respect to tumors and skin. Fluorescence intensity measurements 3 days after receiving 0.5 mg/kg free mTHPC administration revealed no significant difference between tumor and skin ($P = 0.2$) with a ratio of tumor to skin fluorescence intensity of 0.94. Animals receiving an equimolar dose of pegylated mTHPC resulted in a higher fluorescence intensity in tumors than in skin ($P<0.001$) with a tumor to skin intensity ratio of 1.73 (Fig. 5).

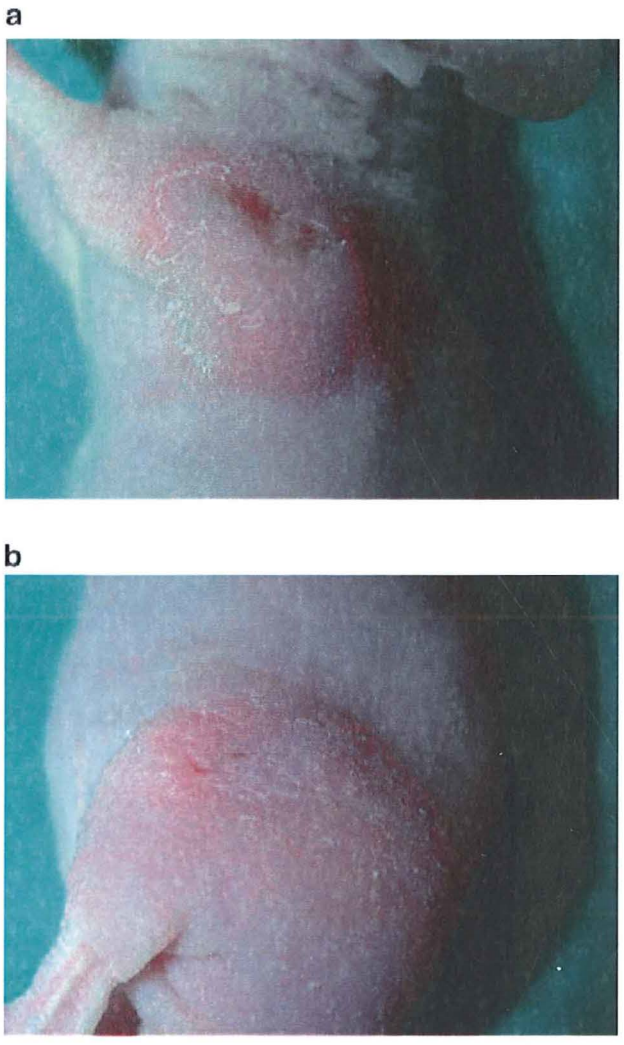

Fig. 1. PDT-related skin phototoxicity 3 days after photoirradiation with free mTHPC (0.15 mg/kg); (**a**) skin overlying the tumor; (**b**) skin overlying the hind leg.

