Inactivation of bovine immunodeficiency virus by photodynamic therapy with HMME

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To investigate the effect of photodynamic therapy (PDT) with hematoporphrin monomethyl ether (HMME) on bovine immunodeficiency virus (BIV) can provide the basis theory for photoinactivation of human immunodeficiency virus (HIV). To assess the protection of HMME-PDT on the cell line Cf2Th infected with BIVR29 by 3-(4,5)-dimethylthiahiazol-2-y1-3,5-di-phenytetrazolium bromide (MTT) with power density of 5 and 25 mW/cm^2 and energy density from 0.6 to 3 J/cm^2. To observe the inhibition of membrane fusion using a new reporter cell line BIVE by fluorescence microscope. HMME-PDT has significant protectant effects on Cf2Th-BIVR29 with both power densities, especially in the group of high power density. Fluorescent microscope shows that there is no significant difference between the group of PDT and control, which means PDT could not inhibit the BIV-mediated membrane fusion.

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According to the latest UNAIDS (uniting the world against AIDS, http://www.unaids.org) statistics, people living with human immunodeficiency virus /acquired immure deficiency syndrome (HIV/AIDS) worldwide were 33.2 million in 2007[1]. Since the occurrence of the first AIDS cases in 1981, remarkable efforts have been made to learn more about HIV/AIDS and to discover effective drugs to contain the dramatic consequences of the pandemic[2]. However, there is no a drug or device could cure AIDS completely so far. Photodynamic therapy (PDT) is a treatment for cancer and for certain benign conditions that is based on the use of a photosensitizer and light to produce reactive oxygen species in cells[3]. Since the renaissance in PDT as a novel approach to treat cancer 20 years ago, a lot of achievements have appeared in the literature. Moreover, Phenothiazine-based photosensitisers have been employed in photoantimicrobial research for nearly 80 years. Because of the serious side effects of these photosensitisers, the photoantimicrobial action just apply to blood disinfection. Fortunately, many efforts have been made to develop new photosensitisers. Hematoporphyrin monomethyl ether (HMME) is a novel porphyrin-related photosensitizer[4]. Experimental studies and clinical trials have shown that HMME has higher selective uptake by tumor tissue, stronger photodynamic effect, lower toxicity, and shorter-term skin photosensitizations than hematoporphyrin derivative (HpD), and is a promising photosensitizer for PDT. Bovine immunodeficiency virus (BIV) was first isolated in 1969 from a cow, R-29, with a wasting syndrome[5]. It was demonstrated that the bovine R-29 isolate was indeed a lentivirus with striking similarity to the HIV, and it was an attractive alternative to HIV in preclinical drug testing. In our study, we use HMME as a photosensitiser to detect the photoantivirual effect in BIV.

HMME is purchased from Shanghai Fudan-Zhangjiang Bio-Pharmaceutical Co., Ltd. (Shanghai, China). MTT and dimethyl sulfoxide (DMSO) were purchased from Sigma. The cell lines, host cell Cf2Th, and reporter cell BIVE, and the virus BIVR29 were gifted by Professor Wentao Qiao.

The diode laser device used was made in Laser Medicine Laboratory of Tianjin Medical University (Tianjin, China) with the wavelength of 635 nm and the output power of 10 mW.

Cf2Th and Cf2Th-BIVR29 were inoculated in 24-well plate at 1:2 with the concentration 2×10^5 cell/mL, 500 μL/well, and then cultured in incubator about 30 h until the syncytia were found. HMME with various concentration were added to the wells respectively at dark, and incubated for 2 h at 37 °C. Then the cells were washed in phosphate buffered saline (PBS) for three times before fresh medium was added. After that, the wells were illuminated using a 635-nm diode laser at power density of 5 and 25 mW/cm^2 for various times respectively. The energy density ranged from 0.6 to 3 J/cm^2. And then the plates were returned to the incubator. Twelve hours after irradiation, the response of cells to HMME-PDT was measured by the MTT assay. Cell survival rate was expressed as the fraction of control samples. Data processing was performed with the software package SPSS (release 11.0). P values less than 0.05 were considered significantly. Experiments were performed at least three times with representative data presented.

The reporter cells BIVE were inoculated in 24-well plate at 1.5×10^4 cells/mL and cultured in incubator overnight for adherence. Meanwhile prepare Cf2Th-BIVR29 in which the syncytia appears, add HMME 5 μg/mL to Cf2Th-BIVR29 and incubate for 2 h at 37 °C. Then the cells were washed, harvested, and resuspended. The final concentration was 1×10^6 cells/mL. 20-μL cell suspension was added to BIVE every well and irradiated immediately or at 10 min, 30 min, 1 h, 2 h, and 4 h after infection. Two days later, the cells were observed

by fluorescence microscope for green fluorescent protein.

We had detected the effects of HMME-PDT on Cf2Th-BIVR29 with two power densities, 5 and 25 mW/cm^2. Results show HMME-PDT has significant protection on BIVR29-infected cells with both power densities (Figs. 1 and 2). However, the laser irradiation without HMME could also improve the cell survival. At 5 mW/cm^2, the irradiation without HMME had similar effects with PDT; at 25 mW/cm^2, the effects of PDT are stronger than irradiation without HMME. The results indicted biological effect by week laser had an important role on the protection on Cf2Th-BIVR29 at 5 mW/cm^2, but PDT played a key role at 25 mW/cm^2. The highest effect of protection was 137.4% when HMME is 5 μg/mL and energy density is 1.5 J/cm^2 at 25 mW/cm^2.

