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Elimination of primary tumours and control of metastasis with rationally designed bacteriochlorin photodynamic therapy regimens

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Abstract Photodynamic therapy (PDT) with current photosensitisers focuses on local effects and these are limited by light penetration in tissues. We employ a stable near-infrared (NIR) absorbing bacteriochlorin with ca. 8 h plasma half-life to increase the depth of the treatment and elicit strong systemic (immune) responses. Primary tumour growth delays and cures of BALB/c and nude mice bearing CT26 mouse colon carcinoma are related to the parameters that control PDT efficacy. The systemic anti-tumour protection elicited by the optimised PDT regimen is assessed by tumour rechallenges and by resistance to the establishment of metastasis after intravenous injection of CT26 cells. The optimised treatment regime offered 86% cure rate in BALB/c mice but no cures in BALB/c nude mice. Cured mice rechallenged over 3 months later with CT26 cells rejected the tumour cells in 67% of the cases. PDT of a subcutaneous CT26 tumour 5 days after the additional intravenous injection of CT26 cells very significantly reduced lung metastasis. The PDT regimen optimised for the bacteriochlorin leads to remarkable long-term survival rates, effective immune memory and control of lung metastasis.

1. Introduction

Photodynamic therapy (PDT) is a promising cancer treatment owing to its selectivity and absence of adverse drug reactions [1]. PDT is based on the photosensitiser administration, its accumulation in tumours and then illumination with light. Photosensitisers absorbing light

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in the NIR, where tissues have higher optical penetration depths ($\delta = 2.3$ mm at 750 nm) [2], increase the treatment depth. Excited photosensitiser molecules transfer energy or electrons to oxygen leading to singlet oxygen or hydroxyl radicals [3,4], respectively, that trigger various biological mechanisms (vascular shutdown, [5,6] apoptosis/necrosis of tumour cells [7,8] and immunogenic cell death [5,9]) eventually leading to tumour remission.

Photosensitisers characterised by long plasma half-lives, such as temoporfin, $t_{1/2} = 45.4$ h, are prescribed with drug-to-light intervals (DLIs) of 4–6 days [10,11]. Long exposure to temoporfin is associated with high tumour selectivity but prolonged skin photosensitivity. The period of photosensitivity is reduced using verteporfin ($t_{1/2} = 5$–6 h) [12]. Verteporfin first used in age-related macular degeneration (ARMD) is currently in clinical trials on pancreatic cancer [13]. Verteporfin is irradiated at DLI = 15 min in ARMD or 60–90 min in pancreatic cancer treatments. Table 1 presents this and other factors that contribute to the treatment outcome. Finding the best combination of drug dose, light dose, DLI, radiant exposure R, irradiance E and tumour margin is crucial for primary tumour destruction. Additionally the PDT protocol may determine antitumour immune responses [14]. Thus, the success of PDT depends on the development of photosensitisers and treatment regimens.

We recently described a photostable bacteriochlorin (redaporfin) with intense infrared absorption, high yield of ROS generation, high phototoxicity [15], low skin photosensitivity and favourable pharmacokinetics [16,17]. This work uncovers relationships between PDT regimens, cure rates, antitumour immune memory and resistance to metastasis using redaporfin. Our results supported to regulatory approval to conduct a phase I/II clinical study of redaporfin (ClinicalTrials.gov identifier: NCT02070432).

Table 1
Factors that limit the range of the parameters controlled in photodynamic therapy (PDT).

Parameter	Lower limit range	Higher limit range
Drug-to-light interval (DLI)	Selectivity	Drug clearance
Irradiance	Sub-lethal damage	Oxygen depletion
Light dose	Depth of treatment	Photosensitiser bleaching
Drug dose	Photosensitiser bleaching	Inner filter
Margins	Re-supply of nutrients	PDT-induced lethality

2. Materials and methods

2.1. Chemicals and Cells

Bacteriochlorin (F_2BMet or redaporfin) was recently described [15]. CT26 colon carcinoma cells lead to subcutaneous tumours in BALB/c mice that are considered minimally to moderately immunogenic tumours [18]. Female 8–10 week old mice (17–22 g) were organised in groups as shown in Table 2. Details on drug formulation, cell cultures and mice are presented in Supplementary Materials.

2.2. Antitumour immune memory

Mice cured with the optimised PDT protocol were subcutaneously rechallenged with 350,000 CT26 cells in the contralateral thigh more than 90 days after the treatment. An age-matched group of BALB/c mice with CT26 tumours was subjected to surgery. The mice that after the surgery remained tumour free >90 days and an age-matched control group of naive BALB/c mice were inoculated with 350,000 CT26 cells.

2.3. Lung metastasis

BALB/c mice were subcutaneously inoculated with 350,000 CT26 cells and 7 days later 500.000 CT26 cells were injected in the tail vein. On day 12, one group with subcutaneous tumours was submitted to the optimised PDT regimen, and 11 days later all the mice were sacrificed, the lungs were harvested, fixed with Bouin's solution, weighted and the metastases were counted by two researchers.

2.4. Immunohistochemistry

Four micrometer paraffin slices from tumours were deparanized and hydrated. Antigen retrieval was done in 0.1 M citrate buffer upon microwave treatment. Samples were blocked with 10% goat serum and incubated, overnight at 4 °C, with a CD3 antibody (Dako). After washing, sections were incubated with anti-rabbit EnVision + System-HRP Labelled Polymer (Dako), revealed with 3,3′-diaminobenzidine (DAB), counterstained with Harris' Haematoxylin and examined by light microscopy.

3. Results

3.1. Intermediate DLI have low phototherapeutic indexes

Table 2 reveals that the protocol parameters tested for vascular-PDT (DLI = 0.25 h) covered observations ranging from the absence of cures to 100% PDT-induced lethality. High light doses (>70 J) associated with large drug doses ($\geqslant$0.75 mg/kg) delivered to large areas (>1 cm^2) led to lethality within the two days after treatment. For comparable doses (1.5 mg/kg, 78 or 95 J), PDT-induced lethality and efficacy decreased as

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