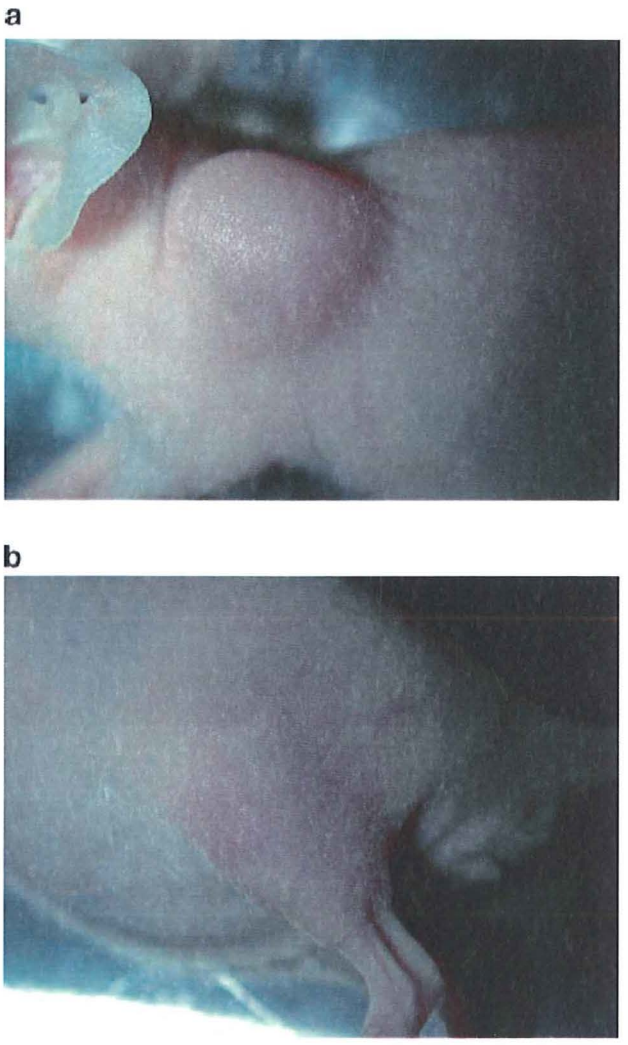

Fig. 2. PDT-related skin phototoxicity 3 days after photoirradiation with equimolar dose of pegylated mTHPC; (**a**) skin overlying the tumor; (**b**) skin overlying the hind leg.

DISCUSSION

PDT has emerged as a new treatment modality for superficially located tumors. It has also been investigated as an intraoperative adjunct following surgery in situations where a wide resection of tumor from otherwise healthy tissues is not feasible. This holds particularly true for malignant pleural mesothelioma. The tumor spreads along the pleural surfaces overlying the lung, the diaphragm, the chest wall, and the mediastinum and is characterized by a relentless local progression despite concerted treatment efforts including surgery, chemotherapy, and radiation [23–25]. Novel concepts for the treatment of mesothelioma have been investigated such as intraoperative PDT of the chest cavity following surgery, which has shown to be feasible and effective to a certain degree [26]. However, intraoperative PDT for mesothelioma was associated with substantial morbidity under clinical conditions with a hematoporphyrin derivative photosensitizer.

Efficacy and tumor selectivity of PDT depends on the photosensitizer, the treatment conditions, and the biological and photophysical properties of the target tissues [27]. New sensitizers such as mTHPC were assessed in order to optimize PDT for the treatment of mesothelioma. mTHPC has shown a powerful anti-tumor activity at relatively low drug and light doses and a fast reduction of skin photosensitivity in experimental and clinical settings. Additionally, the activation wavelength of 652 nm wavelength light penetrates tissue deeply [8]. However, intracavitary mTHPC-PDT of the chest cavity was associated with relevant side effects and normal tissue damage indicating poor selectivity under unfavorable treatment

