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Evaluation of the effects of systemic photodynamic therapy in a rat model of acute myeloid leukemia

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ABSTRACT

Systemic PDT (SPDT) approach is developed to treat a variety of hematological diseases, including cancers and blood-borne infections. We evaluated the efficacy of an SPDT method for treating leukemia using a Brown Norway myeloid leukemia (BNML) rat model with the LT12 cells engineered to express GFP. The survival times of animals receiving SPDT at 5 (early-SPDT) and 10 (mid-SPDT) days post-LT12 injection were prolonged by 2 days, the rats in the late-SPDT group (15 days) exhibited a 6-day increase in life span ($p < 0.05$). The percentages of GFP-LT12 cells in the bone marrow of the late-SPDT rats decreased from 61.6% to 56.5% on day 17. Likewise, there was a decrease in the serum expression levels of IL-1β, IL-10, TNF-α, and IFN-γ in the late-SPDT rats ($p < 0.05$). Our findings indicate that SPDT could be an effective method for the treatment of leukemia, and that antitumor immunity may play a key role in this process.

1. Introduction

Classic photodynamic therapy (PDT) is widely used for treating tumors on the surface of skin or body cavity, including tumors of the skin, head, and neck areas, as well as solid tumors that can be approached with relative ease, such as tumors of the esophagus, lungs, larynx, and uterine cervix [1–3]. This therapeutic approach involves the administration of a tumor-localizing photosensitizer (PS), by intravenous injection or external application, and the subsequent targeted activation of this photosensitizer by exposure to light of a specific wavelength. However, as a result of limitations regarding the depth of light penetration into human tissues, classic PDT is considered to carry out technical improvements for the treatment of hematological diseases, including systemic blood-borne diseases such as HIV and Ebola virus infections and hematological cancers including leukemia, malignant lymphoma, and metastatic cancer, which are difficult to treat despite the availability of chemotherapy and radiotherapy. Some researchers have therefore aimed to develop new PDT approaches such as systemic PDT (SPDT) to treat these hematological diseases. These SPDT approaches employ one of two possible modes of irradiation: irradiation of blood via extracorporeal circulation or internal irradiation of the circulation system via implantation of an optical fiber into a blood vessel. Due to the ease of operation, the latter approach has been utilized previously for treating hematological diseases. For example, Ahn et al. [4] demonstrated that optical fiber implantation-mediated SPDT resulted in increased survival rates in a leukemia animal model. However, there are several disadvantages associated with the internal irradiation approach, including a small lighting area, less precise lighting times, and unstable PS concentration [5–8]. While the irradiation of extracorporeal blood could overcome these problems, there have been no reports that have examined the efficacy of this method.

In a previous study, our team developed an SPDT approach for irradiating blood during extracorporeal circulation. For this method, the animals are subjected to extracorporeal circulation bypass and administered a PS by continuous intravenous infusion to obtain a stable blood drug concentration. The blood in the extracorporeal circulation bypass is then irradiated ex vivo with low doses of light using a specifically designed chamber. Using this approach, the blood targets gained enough PS-uptake time and targets incubated PS gained enough irradiation during extracorporeal circulation by the controlled flow speed and irradiation time. In a previous study, we evaluated the safety of this procedure in blood parameters and in the structure and function of major organs in rabbits [9]. In the current study, we studied the efficacy of our SPDT method in a rat leukemia model.

Abbreviations: BNML, Brown Norway myeloid leukemia; FCM, flow cytometry; GFP, green fluorescent protein; HMME, hematoporphyrin monomethyl ether; PS, photosensitizer; PTD, photodynamic therapy; SPTD, systemic photodynamic therapy; WBC, white blood cells.

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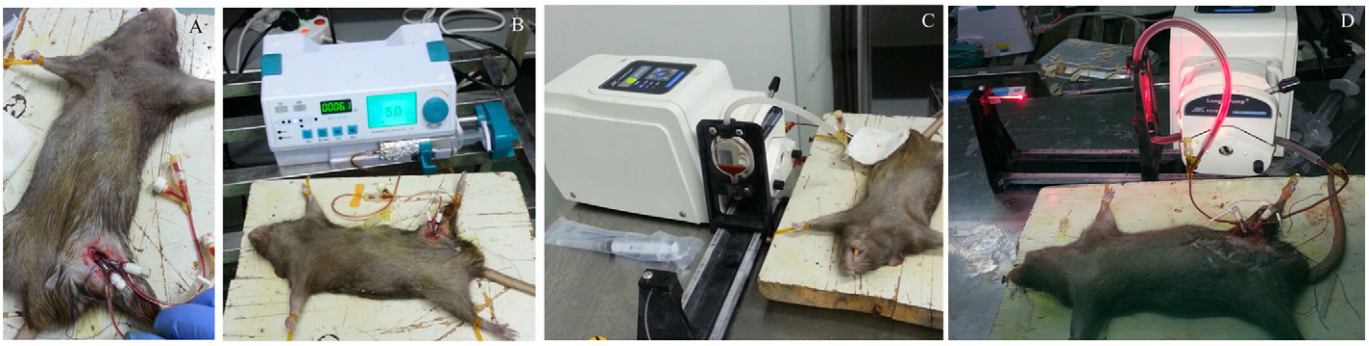

Fig. 1. Depiction of the systemic photodynamic therapy (SDPT) procedure as described in 2.4. (A) Vessel intubation. (B) Administration of HMME (C) Construction of extracorporeal circulation pathway. (D) Irradiation.

