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Comparative study of two kinds of repeated photodynamic therapy strategies in breast cancer by using a sensitizer, sinoporphyrin sodium

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ABSTRACT

Sinoporphyrin sodium (DVDMS) is a newly identified photosensitizer that was isolated from Photofrin. Experimental and clinical results have demonstrated that repeated application of PDT greatly improved the therapeutic efficacy. Here, we comparatively studied two kinds of photodynamic therapy (PDT) strategies by using DVDMS (2 mg/kg) in murine breast cancer 4T1 xenograft model to provide evidence which strategy exerts a better antitumor effect. Regimen (1): DVDMS was injected one time into tumor-bearing mice, which were then repeatedly exposed to 50 J/cm^2 light 24 h, 30 h and 36 h later. Regimen (2): DVDMS was injected 3 times and mice exposed to 50 J/cm^2 light 24 h after each injection, with 5 days intervals between each DVDMS injection. On day 21 after the tumor cell injection, in regimen (1) the tumor volume inhibition ratio was reached to 85.75 ± 7.60%. While at the same day the inhibition ratio was 65.74 ± 8.64% of regimen (2). Additionally, regimen (1) appeared to more effectively initiate tumor tissue destruction and cancer cell apoptosis, inhibit lung metastasis, suppress cancer cell proliferation and angiogenesis. Moreover, no obvious effect on body weight and other side effects were observed in the treated mice. These results suggest that regimen (1) might be a potentially efficient strategy against breast cancer.

1. Introduction

Photodynamic therapy (PDT), a non-ionizing, minimally invasive modality for cancer treatment, has shown good therapeutic effects on a variety of tumors [1–4]. PDT consists of the intravenous administration of a photosensitizer, which preferentially localizes within the tumor, followed by activation with a specific wavelength of light [5]. Activation of the photosensitizer causes the conversion of molecular oxygen into various reactive oxygen species (ROS) that directly induce the death of the tumor cells or damage the tumor-associated vasculature [6]. Compared with other standard treatment, such as surgery, radiation therapy and chemotherapy, which may lead to significant side effects and drug resistance, PDT is essential to focus on innovative therapeutic research [7]. What is more, PDT has a lower systemic toxicity because irradiation and activation occur only at the tumor site [6,8].

Photosensitizers are critical components of PDT. One of the earliest clinical porphyrin-based photosensitizers is Photofrin® (PF). It has been approved by the FDA for use as a sensitizer in PDT of cancer and is the most widely used photosensitizer thus far. However, PF is known to be a mixture of hematoporphyrin in which porphyrin molecules are linked by ether, ester, and C–C bonds [9]. In addition, the patients should be warned to avoid exposure of skin and eyes to direct sunlight or bright indoor light for at least 30 days following injection with PF. Many investigators have synthesized dimeric or trimeric porphyrin photosensitizers to seek for the more ideal photosensitizers [10–15]. Pandey et al. have synthesized certain porphyrin dimers with ester or ether linkages and found that the dimers have better tumoricidal activity than PF [10]. However, these compounds were found to be unstable at RT either as solids or in solution. The weakness resulted in these agents couldn't be clinically applied.

Recently, Sinoporphyrin sodium, referred to as DVDMS (Fig. 1A), is a newly identified photosensitizer that was isolated from PF. This was in accordance with the study that the active fraction of photofrin is mainly porphyrin dimers or oligomers [16–18]. DVDMS was prepared from DVDME (porphyrin dimer esters) by hydrolyzing it in alkaline solution. The chemical structure of DVDMS was ascertained by Cold Spray-MS and HR-ESI-MS. The purity was monitored by HPLC. DVDMS has gained China's independent intellectual property [19]. The novelty of DVDMS has been approved by the State Intellectual Property Office of the People's Republic of China (a specialized and authoritative organization about intellectual property). DVDMS shows highsolubility in water, clear chemical structure, 98.5% chemical purity and results in relatively short-time skin sensitivity [19]. These characteristics give a lot of convenience in its clinical PDT. Previously, DVDMS was found to generate high singlet oxygen production and selectively accumulate in tumor cells and tumor tissue, both of which are necessary for targeted therapy [20–22].

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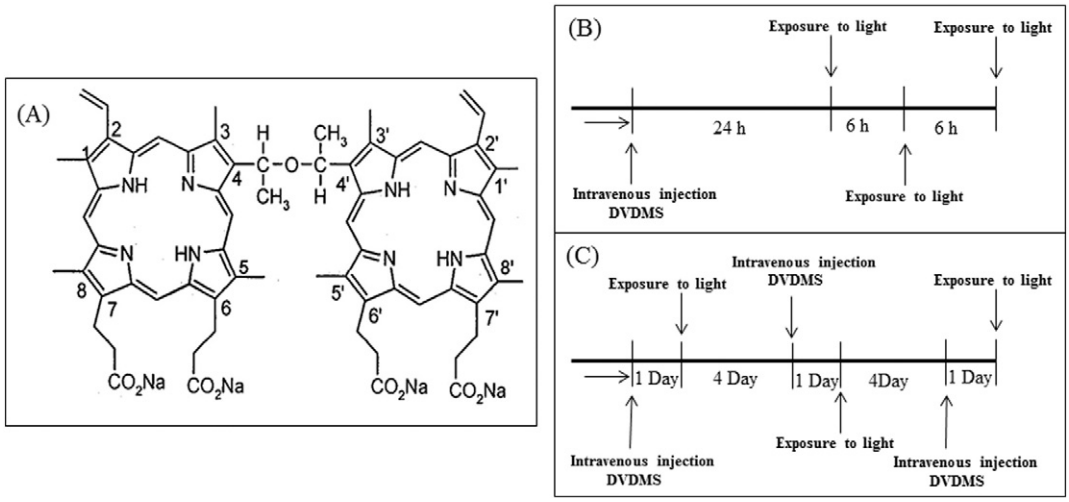

