Photodiagnosis and Photodynamic Therapy (2015) xxx, xxx–xxx

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/pdpdt

Studies of the photosensitizer disulfonated meso-tetraphenyl chlorin in an orthotopic rat bladder tumor model

Yan Baglo Ph.D. [a,*], Qian Peng [b], Lars Hagen [a], Kristian Berg [c], Anders Høgset [d], Finn Drabløs [a], Odrun A. Gederaas [a]

[a] *Department of Cancer Research and Molecular Medicine, Faculty of Medicine, Norwegian University of Science and Technology, N-7491 Trondheim, Norway*
[b] *Department of Pathology, The Norwegian Radium Hospital, Oslo University Hospital, Montebello, N-0310 Oslo, Norway*
[c] *Department of Radiation Biology, The Norwegian Radium Hospital, Oslo University Hospital, Montebello, N-0310 Oslo, Norway*
[d] *PCI Biotech AS, Strandveien 55, N-1366 Lysaker, Norway*

KEYWORDS
Photochemical internalization;
Photodynamic therapy;
Rat orthotopic bladder tumor model;
Non-muscle invasive bladder cancer;
Disulfonated meso-tetraphenyl chlorin

Summary
Background: Photochemical internalization (PCI) is a novel technology for the release of a therapeutic molecule from endocytic vesicles into the cytosol of a cell. The release of molecules occurs after activation of an endocytic membrane-embedded photosensitizer by light. In this study uptake and localization of the photosensitizer disulfonated tetraphenyl chlorin ($TPCS_{2a}$) were explored to optimize a PCI protocol in an orthotopic rat bladder tumor model.
Methods: Female Fischer F344 rats were intravesically instilled with 0.4×10^6 AY-27 transitional carcinoma cells before allowing tumor growth for 14 days. The photosensitizer $TPCS_{2a}$ was intravesically instilled at different concentrations, and bladders were excised after different time intervals. The retention, penetration, and localization of intratumoral $TPCS_{2a}$ were explored *ex vivo* using fluorescence spectroscopy and fluorescence microscopy to determine an optimal PCI protocol. These results were compared to histological analysis of necrotic areas after activation of intratumoral $TPCS_{2a}$ by red light (652 nm, 0.5 J/cm^2).
Results: A superficial distribution pattern of the photosensitizer $TPCS_{2a}$ was seen in bladder tumor tissue, and $TPCS_{2a}$ was almost cleared from the tumors after 72 h. The highest retention of $TPCS_{2a}$ was found at 24 h after instillation when using a concentration of 3 mg/ml.

* Corresponding author at: Department of Cancer Research and Molecular Medicine, Faculty of Medicine, NTNU Postbox 8905, N7491 Trondheim, Norway. Tel.: +47 72573333; fax: +47 72576400.
E-mail address: yan.baglo@ntnu.no (Y. Baglo).

http://dx.doi.org/10.1016/j.pdpdt.2014.12.005

Please cite this article in press as: Baglo Y, et al. Studies of the photosensitizer disulfonated meso-tetraphenyl chlorin in an orthotopic rat bladder tumor model. Photodiagnosis and Photodynamic Therapy (2015), http://dx.doi.org/10.1016/j.pdpdt.2014.12.005

Conclusion: An optimal PCI protocol was defined for the tumor model, including a 24-h $TPCS_{2a}$-to-light interval and a dose of 3 mg/ml $TPCS_{2a}$. This protocol will be utilized for the study of PCI-enhanced therapeutic effects on non-muscle invasive bladder cancer, using a potent chemotherapeutic under an optimal light dose.

Introduction

In Norway, bladder cancer has been one of the five most common cancer types for men during the past decade [1]. Approximately 70—80% of diagnosed bladder cancers worldwide are non-muscular invasive bladder cancer (NMIBC) consisting of carcinoma *in situ*, stage Ta and T1 tumors. NMIBC is normally removed by surgery during a cystoscopy under general anesthesia, known as transurethral resection of bladder tumors (TURBT). Unfortunately, the high rate of recurrence and progression after TURBT means that adjuvant treatments may usually be required, such as intravesical chemotherapy using mitomycin C, epirubicin, or doxorubicin and/or immunotherapy using Bacillus Calmette—Guérin (BCG) vaccine [2—5]. However, recurrence rates still remain high, and progression is not eliminated after the combined therapies. Side effects including bladder irritation, skin rash, frequency and pain are often seen with the chemo- and immunotherapy [6]. To improve the total intravesical agent uptake and hence chemotherapeutic efficacy, various experimental approaches have been evaluated, including enhancing the agent delivery to bladder tumor tissue using electromotive therapy and photochemical internalization (PCI), enhancing cell membrane permeability using intravesical hyperthermia [7—9]. Hexaminolevulinate-guided transurethral resection has been shown to significantly improve detection of Ta and T1 lesions and reduce recurrences [10]. In this study, photochemical internalization (PCI) [11] have been studied *in vivo* to enhance the effect of therapeutic agents for NMIBC.