The reporter cell line BIVE was made by hamster kidney BHK-21 containing a plasmid encoding the green fluorescent protein driven by the BIV-1 long terminal repeat. When BIVE was infected with BIV, the green fluorescence could be observed in cells by fluorescence microscope. In our study, we observed the whole course of membrane fusion. The results showed PDT had no effect on the membrane fusion, as shown in Fig. 3.

The treatment of AIDS is always a focus issue of virology and many effords have made on this field. As known, the culture of HIV in vitro need very strict enviroment, just as P3 laboratory, which limits the development of AIDS study. The BIV and HIV types 1 and 2 are members of the lentivirus genus of retrovirus. Although deoxyribonucleic aid (DNA) sequences of these viruses have diverged considerably, the BIV genome organization, function of structural and regulatory genes, and replication cycle are very similar to that of HIV-1, making BIV a potentially useful model to study compounds with anti-HIV-1 activity. Moreover, the group of professor Qiao have achieved a reporter cell lines BIVE which could provide a simple, rapid, and direct method for monitoring BIV infectivity titers.

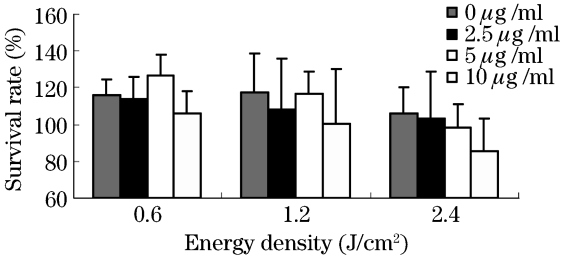

Fig. 1. Protection of HMME-PDT with power density of 5 mW/cm^2 on Cf2Th-BIVR29.

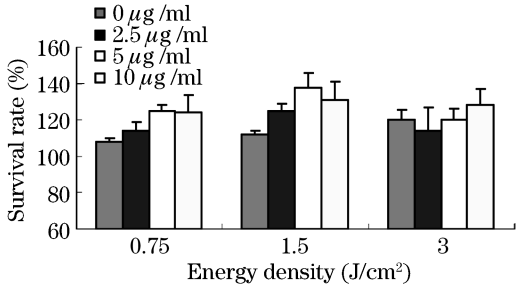

Fig. 2. Protection of HMME-PDT with power density of 25 mW/cm^2 on Cf2Th-BIVR29.

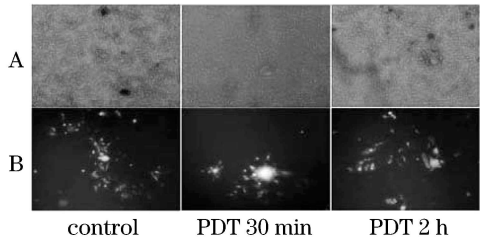

Fig. 3. Inhibition of membrane fusion by PDT with HMME on BIVR29 (A: optical microscope; B: fluorescence microscope).

Although the photodynamic effect was demonstrated against viral targets more than seventy years ago, the use of photosensitisers as antivirals in vivo has been slow in gaining acceptance. Currently, the clinical use of photosensitisers in this field is limited to the treatment of laryngeal papillomata. However, considerable progress has been made in the photodynamic disinfection of blood products. Many photoantiviral protocols with series of photosensitisers and corresponding light irradiation are studied such as Phenothiazine derivatives[6,7], Psoralens [8] and Phthalocyanines[9]. These studies indicate PDT indeed has the antiviral activity, including HIV, hepatitis B virus (HBV), hepatitis C virus (HCV), and so on. However, photoantivirals have traditionally been targeted at cell-free virus. There is little study of PDT about cell-associated virus and virus-infected cells. For the goal of AIDS treatment, we had studied the protection of PDT on BIV-infected cells and inhibition on membrane fusion. The results demonstrated HMME-PDT could improve the survival of the BIV-infected cells, but could not inhibit the membrane fusion when BIV infected. The study by Dimitrov *et al.*[10] demonstrated HIV-1 envelope glycoprotein-mediated fusion could be blocked by photoinactivation of a membrane-solube fluorescent dye. Moreover, the work of Lenard *et al.*[11] suggested that the mechanism of photoinactivation of HIV-1 and other enveloped viruses by hypericin and rose Bengal is by inhibition of fusion. In our study, the reason of no inhibition may be the mechanism of BIV-mediated membrane fusion which is unknown is different from that of HIV-1. It has been universally established that reactive oxygen species (ROS) generated in the collisional energy transfer from the exited triplet state of photosensitizer to the ground triplet state of oxygen is vital for PDT effects[12]. The protective effects of HMME-PDT on BIV-infected cells in the study may be made by ROS destroying the course of virus replication. We believe the conclusion could surport important information for the treatment of AIDS by PDT. To contrabute to AIDS treatment, we have a long way to grope in photoantivirals.

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