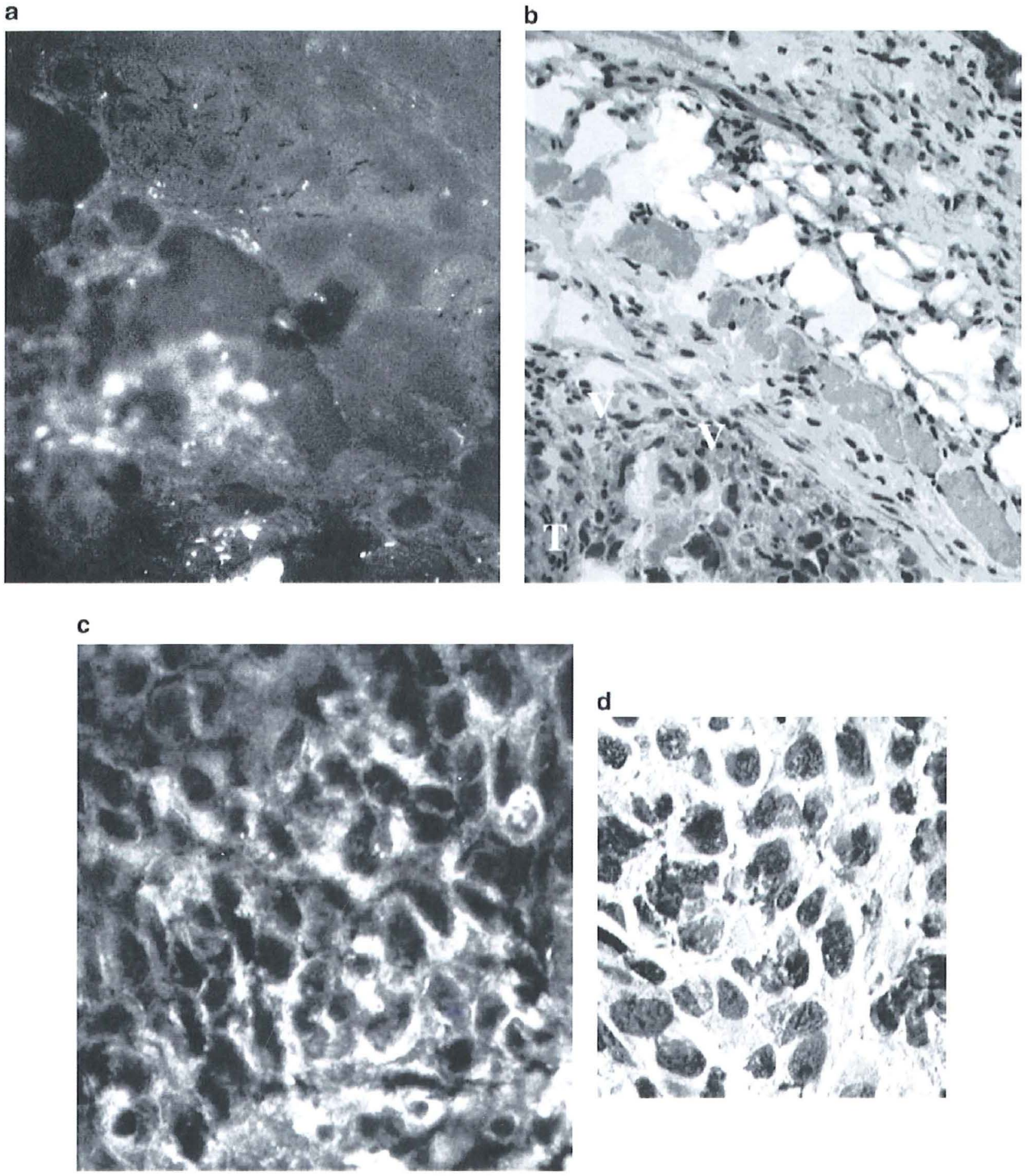

Fig. 3. Fluorescence photomicrograph showing the localization of free mTHPC in mesothelioma xenografts 3 days after i.v. administration (0.5 mg/kg); (**a**) heterogeneous photosensitizer distribution confined to perivascular areas and (**b**) same slice after H&E staining (T = tumor, V = vessels) (original magnification 400×); (**c**) homogenous intracellular localization in the cytoplasm with absent signaling from the nucleus in areas with sensitizer captation, and (**d**) same slice after H&E staining (magnification 400×).

