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Photosensitization of Chinese hamster V79 cells to photoactivated alpha-terthienyl involving membrane damage and oxidative stress

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ABSTRACT

Photosensitization of V79 Chinese hamster lung fibroblasts was tested to investigate if the cells can fit the photoactive effect of alpha-terthienyl for safety application. Using 15-min photoirradiation of a black light (320–400 nm, 40 W), alpha-terthienyl was significantly photoactivated and caused V79 cells to be shrinkage, detachment and necrosis. The photoactivated alpha-terthienyl played a concentration-dependent stress to decrease cell survival and to induce cell death with median inhibitory concentration (IC_{50}) as 4.78 μg/ml. Cell viability in MTT assays also fell down to 10.58% of the control in the treatment of 10.0 μg/ml photoactivated alpha-terthienyl. As the irradiation time prolonged and the concentration of photoactivated alpha-terthienyl increased, cell death increased significantly, the intracellular level of reactive oxygen species (ROS) and the content of extracellular malondialdehyde were gradually increased. The changes of peroxidase, superoxide dismutase and catalase activities in V79 cells were positively responsive to the oxidative stress caused by photoactivated alpha-terthienyl. Moreover, using non-photosensitizing condition, the increased cell death and oxidative stress in the treatment of alpha-terthienyl at > 7.0 μg/ml were also observed. The results showed the maladjustment response of V79 cells with membrane damage and cell death, clearly demonstrating the photosensitization of animal cells to the photoactivated cytotoxic effect of alpha-terthienyl.

1. Introduction

Photodynamic sensitizers have received considerable attention as useful agents for controlling pest population [1, 2]. Many types of photodynamic sensitizers including thiophenes, porphyrins, acridines, polyacetylenes, xanthenes, phenothiazines, and furanocoumarins have been developed [3, 4]. It has been reported that, when being exposed to light, the photosensitizers undergo rapid activation to destroy insect tissues, thereby exerting high efficiency in killing insect pests, and then transform into non-phototoxic and environmental pollution-free residues [5, 6].

The exposure of animals, even animal cells and tissues, to these photodynamic sensitizers has put forward the problem of biology safety. Porphyrin derivatives after light illumination are used for photodynamic therapy to treat various diseases involving cell hyperproliferation and especially cancer based on the production of reactive species such as singlet oxygen ($^1O_2$) [7, 8], and thiophenes have been shown to possess various biological activities including anti-viral and anti-tumor activities [9], but O'Connor et al. (2009) reported that excessive exposure of skins to exogenous carcinogens or a condition that weakens the immune system caused cellular DNA damage, even the generation of cancer [10]. The reason may be that, singlet oxygen and/or related reactive oxygen species interact with biomolecules to damage cellular organelles, which leads to cellular death and tumor degeneration, thereby promoting these photosensitizers to act on multiple targets [11, 12].

Alpha-terthienyl, a thiophene compound isolated from many plant species including marigolds (Tagetes species) and members of the Asteraceae family, has been developed as an effective photoactivated insecticide. It can reduce the populations of mosquitoes, caterpillars, tobacco hornworms, European corn borers and other pests, and induce the development of larvae into abnormal pupae through targeting on the cuticle enzymes, the neuromuscular sheath, and the midgut membranes [13–15]. Topical alpha-terthienyl after light illumination also shows phototoxicity with resultant disruption of cell membranes of human skin [16], and alpha-terthienylmethanol inhibits the growth of

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human ovarian cancer cells by S phase cell cycle arrest via induction of ROS stress and DNA damage [17]. Thus, the photosensitization of animal cells towards the alpha-terthienyl under photoactivated condition is gaining attention.

Hence, the photosensitization of V79 Chinese hamster lung fibroblasts, that are near diploid cells with fast proliferation and high rate of colony formation, to alpha-terthienyl under light illumination was investigated by performing cytotoxicity assays like trypan blue dye exclusion test, neutral red dye uptake test, MTT assay, estimation of markers of oxidative stress and determination of the activity of oxidative related enzymes. The aim was to bring insight if animal cells like V79 Chinese hamster lung fibroblasts could fit the photoactive effect of alpha-terthienyl.

2. Materials and Methods

2.1. Chemicals

Trypan blue, neutral red, MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] and H_2DCF-DA (dihydrodichlorofluorescein diacetate) were purchased from Sigma-Aldrich Chemicals (Bornem, Belgium). Dulbecco's Modified Eagle's medium was from HyClone. Fetal bovine serum (FBS) was from PAA Laboratories. All other reagents were analytically pure and were purchased from Sinopharm Chemical Reagent Corporation (Shanghai, China).

Alpha-terthienyl (> 98% purity) obtained from Sigma was dissolved in acetone to prepare a stock solution with a concentration of 1000 μg/ml, and placed at 4 °C away from light. The solution was then diluted in culture media to desired concentrations for experimental use.

2.2. Cell Cultures

The V79 Chinese hamster lung fibroblasts culture obtained from Cell Bank (Chinese Academy of Sciences, Shanghai, China) were maintained with twice weekly subculture in DMEM supplemented with 10% foetal bovine serum (FBS), 100 mg/ml penicillin/streptomycin and 1% L-glutamine. The cells were grown at 37 °C in the presence of 5% CO_2.

