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Phototoxicity in Human Lens Epithelial Cells Promoted by St. John's Wort¶

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ABSTRACT

St. John's Wort (SJW), an over-the-counter antidepressant, contains hypericin, which absorbs light in the UV and visible ranges and is phototoxic to skin. To determine if it also could be phototoxic to the eye, we exposed human lens epithelial cells to 0.1–10 μ*M* hypericin and irradiated them with 4 J/cm^2 UV-A or 0.9 J/cm^2 visible light. Neither hypericin exposure alone nor light exposure alone reduced cell viability. In contrast, cells exposed to hypericin in combination with UV-A or visible light underwent necrosis and apoptosis. The ocular antioxidants lutein and *N*-acetyl cysteine did not prevent damage. Thus, ingested SJW is potentially phototoxic to the eye and could contribute to early cataractogenesis. Precautions should be taken to protect the eye from intense sunlight while taking SJW.

INTRODUCTION

The human eye is exposed to ambient radiation that serves the fundamental biological functions of directing vision (1) and circadian rhythm (2). Light damage to the eye is attenuated by protective chromophores (*i.e.* 3-OH kynurenine glucoside) (3,4) that absorb light but do no cause harm and by an efficient antioxidant system (5). The cornea cuts off all light less than 295 nm. Long UV-B (280–315 nm) and UV-A (315–400 nm) are absorbed by the human lens (6). Any substance that absorbs light greater than 295 nm and produces reactive oxygen species has the potential to damage the human lens. If the substance absorbs light in the visible range (above 400 nm), it can potentially damage both the human lens and retina (7). Such damage is seen in patients taking phototoxic prescription drugs or over-the-counter herbal medications (7). This can lead to permanent or transient loss of vision due to early cataract formation.

St. John's Wort (SJW) is a dietary supplement that has antidepressant properties (8,9) but has been associated with side effects and drug interactions (10). One component of SJW, hypericin, produces significant reactive oxygen species upon irradiation with either UV light or visible light (11). It is known to induce photosensitized erythema in the skin (12,13). Hypericin is so photoactive that it is currently being tested as a photodynamic agent in the treatment of serious bacterial and viral infections and tumors (12,14). Hypericin crosses blood ocular barriers whether ingested or when given by introvitreal injection for treatment of retinal neovascularization and has been detected in the retina in concentrations from 1 to 100 μ*M* (15). The common effective dosage of SJW of 1000 mg produces a serum level of 43 ng/mL of hypericin (16), which is equivalent to a concentration of approximately 0.1 μ*M*. *In vitro* studies have determined that hypericin inhibits the growth of retinal pigment epithelial cells (17). Our previous studies have shown that hypericin is taken up by the intact lens (18), photooxidizes lens proteins (19) and distorts the transmission of light through the lens (20). Therefore, hypericin fulfills the criterion of a potential ocular phototoxic agent.

MATERIALS AND METHODS

Materials. Hypericin, lutein (21), *N*-acetyl cysteine (22) and buffering materials were purchased from Sigma Chemical Co. (St. Louis, MO).

Cell lines. An extended life span human lens epithelial cell line (HLE B-3) was used in these studies (23). Human lens epithelial cells were cultured by isolating epithelium fragments from infant human lenses and from patients who underwent treatment for retinopathy of prematurity and by allowing epithelial cells to grow from explants. Cells were infected with an adenovirus 12–SV40 hybrid virus to increase their ability to propagate in culture.

Culture medium. Cells were grown in Eagle's minimum essential medium (Sigma) containing 50 μg/mL gentamycin and 20% fetal bovine serum in an atmosphere of 5% CO_2 and air at 37°C. The pH of the medium was adjusted to 6.8–7.0, before sterile filtration and addition of serum. Cells were fed twice a week and after attaining confluence were passaged using trypsin–ethylenediaminetetraacetic acid.

UV-A–visible light treatment. Hypericin was dissolved in dimethyl sulfoxide (1 mg hypericin/100 μL) and then diluted into culture medium. Human lens epithelial cells were then incubated in the dark with 0.1–10 μ*M* hypericin in the medium at 37°C for 1 h. The medium was removed, and cells were washed twice with sterile phosphate-buffered saline (PBS) (calcium/magnesium free). After the addition of sterile PBS, the cells were irradiated with fluorescent lamps (Houvalite F20T12BL-HO PUVA, National Biological Corp., Twinsburg, OH) with the dish lid on. The UV-A dose was monitored with a Goldilux UV meter equipped with a UV-A detector (Oriel Instruments, Stratford, CT). For visible light treatment, cells were irradiated with cool-white visible light (Phillips F40 AX50, 5000K Advantage) that was filtered to transmit only wavelengths greater than 400 nm. The UV-A dose was measure to be 4 J/cm^2, and the visible light dose was measured to be 0.9 J/cm^2. Neither the UV-A radiation nor the visible light dosages had an effect on the viability of the human lens epithelial cells. Control samples were kept in the dark under the same

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Abbreviations: HLE B-3, human lens epithelial cell line; PBS, phosphate-buffered saline; PI, propidium iodide; SJW, St. John's Wort.