2. Materials and Methods

2.1. Cell Lines

The LT12 rat nonimmunogenic acute promyelocytic leukemia cell line, an immortalized cell line derived from Brown Norway myeloid leukemia (BNML) tissue, was a generous gift from Dr. Anton Martens (University Medical Center Utrecht, The Netherlands). The 293 T kidney cell line was purchased from the American Type Tissue Culture Collection (ATCC, Manassas, VA, USA).

2.2. Construction of the GFP-LT12 Cell Line

The 293 T cells were maintained in DMEM medium containing 10% fetal bovine serum, while the LT12 cell line was maintained in RPMI 1640 medium containing 10% fetal bovine serum. Both cell lines were cultured at 37 °C with 5% CO_2. To generate the GFP reporter cell line, 293 T cells were first transfected with the pLenti CMV GFP Puro lentiviral vector and the packaging plasmids pCM-VSV-G, pMDLg/pRRE, and pRSV-REV. The resulting GFP-expressing lentiviruses were harvested from the culture medium and used to infect LT12 cells. The infected cells were then subjected to puromycin selection to generate a stable BNML GFP-reporter cell line. This cell line is hereafter referred to GFP-LT12.

2.3. Development of the Brown Norway Myeloid Leukemia Rat Model

Inbred male Brown Norway (BN/Bi) rats bred under specific pathogen-free conditions were purchased from Vital River Laboratories (Beijing, China) and were used at 12–16 weeks of age (body weight 200–250 g). Rats were housed in plastic cages with a 12 h light/dark cycle and had access to purified water and breeding food. To establish the BN rat syngeneic model of AML, 5×10^5 GFP-LT12 cells were suspended in 500 μL phosphate buffered saline (PBS) and introduced into the animals by tail vein injection. The time of injection was considered day 0. After injection, 300 international units (IU)/mL roxithromycin and 320 IU/mL gentamicin were added to the animals' drinking water to prevent bacterial infection. All experiments were approved by the local animal welfare committee.

2.4. Systemic Photodynamic Therapy Protocol

The SPDT protocol is summarized in Fig. 1. Rats were anesthetized via intraperitoneal injection of 10% chloral hydrate (0.35 mg/kg of body weight), the surgical site was prepared, and disposable indwelling needles were inserted into the femoral artery and vein and fixed, respectively. The PS hematoporphyrin monomethyl ether (HMME) was then administered through the venous pathway as follows: an initial HMME loading dose of 5.2 mg/kg body weight was injected, followed by the administration of the PS via an intravenous (iv) infusion pump at a rate of 4.9 mg/h for 30 min. At this moment, the stable concentration of PS at 20 μg/ml in blood system was achieved. The doses two-step PS delivery was calculated according table 1 in our published paper [10] based on the intravenous infusion of drugs (three compartment models). After PS administration, the extracorporeal circulation pathway was constructed by connecting high-pressure sterilized silicone tubes to both ends of a sterilized quartz irradiation chamber that was customized in our laboratory. Irradiation chamber is flat cylindrical, 37.5 mm in diameter, 3 mm in high, so the volume is 3312 mm^3 and blood volume is about 3 ml. The two silicone tubes were then connected to the indwelling needles inserted into the femoral artery and vein, respectively, and the silicone tube near the artery was fixed to a peristaltic pump. The tubes were filled with saline and the pump was initiated. The blood was pumped from the femoral artery, through the saline-filled circulation bypass, and subsequently back into the body through the femoral vein at a speed of 1 mL/min (controlled by pump). So the blood in the chamber could be irradiated for 3 min. The blood within the irradiation chamber was irradiated outside immediately after the extracorporeal circulation pathway was prepared using a 630-nm semiconductor laser with a 0.44-μm quartz fiber and a power density of 40 mW/cm^2 for a total of 30 min. Illuminance area is about 10 cm^2, just covering the bottom of the chamber. After irradiation, the femoral artery and vein were tied off, the bypass was removed, and the wound was stitched. Rats were returned to normal feeding after recovery from the anesthesia described above, and were provided penicillin (48,000 IU) via abdominal injection for 3 days to prevent infection.

2.5. Safety Experiments

Six healthy BN rats were treated by SPDT, as described above. Peripheral venous blood samples were collected both before and immediately after surgery, and again at days 1, 3, and 7 post-surgery. Red blood cell, white blood cell (WBC), platelet, and hemoglobin counts were then evaluated using a whole blood analyzer, while liver and kidney functions were assessed by measuring the blood levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, γ-glutamyl transpeptidase, uric acid, blood urea nitrogen, and creatinine using an automatic biochemical analyzer. All animals were sacrificed by cervical dislocation at 7 days post-SPDT. The heart, liver, spleen, lungs, and kidneys were removed from each animal within 10 min and fixed by incubation in 10% formalin for 1 week at room temperature. After fixation, the specimens were subjected to conventional dehydration, paraffin embedding, and sectioning, followed by hematoxylin and eosin (HE) staining. Sections were visualized using an Olympus CX31 light microscope (Olympus, Tokyo, Japan) and photographic