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Inhibitory and immunological effects induced by the combination of photodynamic therapy and dendritic cells on mouse transplanted hepatoma

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ABSTRACT

Objective: To investigate the anti-tumor and immune efficacy of photodynamic immune-therapy (PIT), the combination of photodynamic therapy and dendritic cells (DC), on murine Heps hepatoma.
Methods: DCs were derived from syngeneic mouse bone marrow and then labeled with DAPI in vitro. The hepatoma model was established by subcutaneous inoculation with Heps cells in one hundred and twenty-eight mice. They were then divided into four groups at random: control group, PDT group, DC group and PIT group. Tumors in the control group were injected with normal saline. Mice in the PDT group were injected with the photosensitizer Deuteporfin 24 h before irradiation. Mice in the DC group were injected with DAPI labeled dendritic cells intratumorally. Mice in the PIT group were further given an injection of DCs after photoirradiation. Tumor growth and survival time were recorded after treatment. Fluorescence of tumor draining lymph nodes was evaluated under fluorescence microscope. Cytotoxic activity of splenocytes was tested by standard lactate dehydrogenase (lactate dehydrogenase, LDH) release assay.
Results: (1) Tumor growth was significantly slowed down in the PDT and PIT group compared to the control group ($P < 0.01$). (2) The mean survival time was significantly prolonged in the PDT and PIT group. (3) The number of fluorescent cells in the draining lymph nodes from DC group was higher than that of the PIT group. (4) The anti-tumor activity of splenocytes in the PDT and PIT group was significantly higher than that of the DC and control groups ($P < 0.01$, $P < 0.01$).
Conclusions: The present study suggests that PDT can inhibit tumor growth and induce anti-tumour immune response. The combination of PDT induced by Deuteporfiin and dendritic cell is capable of amplifying the antitumor immune response.

1. Introduction

Photodynamic therapy (PDT) is a promising treatment of various malignant and nonmalignant disorders. It's a two-step procedure involving the local or systemic administration of a photosensitizer, followed by illumination of the neoplastic lesion with a light of appropriate wavelength able to trigger photochemical reactions that lead to the generation of singlet oxygen and other reactive oxygen species (ROS) [1]. PDT-based anti-tumour effects are multifactorial. PDT has an direct affect on cancer cells, inducing cell death by necrosis and/or apoptosis [2]; PDT also has an affect on the tumour vasculature, causing tumor ischemia [3,4]. PDT also has an immunotoxicity effects towards tumour-infiltrating immune cells and rapid recruitment and activation of immune cells that can facilitate development of anti-tumour adaptive immunity [1,5,6].

A large body of evidences affirm that the outcome of tumor PDT is critically dependent on the contribution from the host [7]. Foremost, the PDT can stimulate both the innate immune response and adaptive immune response. The PDT-induced host response is instigated and promoted by an extensive release/expression of various pro-inflammatory mediators from the treated site including complement proteins, heat shock proteins, cytokines and chemokines, and arachidonic acid metabolites [8,9,10,11]. The key elements of innate immune system, the complement system and Toll-like receptors, become engaged in sensing PDT-generated altered self -danger signals and propagating the ensuing inflammatory and

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immune responses [7,11]. The innate immune effectors participating in tissue destructive action in PDT-treated tumors include the components of activated complement system, neutrophils, mast-cells, macrophages and natural killer cells [8,12,10]. The activity of these elements of innate immunity culminates in the orchestration of the development of adaptive immune response based on the recognition of antigens of PDT-treated tumors. The recovery of immune memory cells from distant lymphoid sites underlying the existence of long -lasting systemic immunity raised against even poorly immunogenic PDT-treated tumors [13,14].

Further development of PDT application in cancer treatment includes the establishment of strategies in combination with other anticancer therapies. Because of the inflammatory/immune response triggered by PDT, this therapy was shown to be particularly suitable for combining with various types of immuotherapy [15]. The combination of PDT with the cytokines, immun-cells, adjuvants, immune response modifier can be effectively promoted the host anti-tumour adaptive immunity [16]. Dendritic cell (DC) is the strongest Antigen presenting cell(antigen presenting cell, APC) in human body, it play an important role in anti-tumor immunity [17]. In this study, we investigate the anti-tumor and immune efficacy of the combination of photodynamic therapy and intratumoral injection of DCs.

2. Materials and methods

Mice: Male Kunming mice, 6–8 weeks of age, were purchased from Xuzhou Medical College. The mice were fed on a basal diet and housed at a temperature of 20–25 °C. Animals used in this study were maintained in accordance with the Guide for Care and Use of Laboratory Animals the National Institutes of Health of China (document number 55, 2001) and the Policy of Animal Ethical and Welfare Committee of the 97th Hospital PLA (For details, see the Appendix).

Tumor cell line: The Heps tumor cell were presented by the Shanghai Institute of Cell Biology, Chinese Academy of Sciences, and maintained in vivo by weekly intraperitoneal passage of 1×10^6 cells in kunming mice by the 97th Hospital of PLA (Jiangsu,China).

