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Hematoporphyrin monomethyl ether-mediated photodynamic effects on THP-1 cell-derived macrophages

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ABSTRACT

Photodynamic therapy (PDT) has been shown to attenuate atherosclerotic plaque progression and decrease macrophage-infiltration. The effectiveness of PDT depends strongly on the type of photosensitizers. Hematoporphyrin monomethyl ether (HMME) is a promising second-generation porphyrin-related photosensitizer for PDT. This study is designed to characterize effects of HMME-based PDT on THP-1 cell-derived macrophages and define the cell-death pathway. HMME was identified to accumulate in the macrophages by fluorescence microscopy and confocal scanning laser microscope. Our data demonstrated that the intensity of laser-induced HMME fluorescence in macrophages steadily increased with the increasing incubation concentration of HMME. The survival rate of macrophages determined by MTT assay decreased with the increasing HMME concentration and irradiation time. HMME-based PDT induced macrophage apoptosis via caspase-9 and caspase-3 activation pathway detected by caspase fluorescent assay kit and flow cytometer. The PDT increased the number of apoptotic macrophages by 14-fold at 12 h post irradiation by 9 J/cm^2 635 nm diode laser. These results imply that photodynamic therapy with HMME may therefore be a useful clinical treatment for unstable atherosclerotic plaques.

1. Introduction

Atherosclerosis is the leading cause of morbidity and mortality in developed and some developing countries, largely due to events caused by the sudden rupture of atherosclerotic plaques [1]. Such rupture-prone plaques are characterized by large necrotic lipid cores, thin fibrous caps, and dense macrophage-infiltration. The pathology of vulnerable plaques has been investigated extensively, and ample evidences suggest that macrophages play a crucial role in the instability of such plaques [2–5]. Therefore, the therapeutic targets for these plaques should be the local inflammatory cells, particularly activated macrophages [6,7].

Recently, photodynamic therapy (PDT) has evolved as a promising treatment for cardiovascular pathologies, including atherosclerosis and restenosis [8]. Photoangioplasty using a photosensitizer, such as ALA [9], motexafin lutetium [10] or MV0611 [6], has been used in a hyperlipidemic rabbit model of atherosclerosis. It was found that PDT attenuated the progression of plaque, and promoted the stabilization of plaque, vessel healing and repair [11]. The number of macrophages and foam cells also decreased after PDT. One possible reason for the dropout of vascular cells without the promotion of inflammatory responses during PDT is apoptosis triggered by the redox-sensitive pathway and the activation of caspases [12].

Hematoporphyrin monomethyl ether (HMME) is a second-generation, porphyrin-related photosensitizer that has recently been developed [13]. HMME consists of two monomeric porphyrins, i.e., 3-(1-methyloxyethyl)-8-(1-hydroxyethyl) deuteroporphyrin IX and 8-(1-methyloxyethyl)-3-(1-hydroxyethyl) deuteroporphyrin IX (Fig. 1). Experimental studies and clinical trials have demonstrated that HMME which can be selectively taken by tumor tissues has a stronger photodynamic effect, lower toxicity and shorter-term skin photosensitizations. Moreover, HMME is less costly compared with other drugs [14–18].

It is therefore of interest to know whether HMME is capable of inducing photodynamic cytotoxic effects on macrophages. In this work, THP-1 cell-derived macrophages were used to examine the possible effects and mechanisms of HMME-mediated PDT in vitro.

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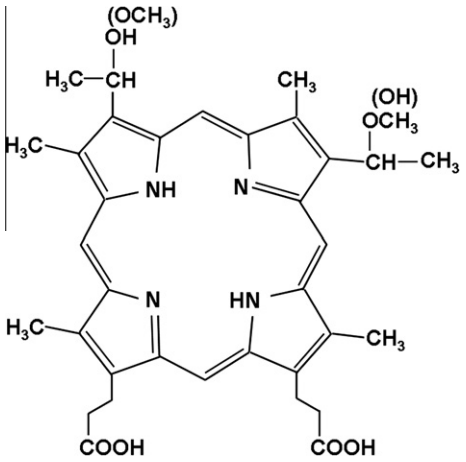

Fig. 1. The chemical structure of hematophorphyrin monomethyl ether.

2. Materials and methods

2.1. Chemicals

HMME was provided by the Pharmacology Laboratory of the Second Military Medical University (Shanghai, China). A stock solution was made in ethanol at a concentration of 10 mg/ml and kept in the dark at −20 °C. Fetal bovine serum (FBS) and RPMI 1640 were bought from Hyclone Laboratories, Inc. (HyClone, Logan, UT, USA). Phorbol-12-myristate-13-acetate (PMA) was purchased from EMD Biosciences, Inc. (La Jolla, USA). The ApoAlert Annexin V-FITC kit and the ApoAlert fluorescent assay kits of caspase-9 and caspase-3 were purchased from BD Bioscience. All other drugs and chemicals used for this study were purchased from Sigma Chemical Co., Ltd.