For the alpha-terthienyl treatment, the V79 cells in the logarithmic growth phase (2.0×10^5 per ml) were preincubated in a humidified air incubator containing 5% CO_2 at 37 °C for 24 h, then the monolayer cultures were exposed to 1.0, 2.0, 5.0, 7.0 and 10.0 μg/ml alpha-terthienyl in dark condition or under photo irradiation for 0, 5, 15 or 30 min. After the next 2 h co-incubation in dark, the cells were digested using 0.25% trypsin and re-suspended in 0.1 M cold PBS buffer (8.00 g NaCl, 0.20 g KCl, 1.29 g $Na_2HPO_4{\cdot}3H_2O$, 0.20 g KH_2PO_4, 1000 ml ddH_2O, pH 7.4) for next determination. Fresh medium containing 0.1% acetone was used as the control.

2.3. Photo Irradiation

A black light, at wavelengths (λ) of 320–400 nm and power (P) of 40 W, was used to irradiate cells. The alpha-terthienyl exposed cells were placed under the light at 17 cm distance to achieve maximum energy intensity of UV 250 mW cm^{-2} [18]. The irradiation protocols were performed at 37 °C with the lights off so as to eliminate any other external light interference. The irradiation time was set as 5, 10, 15, 20, or 30 min, respectively. After the irradiation, all cultures were allowed to incubate in dark for 2 h, and then used for next determination.

Another irradiation using a fluorescent light with wavelengths (λ) of 400–700 nm or an ultraviolet light with wavelength of 253.7 nm instead of the black light was performed to compare the effect of light source on the susceptibility of V79 cells to alpha-terthienyl exposure.

2.4. Trypan Blue Exclusion Assay

Trypan blue exclusion was taken to count the number of dead cells [19]. Briefly, 10 μl Trypan blue reagents (0.4%) were added to 10 μl cell suspension of the alpha-terthienyl exposed cells (5×10^4 cells per ml). After the mixing, the cells were then loaded onto a cell counting chamber slide under optical inverted microscope. In the Trypan blue assay, cells with an intact cellular membrane do not take up the dye and maintain a clear appearance under the microscope, while damaged cells take up the dye and so appear blue in colour. Values are the mean ± SD of triplicates and normalized to that of control group to determine the % of viability.

2.5. Neutral Red Uptake Assay

Neutral red uptake was used to quantitatively estimate the number of viable cells [20]. The alpha-terthienyl exposed cells (5×10^4 cells per ml) were washed with PBS, and then incubated with medium containing 0.05 μg/ml neutral red dye for 2 h. The cells were then fixed with 100 μl fixative solution containing 1% formaldehyde and 1% calcium chloride for 1 min. After removal of the fixative, the neutral red dye taken up by the viable cells was extracted by adding 50% ethanol containing 1% (*v/v*) acetic acid for 10 min. The optical density (OD) at 540 nm was measured by using a UV/visible spectrophotometer. Percent cell survival rate was calculated by using formulas as cell survival rate (%) = [(OD in treatment − OD in background) / (OD in control − OD in background)] × 100% [**I**].

2.6. Cells Viability Assay

The cell viability was determined by succinic dehydrogenase activity of active mitochondria that catalyzes the reduction of MTT to formazan [21]. The alpha-terthienyl exposed cells (5×10^4 cells per ml) in each well were mixed with 10 μl medium containing 5 mg/ml of MTT in a volume ratio 1:1 (*v:v*). After the incubation at 37 °C for 4 h, the medium was removed and 100 μl of DMSO was added to dissolve the formazan crystals. The absorbance was measured at double wavelengths at 550 nm and 655 nm in a Multiskan MK3 microplate reader (Thermo Scientific). Percent cell viability relative to the negative control was calculated by using above **I** formulas.

2.7. Reactive Oxygen Species (ROS) Detection

Cellular ROS was assayed using H_2DCF-DA dye as described previously [18]. It can be deacetylated by esterase and then oxidized to fluorescent DCF by ROS in the viable cells [22]. The alpha-terthienyl exposed cells (1.0×10^5 cells per ml) were loaded with 5 μg/ml H_2DCF-DA at 37 °C in the dark for 2 h, and then they were washed with PBS to remove unincorporated dye. The fluorescence intensities were measured by using a Fluorescent microtiter plate photometer (Synergy2 BioTek) at 488 nm for excitation and 530 nm for emission. The treatment only with fresh medium and 0.1% acetone was used as the negative control.

2.8. Malondialdehyde (MDA) Measurement

The level of MDA was determined in order to measure lipid peroxidation [23]. The alpha-terthienyl exposed cells (1.0×10^5 cells per ml) were treated using the previously described method [4]. The MDA level was calculated by the coefficient (1.56×10^5 l/mol·cm) of the absorbance at 532 nm of MDA-thiobarbituric acid complex and expressed in nanomoles per liter.

2.9. Oxidative Enzyme Assays

The alpha-terthienyl exposed cells (1.0×10^5 cells per ml) were