Fig. 1. (A) The chemical structure of DVDMS. (B) The schedule of frequency photodynamic therapy. (C) The schedule of versus photodynamic therapy. Tumor bearing mice (on 5 days after tumor implantation) were injected with DVDMS (2 mg/kg) by tail vein, 635 nm light was delivered to the tumor with fluencies of 50 J/cm^2 at 24 h post injection.

Furthermore, DVDMS-mediated PDT was preliminarily confirmed to exhibit stronger tumoricidal activity than PF-PDT in S180, H460-tumor bearing mice [23]. These indicate that as a good sensitizer, DVDMS is a potential candidate for PDT clinical usage.

Unlike traditional radiotherapy, PDT uses non-ionizing irradiation and can be administered repeatedly without cumulative long-term complications. Clinical and experimental results have demonstrated that repeated applications of PDT effectively improved therapeutic effect [24–28]. Moreover, DVDMS mediate repeated PDT has not been carefully investigated. Therefore, we design two strategies of repeated PDT in this study (Fig. 1). In our previous study, we found that the pharmacokinetics of DVDMS in tumor reached peak at 12 h, and at 24 h the DVDMS concentration was about 95% of the peak in the tumor [29]. At 48 h and 72 h, the concentration of DVDMS decreased to a low level. The pharmacokinetics of DVDMS in normal organs reached peak in the first 2 h, then decreased rapidly in the next 22 h. At 24 h, the DVDMS concentration in these organs all decreased to 50% or lower to their corresponding peak. In order to have a therapeutic effect on tumors (high concentration in tumors) and to minimize side effects on healthy tissues (low concentration in surrounding healthy tissues), 24 h after DVDMS administration might be an appropriate time point for light radiation. More than that, we found that at 30 h and 36 h the concentration of DVDMS arrived approximately at 85% and 80% of the maximum concentration in tumor. Therefore, it is possible that multiple light exposures using DVDMS within 36 h after injection might enhance treatment efficacy. Namely is our regimen (1) in the paper. Fang et al. has reported that the skin side toxic effect disappeared after 96 h post DVDMS administration, implying that exceed 4 days intervals between each PDT wouldn't produce the interference effect. Additionally, our previously investigated PDT treatment in 4T1 tumor bearing mice found that after PDT irradiation tumor site gradually appeared scab (the damaged tumor cells formed). The scab would defulvium after 5–6 day post PDT when we almost couldn't touch the tumor shape. While after the scab falls off, the residual tumor cells started to grow. Therefore, we choose 5 day intervals between each DVDMS injection, in regimen (2).

We focused our investigation on comparing the anti-tumor effect of DVDMS combined with two PDT strategies to provide evidence which strategy had a better antitumor effect in 4T1 xenograft model. The BALB/c-derived mouse mammary carcinoma cell line 4T1 was used. The cancer cells share numerous characteristics with human mammary carcinoma, including immunogenicity, growth characteristics, and metastatic properties. One strategy is single-injection and in the short term with multiple light exposure. Another is in the long-term with multiple-injections plus multiple light exposure. What's more, we examined the tumor metastasis, proliferation, apoptosis, and angiogenesis to explore the possible antitumor mechanisms. To the best of our knowledge, the present study is the first in vivo preclinical study that assesses the anti-tumor effects of DVDMS combined with repeated PDT. These findings may have important implications for DVDMS application in clinical PDT therapy.

2. Materials and Methods

2.1. Chemicals

Sinoporphyrin sodium (DVDMS, molecular formula: $C_{68}H_{66}N_8O_9Na_4$, Molecular weight: 1230.265) was kindly provided by Professor Qicheng Fang from the Chinese Academy of Medical Sciences (Beijing, China). It has a purity of 98.5%. It was dissolved in a physiological saline solution to a final storage concentration of 1.25 mg/ml and was stored in the dark at −20 °C. The chemical structure of DVDMS is shown in Fig. 1A.

2.2. Reagents

Primary antibodies against proliferating cell nuclear antigen (PCNA) and CD34 were purchased from Abcam (Cambridge, UK). Secondary antibodies were obtained from Zhong Shan Golden Bridge Biotechnology (Beijing, China). The terminal deoxynucleotidyl transferasemediated dUTP nick-end labeling (TUNEL) assay kit was purchased from Roche (Basel, CH).

2.3. Tumor Cell Lines

The 4T1 murine breast cancer cell line was obtained from the Department of Basic Medicine, Union Medical College, Beijing, China. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco, Life Technologies, Inc.) supplemented with 10% fetal bovine serum (FBS, Hyclone, USA), 100 U/ml penicillin, 100 μg/ml streptomycin, and 1 mM L-glutamine. Cultures were maintained at 37 °C with humidity and 5% CO_2.

2.4. Animals

The BALB/c mice (female, 18–20 g body weight) were supplied by the Experimental Animal Center of Fourth Military Medical University (FMMU) (Xi'an, China). They were housed in an air-conditioned room at 23 °C ± 2 °C with free access to food and water and were maintained