2.2. Cell line and cell culture

Human THP-1 cells, a monocytic cell line (ATCC), were seeded at a density of 0.5×10^6 cells per milliliter in RPMI 1640 medium containing 10% FBS, 20 μg/ml penicillin and 20 μg/ml streptomycin. The cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2 [19]. THP-1 cells were stimulated with PMA (100 ng/ml) for 72 h to induce a macrophage phenotype in 96-well plates or 35-mm Petri dishes.

2.3. Cell uptake of hematoporphyrin monomethyl ether

To investigate the intracellular kinetics of HMME, the cells were put into serum-free medium and incubated with HMME (0–40 μg/ml) in a dark, 5% CO_2 atmosphere at 37 °C for 3 h. Then the medium was removed. The incubated cells were rinsed three times with cold phosphate-buffered saline (PBS) and illuminated with a 405 nm violet light to trigger the fluorescence of HMME inside the cells. The 5 cm × 5 cm square light source was composed of one hundred violet light-emitting diodes (Sunlight Shenzhen Opto-Electronic Technology Co. Ltd.) with a nominal operating wavelength of 405 nm and a linewidth of 20 nm. A quartz lens with a focal length of 80 mm was used to focus the light onto the surface of the cells, creating an irradiated light spot with a diameter of 0.2 cm. The resulting fluorescence was detected by a multimode optical fiber (Ocean Optics Inc. OFLV-200-1100), which was coupled to an 8 nm-resolution spectrometer composed of a monochromator and a 2048-element CCD-array detector (Ocean Optics Inc. USB2000). All spectral measurements were performed at room temperature.

The intracellular localization of the fluorescence was determined by an OLYMPUS IX81 fluorescence microscope, and the images were captured by an UPLSAPO objective and a CCD camera, and subsequently processed using Image-Pro software (Media Cybernetics, USA). To determine the intracellular localization of HMME in the cells further, a sterile quartz coverslip (0.5 mm in diameter, 0.2 mm thick) was placed onto the bottom of a 35 mm Petri dish. During the treatment with HMME, the medium was aspirated and replaced with medium containing HMME (30 μg/ml). After 3 h incubation, the cells were rinsed three times with PBS. The cells were then incubated with Hoechst 33342 dye (10 μg/ml) for 5 min and examined immediately by OLYMPUS FLUOVIEW 500 confocal scanning laser microscope (OLYMPUS, Japan). Fluoview (version 4.3) was used to encode and process the fluorescence images.

2.4. Phototoxicity assay

The survival rate of the cells after PDT was measured by MTT assay. During the experiments, 1×10^5/ml cells were incubated with different concentrations of HMME (0–40 μg/ml) in a 96-well culture plate for 3 h. Then the drug-containing medium was aspirated and the cells were rinsed with PBS. The medium was replaced with 200 μl RPMI 1640 before illumination. The laser source was a diode laser device (High Power Devices) with a maximal output of 500 mW. The irradiation was carried out for 0.5–3 min by 635 nm light with an output power of 100 mW/cm^2 (a diode laser device from High Power Devices, Inc., NJ, USA). Following PDT, the medium was replaced with 10% FCS RPMI 1640 and the cells were able to proliferate. At 12 h post irradiation, 20 ml of MTT (final concentration: 0.5 mg/ml) was added to each well and the cells were incubated for 4 h at 37 °C. Afterwards, the culture medium was replaced with 200 ml DMSO. The optical density (OD) of the 96-well culture plate was examined immediately at 490 nm with a micrometer reader (BioTek ELx800). The cell survival rate was calculated as the ratio of the absorbance of the treated cells over untreated cells.

2.5. Determination of cell death

After illumination at a fluence of 9 J/cm^2 (HMME = 30 μg/ml), the amount of cell apoptosis was assessed by the Annexin V-FITC apoptosis kit according to the manufacturer's instructions. Approximately 0.5×10^6 treated or untreated cells were gently scraped off and washed twice with cold PBS. The cells were resuspended in 480 μl of binding buffer and incubated with 5 μl of Annexin V and 10 μl of propidium iodide (PI) for 20 min at room temperature in the dark. Cells from each sample were then analyzed by FacsCalibur flow cytometer (Becton–Dickinson, USA). The data were analyzed using the CELLQuest software (Becton–Dickinson, USA). The results were interpreted in the following fashion: cells in the lower-left quadrant (Annexin-V^-/PI^-) represent living cells, those in the lower-right quadrant (Annexin-V^+/PI^-) represent early apoptotic cells, those in the upper-right quadrant (Annexin-V^+/PI^+) represent late apoptotic cells and those in the upper-left quadrant (Annexin-V^-/PI^+) represent necrotic cells. The total apoptotic rate is calculated as the ratio of cells in the lower-right quadrant and in the upper-right quadrant to those the other two quadrants.

2.6. Measurement of caspase-9 and caspase-3 activities

The activities of caspase-9 and caspase-3 were measured using the fluorescent assay kit at various times after illumination at a fluence of 9 J/cm^2 (HMME = 30 μg/ml). The treated or untreated cells were collected by centrifugation at various times post-PDT and resuspended in 50 μl cell lysis buffer for 10 min at 0 °C. Then, the