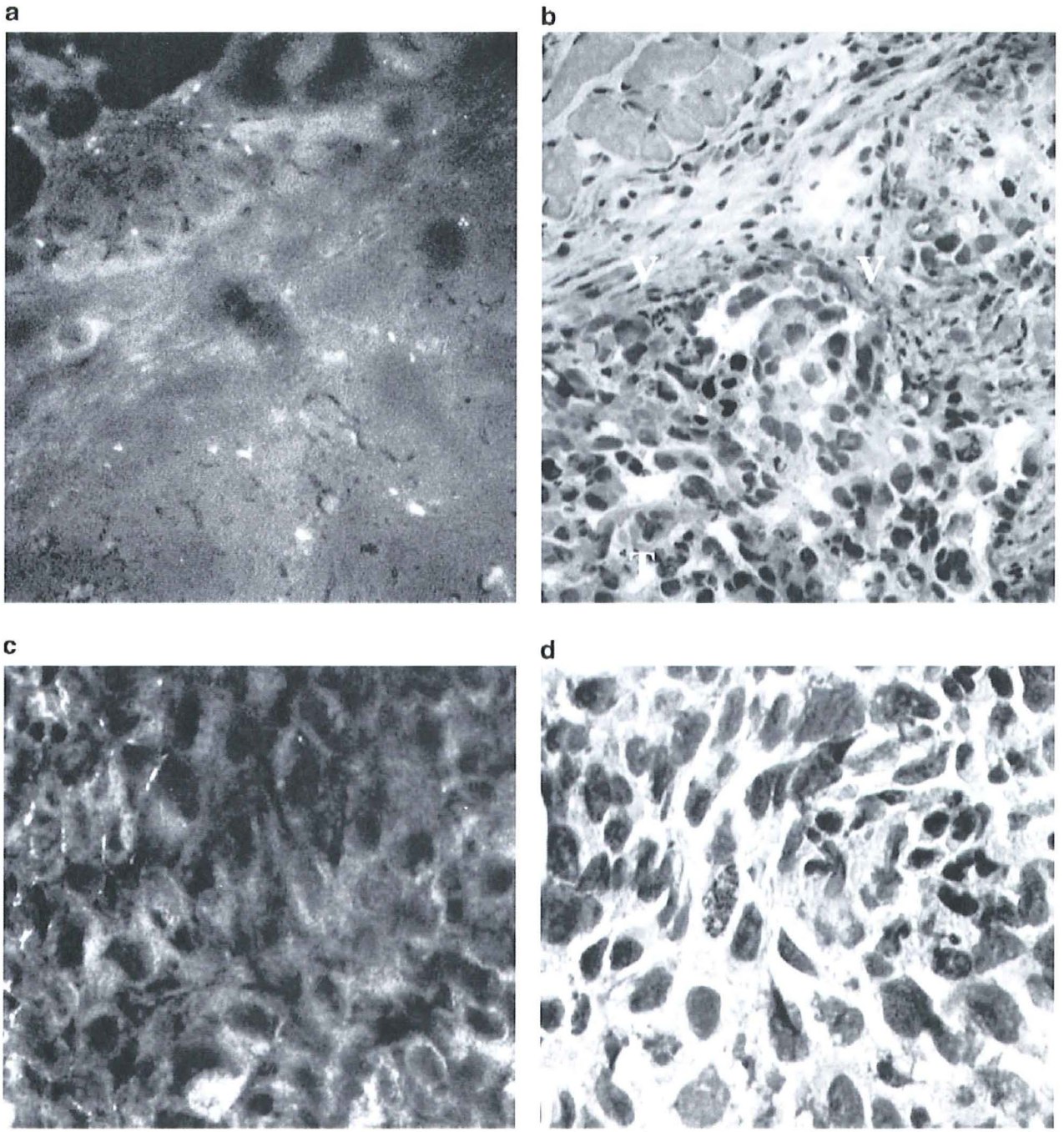

Fig. 4. Fluorescence photomicrograph showing the localization of equimolar dosed pegylated mTHPC in mesothelioma xenografts 3 days after i.v. administration; (**a**) heterogeneous photosensitizer distribution confined to perivascular areas and (**b**) same slice after H&E staining (T = tumor, V = vessels) (original magnification 400×); (**c**) homogenous intracellular localization in the cytoplasm with absent signaling from the nucleus in areas with sensitizer captation, and (**d**) same slice after H&E staining (magnification 400×).