Laser instrument and photosensitizer: The HDJ-500He-Ne laser (purchased from Southeast University, Nanjing, China) with an output wavelength of 623.8 nm was used for light irradiation. The laser was integrated into a quartz fiber 400 μm in diameter. The photosensitizer Deuteporfin purchased from Xianhui pharmaceutical Co. Ltd. (Shanghai, China) was used as the photosensitizer.

Reagents and chemicals: The Roswell Park Memorial Institute (RPMI) 1640 medium was purchased from Gibco (Grand Island, NY, USA). Recombinant murine interleukin-4 (rmIL-4) and recombinant murine granulocyte-macrophage colony-stimulating factor (rmGM-CSF) were obtained from Peprotech Co. (New Jersey, USA). 4,6-diamidino-2-phenylindole (DAPI) was got from Bi Yun Tian Co. (Shanghai,China).

Isolation and culture of bone marrow DCs: Primary DCs were isolated and cultured according to the method described previously. Briefly, murine bone marrow cells were harvested from femurs and tibias of 8-week-old Kunming mice with cold RMPI 1640. Cells (6×10^6/3 ml/well) were then cultured in 6-well in complete RPMI 1640 at 37 °C in a humidified atmosphere of 5% CO2/95% air. 12 h later, non-adherent cells were gently removed, and fresh medium with rmIL-4 (50 ng/ml) and rmGM-CSF (100 ng/ml) was added. The medium exchange procedure was repeated every 2 days. Cell morphology and growth were observed with inverted microscopy. On day 8, the cells were collected for further experiments.

The preparation of DCs labeled with DAPI: The collected DCs were adjusted to a concentration of 2×10^6/ml, then stained with DAPI at a ratio of 1:3 for 5 min at room temperature. The mixture was then washed three times with phosphate buffered saline (PBS), and observed under fluorescence microscope.

Establishment of the subcutaneous Heps tumor model: Mouse Heps tumor cells were inoculated into the abdominal cavity of Kunming mice. 7–9 days later, peritoneal fluid was collected. The fluid was diluted and washed with saline solution and finally resuspended to a concentration of 1×10^7 cells/ml. To establish the tumor model, the mice were depilated on the back and then injected subcutaneously with 1×10^6 Heps cells (0.1 ml). Tumors grew predictably in all mice and reached a size of 8–12 mm in diameter 8–10 days after the inoculation.

Study design: The 128 mice were randomly assigned to the control group, the DC group, the PDT group and the PIT group ($n = 32$). Tumors in the control group were intratumorally injected with 0.1 ml saline solution. Mice in the DC group were injected with 5×10^5 DAPI stained dendritic cells. Mice in the PDT group were administered photosensitizer Deuteporfin intravenously at a dose of 20 mg/kg. After a 24 h drug and light interval, the mice were anesthetized intraperitoneally with ketamine hydrochloride at a dose of 100 mg/kg, then the tumor was photoirradiated at a power density of 200 mW/cm^2 for a 15 min duration to a total energy rate of 180 J/cm^2. Mice in the PIT group received the same PDT procedure and were then given an immediate intratumoral injection of DAPI labeled DCs.

The record of tumor volume after treatment: Tumor growth was assessed to the sixteenth day after treatment by the measurement of the orthogonal tumor dimensions (a and b) with a Vernier caliper every other day. The tumor volume was calculated according to the formula, $V = 1/2 \times a \times b^2$. At last, rendering mice tumor volume growth curve use the Kaplan SPSS16.0 statistical software.

The survival time of mice in different groups: Survival time was recorded in 8 mice of each group, they were observed from the day of treatment until death, then use the Kaplan SPSS16.0 statistical software rendering mice Kaplan–Meier survival curves, comparing the survival time of tumor-bearing mice of each group.

Fluorescence intensity comparasion between the DC and PIT group: 3 mice was sacrificed at 12 h, 24 h and 48 h respectively with the tumor draining lymph node excised. Then the lymph node was cut into halves with the tissue cross-section pressed on the glass slide. The tissue was finally sealed with antifade solution and observed under a fluorescent microscope at magnification of 200 times. Every slide was observed for 30 views with the fluorescent cells counted. Then the average DAPI positive cell was calculated.

The measurement of spleen cell cytotoxicity: The LDH (lactate dehydrogenase) release assay was adopted to determine the cytotoxicity of spleen cells. Three mice were sacrificed 7 days after treatment in each group. Their spleen was excised and processed into suspension under sterile conditions. The spleen cell and the Heps tumor cell were then mixed at ratio of 10:1, 20:1 and 50:1 with the spleen cell acted as the effector cell and Heps as the target cell. The Encore automatic biochemical analyzer was adopted for the activity measurement of LDH. The cytotoxicity of spleen cells was calculated according to the formula: cytotoxicity (%) = (U value of LDH in the measurement tube-natural release tube)/(U value of LDH in the maximum release tube-natural release tube) × 100% [18].

3. Statistics

SPSS16.0 software package was applied to statistical analysis in this study. The measurement dates were recorded as mean ± standard deviation. One-way ANOVA was performed to compare tumor volume and fluorescence intensity of different groups. Repeated measures was performed to analyze the growth curve of different groups. And Kaplan–Meier was applied to com-