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In vitro assays for evaluating the ultraviolet B-induced damage in cultured human retinal pigment epithelial cells

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Abstract

The present study demonstrates broadband UV-B-induced damage of cultured human retinal pigment epithelial cells as an effort to develop an *in vitro* model that can be used, along with *in vivo* research and other *in vitro* efforts, to evaluate the need for retinal UV protection in humans after cataract removal. The human retinal pigment epithelial cell line, ARPE-19, was cultured in two groups: control and treated. Treated cells were irradiated with three broadband UVB radiations at energy levels of 0.05, 0.1 and 0.2 J/cm^2. After irradiation, cells were incubated for 48 h while cellular viability, morphology, and phagocytotic activity were analyzed using the Alamar blue assay, confocal microscopy, and fluorescent microspheres. Confocal analysis concentrated on the study of the cell nuclei and mitochondria. The Alamar blue assay of UV-B-exposed cells showed dose and time-dependent decreases in cellular viability in comparison to control cells. Loss of cell viability was measured at the two higher energy levels (0.2 and 0.1 J/cm^2), but the cell group exposed to 0.05 J/cm^2 showed no significant viability change at 1-h time point. Morphological evaluation also showed dose and time-dependent degradation of mitochondria and nucleic acids. Cells exposed with 0.05 J/cm^2 UVB did not show significant degradation of mitochondria and nucleic acids during the entire culture period. Phagocytotic activity assay data for UVB-exposed cells showed dose-dependent decreases in phagocytotic activity in comparison with the control cells. The control cells have significantly greater capacities for uptake than the 0.1 and 0.2 J/cm^2 UV-B-exposed cells, while the 0.05 J/cm^2 UV-B-exposed cell group showed no significant difference from the control cell group. The findings suggest that UVB radiation-induced cultured RPE cell damage can be evaluated by assays that probe cellular viability, morphological change, and phagocytotic activity, and that these assay methods together provide a valuable *in vitro* model for ultraviolet radiation-induced retinal toxicology research.

Keywords: UVB-induced retinal damage; RPE; Alamar blue assay; Cellular viability; Confocal microscopy; Mitochondrial damage; DNA damage; Phagocytotic activity

1. Introduction

The neural retina detects the retinal image and transforms light energy into a communicable form of chemical energy. The outermost layer of the retina, the retinal pigment epithelium (RPE) is derived embryologically from the same neural tube tissue that forms the neurosensory retina. Although the neurosensory retina differentiates into several layers of neurons, the RPE remains a melanin-pigmented monolayer that takes on the characteristics of a secretory epithelium [1]. The RPE is not directly involved in the neural events of vision, but it is critical for the normal functioning and well-being of the photoreceptors [2].

Solar UV radiation, having wavelengths from approximately 200 to 400 nm, is capable of penetrating the ozone layer and only UVB and UVA (280–400 nm) reach the terrestrial surface [3]. The depletion of ozone increases the levels of UV radiation, particularly UVB (280–315 nm), reaching the Earth's surface [4]. Exposure to solar UV radiation has been implicated in a spectrum of skin and ocular pathologies. It is well known that visible and ultraviolet (UV) radiation can produce photochemical lesions in the

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neural retina and the RPE. For example, in studies of morphometric cellular changes of human RPE cells after exposure to UV light (200–400 nm), *in vivo* examination of anesthetized rat retina after UV and green light irradiation (320–600 nm), and DNA damage of human RPE cells caused by broadband UVA (315–400 nm) and UVB (280–315 nm) radiation [5–9]. The role of UV exposure in retinal damage has been well established, including retinal degeneration due to the lipid peroxidation [7,10,11]. The cornea and the lens of the eye substantially filter and attenuate UV radiation that enters the eye, so that only a very small proportion reaches the retina [12]. Also, cataract development with age will reduce the amount of UV radiation exposed to the retina due to the opacification of the crystalline lens. However, depletion of the ozone layer, in combination with the increase in outdoor recreation and average span of life, may result in an increase in the accumulative lifetime exposure of the retina to UV radiation [8]. Also, the removal of the crystalline lens by cataract surgery may be associated with a substantial increase in the UVR that reaches the retina [13]. While manufacturers of intraocular implants have responded to the need to filter UV wavelengths, the cut-off wavelength at the blue end of the spectrum is still a matter of dispute. In fact, a human RPE cell line has been used for many *in vitro* study of UV-induced damage [8,9] as well as blue light damage [14,15] to the retina.