PCI has been developed from photodynamic therapy (PDT) [12], but is designed to favor the delivery of a therapeutic molecule without inactivating the molecule or lethally damaging the cell. The cell killing effect therefore results predominantly from the therapeutic molecule, rather than the photodynamic treatment [13]. This is of particular importance for the delivery of genes and siRNA [14]. However, PCI is based on the same photochemical principles as PDT. PCI can be looked upon as a three-stage process. The first stage is the selective accumulation of an amphiphilic photosensitizer in the tumor endosomal or lysosomal membranes *via* endocytosis, in the absence of light. The second stage is the accumulation of a therapeutic molecule in the endocytic vesicles, also in the absence of light. In the last stage, the photosensitizer is activated by light of the appropriate wavelength and intensity. Using a sub-lethal photodynamic dose, activated photosensitizer evokes a response only in the endosomal and lysosomal membranes by reacting with molecular oxygen leading to membrane rupture and release of the molecules [11]. PCI could be combined with different therapeutic macromolecules or molecules with limited ability to penetrate the cell membrane, but PCI has also been shown to enhance therapeutic efficacy of some conventional cytotoxic therapies, with reduced side effects as the therapeutic site is limited to the illuminated area [15,16].

NMIBC is potentially well suited for effective treatment by PDT or PCI because it is easily accessible for both intravesical instillation and illumination. In addition, it is possible to irradiate the whole bladder and hence provide access to multifocal tumors [17]. As no difference in light penetration is observed between tumor and normal bladder tissue, the photodynamic responses upon illumination of the bladder will depend on the localization and accumulation of the photosensitizer within the tumor [18]. In this study, the potent photosensitizer disulfonated tetraphenyl chlorin ($TPCS_{2a}$) was utilized to explore its tissue localization and accumulation in a rat bladder tumor model [19—22], to establish an optimal protocol for the first stage of PCI.

$TPCS_{2a}$ is an amphiphilic chlorin produced from disulfonate tetraphenyl phorphyrin ($TPPS_{2a}$) [15,23]. The absorption spectrum of $TPCS_{2a}$ has been investigated in organic media and shown to be only slightly affected by the properties of the organic solvents. It is observed that $TPCS_{2a}$ has absorption maximum at 650—653 nm and emission maximum at 654—657 nm in organic solvents [24,25]. As a fluorophore, $TPCS_{2a}$ is found to have stronger light absorption in the red wavelength region and to be more efficient for *in vivo* applications than its corresponding porphyrin $TPPS_{2a}$ [23—27]. $TPCS_{2a}$ was approved for use in clinical studies in 2008, and a phase I clinical trial of $TPCS_{2a}$-mediated PCI of bleomycin in head-and-neck cancer showed no severe side effects associated with the treatment. The efficacy and safety of the modality are currently being evaluated in a phase II clinical intervention trial [28,29]. In animal studies, $TPCS_{2a}$-mediated PCI of the chemotherapeutic agent bleomycin has been investigated as an adjunct to radiotherapy and surgery by Norum et al. [30,31], showing that PCI induces synergistic therapeutic effects in a human sarcoma model. PCI is also proposed to have potential competence to bypass the development of drug resistance induced by repeated exposure to therapeutics [15]. Very recently, PCI-enhanced vaccination using $TPCS_{2a}$ was demonstrated to strongly increase stimulation of CD8 T-cell responses preventing tumor growth in a mouse skin tumor model [32]. With rapid development of $TPCS_{2a}$ usage in PCI, it has become of great interest in the possible treatment of bladder cancer [7,9].

Materials and methods

Cell suspension

Rat bladder transitional carcinoma cells (AY-27) [33] were maintained in the same RPMI-1640 culture medium and conditions as described in our earlier study [34]. Immediately

Please cite this article in press as: Baglo Y, et al. Studies of the photosensitizer disulfonated meso-tetraphenyl chlorin in an orthotopic rat bladder tumor model. Photodiagnosis and Photodynamic Therapy (2015), http://dx.doi.org/10.1016/j.pdpdt.2014.12.005