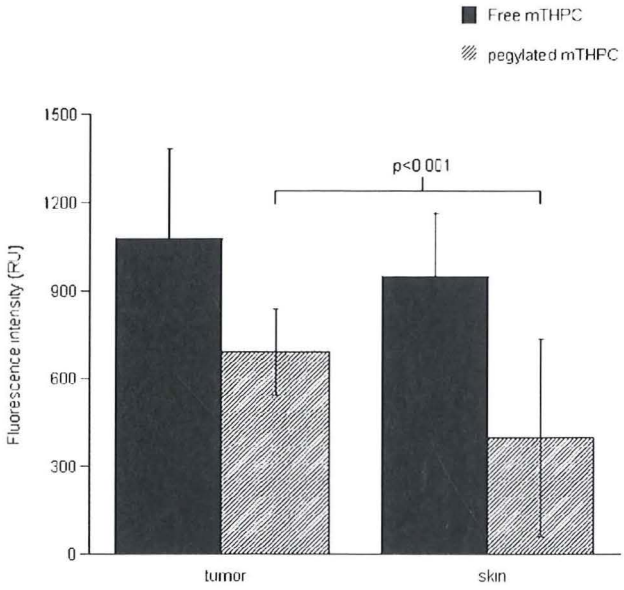

Fig. 5. Fluorescence intensity measurements 3 days after i.v. injection of free mTHPC (0.5 mg/kg) and an equimolar dose of pegylated mTHPC on the tumors and skin.

conditions [9–11,13,17]. In an attempt to increase poor selectivity of free mTHPC for tumor, macromolecular derivatives of mTHPC were arranged by linking hydroxyl (polyethylene glycol) chains of varying lengths to the photosensitizer (i.e., pegylation) [19,20]. Pegylation leads to increased water solubility of many drugs and increases plasma half life due to reduced kidney clearance and a decreased drug uptake by the reticuloendothelial system [28]. The prolonged drug circulation time and a better permeability of the tumor microvasculature for high molecular weight compounds are believed to enhance tumor drug uptake of pegylated compounds [29].

Several studies have compared the photodynamic activity of free and pegylated mTHPC on human mesothelioma xenografts [19,20,30] and have shown a similar extent of tumor necrosis but a lower degree of normal tissue damage with pegylated mTHPC when compared to an equimolar dose of free mTHPC. However, photosensitizer concentration in tissue measured by HPLC did not correlate with the photodynamic activity for both mTHPC formulations [15,19]. The difference in photodynamic activity for free and pegylated mTHPC may be related to a different spatial distribution of the photosensitizers in tumors and normal tissues which may not necessarily be detected by sensitizer concentration measurements. In contrast, fluorescence microscopy allows the localization of a photosensitizer in different tissue compartments by registration of the sensitizer's fluorescence signaling after activation. After recording the fluorescence images, the same slices are stained with H&E and compared with the fluorescence image from the same site in order to localize the sensitizer within the tissues. In addition, the relative fluorescence intensity can be measured which allows an estimation of the sensitizer distribution in different tissues.

Our results revealed a similar photodynamic activity in mesothelioma xenografts but a lower skin phototoxicity with pegylated mTHPC compared to equimolar dosed free mTHPC at a drug-light interval of 3 days. The extent of PDT-related tumor necrosis was significantly higher for both mTHPC formulations than in non-treated tumors but there was no significant difference between free and pegylated mTHPC for the treatment conditions applied. These results are consistent with the findings of previous studies [20]. Fluorescence microscopic analysis revealed no detectable fluorescence signaling at a dose of 0.15 mg/kg free or equimolar dose of pegylated mTHPC, but a heterogeneous fluorescence signaling for both mTHPC formulations in tumors confined to perivascular areas was detected at a dose of 0.5 mg/kg free or equimolar dose of pegylated mTHPC. Similar results have been described by other investigators who assessed the distribution of free and pegylated mTHPC in human adenocarcinoma xenografts [30]. The heterogeneous spatial distribution of free and pegylated mTHPC in xenografts may explain the findings of previous studies showing that for the same treatment conditions are applied, well-vascularized adenocarcinoma xenografts revealed a larger extent of mTHPC-PDT-related tumor necrosis than squamous cell carcinoma or mesothelioma xenografts which were poor in stroma and vessels [31,32]. Fluorescence intensity measurements after administration of free mTHPC (0.5 mg/kg) revealed no significant difference between tumor and skin whereas an equimolar dose of pegylated mTHPC resulted in significantly higher fluorescence intensity in tumors than in skin. Our results suggest that fluorescence intensity measurements may predict the photodynamic effect for both mTHPC formulations in tumors and skin but requires refinements in order to detect sensitizer fluorescence signaling at lower drug doses.

In conclusion, pegylated mTHPC revealed similar photodynamic activity but improved tumor selectivity versus free mTHPC. Fluorescence signaling measurements have the potential to predict the photodynamic activity for both mTHPC formulations.

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