The objective of this study is to evaluate the broadband UV-B-induced damage in cultured human retinal pigment epithelial cell, and to develop an *in vitro* model of retinal UV protection. An *in vitro* model of retinal UV protection is needed as a result of the increase in energy accumulative lifetime exposure of the retina to UV radiation and the fact that crystalline lens protection is lost in cataract surgery without appropriate intraocular lens protection. Thus, an *in vitro* model will also be useful for testing the safety and efficacy of intraocular lens material in protecting the retina from UV damage. This work involves the exposure of a retinal cell culture to UVB radiation. Cellular viability, mitochondrial damage, nucleic acid damage, and phagocytotic activity are quantified after exposure, using the Alamar blue assay, confocal laser scanning microscopy with two different fluorescent stains, and a phagocytotic activity assay.

2. Materials and methods

2.1. Human retinal pigment epithelial cell culture

The human ARPE-19 cell line was derived from the globes of a 19-year-old male donor. Human RPE cell line ARPE-19, originally obtained from American Type Culture Collection (ATCC), was provided by Dr. R. Tchao, University of the Sciences in Philadelphia. ARPE-19 was plated at low density and cultured in DMEM/Ham's F-12 with L-glutamine and 15 mM Hepes (Mediatech, VA, USA). The medium also contained 10% fetal bovine serum (Hycolone, UT, USA), and insulin–transferrin–sodium selenite media supplement (ITS supplement) (Sigma, MO, USA). The cell line was then maintained in a humidified incubator in an atmosphere of 95% air and 5% CO^2 at 37 °C and the culture medium was changed every 48 h. The cells were plated in T75 or T150 flasks (Falcon, NJ, USA), and allowed to grow until the cultures were confluent. After a confluent monolayer appeared (at least 70% confluent), subculturing was carried out using a dissociating agent such as Trypsin/EDTA solution (Cascade Biologics, OR, USA). Subculturing is usually performed during the log phase when the cells are at their healthiest and are able to adapt to the new environment most efficiently [47].

2.2. UVB irradiation of cultured RPE cell

Exposure was produced by filtering banks of UVB fluorescence tubes (Cat. No F15T8/UVB; Microlites Scientific, ON, Canada) in a custom designed UV irradiation unit with 4% CO^2 and 96% membrane filtered air. The spectral distribution of the UVB fluorescent tubes used extends from 290 to about 370 nm wavelengths, with a peak at around 315 nm. However, since it was found that the contribution of the wavelengths within the 320 to 370 nm waveband was only 0.1%, this was ignored. Before irradiation, the UV source was calibrated with an Instaspec II diode-array spectroradiometer (Oriel, CT, USA). After two to three days of pre-incubation at 37 °C, 4% CO^2/96% air to form a confluent cell monolayer, the cells were irradiated with 0.2, 0.1 and 0.05 J/cm^2 of broadband UVB (280–315 nm). The UV source was positioned directly above the cell. In order to minimize absorption of the radiation by phenol red in the medium, the medium above the cell was removed during UV exposure. A thin layer of medium, 1.0 mm thick was left and thus, a minor phenol red effect may have influenced the results. Exposure time was calculated with the formula: $H_\lambda = t \times E_\lambda$, where H_λ is the energy level indicated as exposure per unit area (J/cm^2), t is the exposure time (second), and E_λ is the irradiance (W/cm^2) (ACGIH, 2000). The irradiance, measured with a calibrated Instaspec II diode-array spectroradiometer, was 0.000306 W/cm^2, and the exposure times were 10 min 54 s, 5 min 27 s, and 2 min 44 s, respectively. The solar broadband UVB irradiance level in June 1999, measured in Waterloo, ON, Canada, was 0.000276 W/cm^2 [48]. Thus, the irradiance level used for this study is comparable to solar spectral irradiance. For comparison to other studies, it was necessary to calculate the biologically effective UVB (280–315 nm) dose of the three radiant energy levels. The calculation was carried out using the American Conference of Governmental Industrial Hygienist (ACGIH) spectral weighting functions using the formula: Biologically effective dose $= {}^{320}\sum{}^{290} H_\lambda \times \Delta_\lambda \times S_\lambda$, where H_λ is the actual radiant exposure (J/cm^2) at a specific wavelength as obtained from the spectroradiometer, Δ_λ is the wavelength interval (nm), and S_λ is the UVR spectral weighting