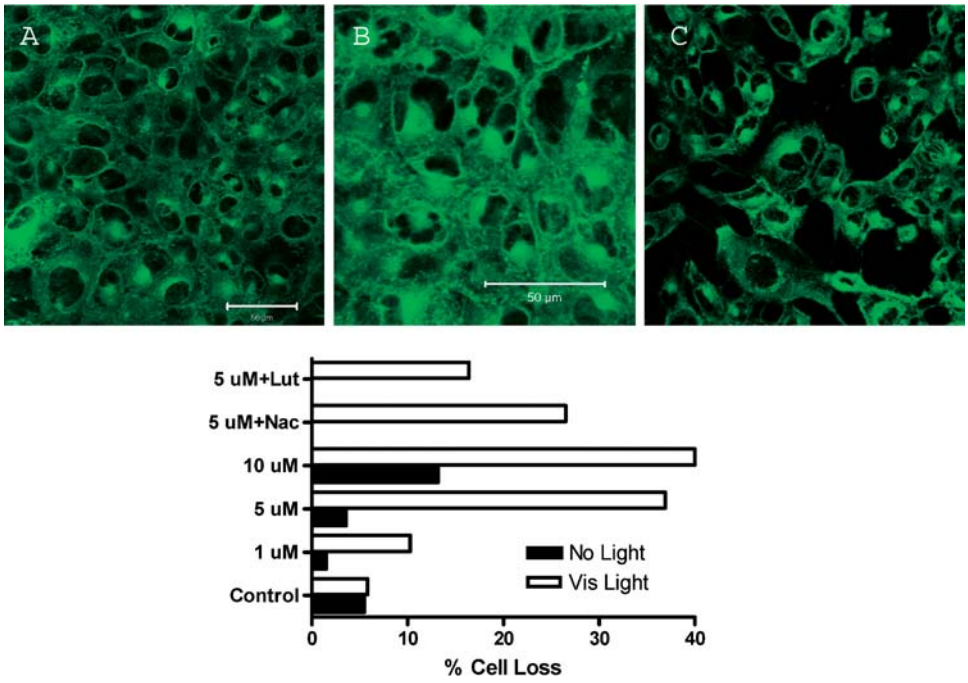

Figure 1. Hypericin effects on HLE B-3 cells. Cells at near confluence were incubated for 1 h in PBS with 0 µ*M* (control) to 10 µ*M* hypericin and then exposed to 0.9 J/cm^2 visible light. Natural hypericin fluorescence was used to image the cells (Zeiss CLSM 510) and determine subcellular distribution of the naturally fluorescent compound and cell density. A,B: Cells exposed to 5 µ*M* hypericin in the dark. The compound accumulates in surface and intracellular membranes but not in the nucleus. C: Cells exposed to 5 µ*M* hypericin plus visible light. Note the areas where cells have detached from the surface. D: Cell loss as a function of hypericin exposure. For each treatment, 2.1×10^5 µm^2 of culture surface area was analyzed.

conditions. After treatment, the supernatant was removed, fresh medium was added after exposure and the cells were incubated at 37°C. In selected experiments, cells were pretreated with lutein (20 µ*M*) or *N*-acetyl cysteine (1 m*M*) at 37°C for 1 h before irradiation.

Analysis of cell death. Flow cytometric analysis was used to evaluate apoptosis and necrosis (24,25). After incubation, control or treated cells were harvested by trypsinization and collected by centrifugation at 300 ***g*** for 5 min at room temperature. Cells were washed with cold PBS and stained with TACS™ Annexin V kits according to the manufacturer's instructions (Trevigen, Gaithersburg, MD). Cells positive for propidium iodide (PI), Annexin V–fluorescein isothiocyanate or both were quantified by flow cytometry using a Becton Dickinson FACSort (Becton Dickinson, Mountain View, CA). In the dot plot histogram, the lower left quadrant in the fluorescence dot plot of Annexin V-PI–stained cells shows normal viable cells that are negative for both Annexin V and PI, the lower right quadrant shows early apoptotic cells that are positive for Annexin V, the upper left quadrant shows necrotic cells that are positive for PI and the upper right quadrant shows late apoptotic cells that are positive for both Annexin V and PI (24,25).

RESULTS AND DISCUSSION

As part of a screening for ocular phototoxicity of psychoactive agents (26), we report in this study the effects of hypericin on human lens epithelial cells irradiated with UV-A and visible light. We exposed human lens epithelial cells (HLE B-3 cells) to 0.1–10 µ*M* hypericin for 1 h at 37°C and then irradiated the cells with either 4 J/cm^2 UV-A or 0.9 J/cm^2 visible light. These levels of illumination were chosen to simulate ambient UV-A exposure and visible light exposure and they induced no detectable damage to the human lens epithelial cells. This is the energy of light one would expect on a bright sunny day (27–29). Hypericin absorbs light both in the UV-A (λ_{max} = 342 nm) and visible (λ_{max} = 555 and 599 nm) regions and fluoresces in the visible region (19). In initial experiments, we took advantage of the compound's natural fluorescence to determine its distribution within HLE B-3 cells and to measure the effects of hypericin and visible light on cell adhesion to the culture dish. Confocal microscopy showed that hypericin accumulates within both surface and interior membranes of the cells (Fig. 1A,B). In cells not exposed to hypericin, exposure to light had no effect on cell density. In the dark, hypericin did not increase cell loss from the monolayer (Fig. 1A,D). However, exposure to hypericin and visible light caused concentration-dependent loss of cells from the monolayer; with 5–10 µ*M* hypericin, about 40% of the dish surface was devoid of cells (Fig. 1C,D). Pretreatment of cells with the ocular antioxidants lutein and *N*-acetyl cysteine reduced but did not prevent cell loss (Fig. 1D).

We used flow cytometry to quantify necrotic and apoptotic HLE B-3 cells after exposure to hypericin and visible light or UV-A. In the absence of light, hypericin concentrations up to 10 µ*M* had no effect on apoptosis or necrosis (data not shown). However, when we pretreated cells with 5 or 10 µ*M* hypericin and then exposed them to visible light, apoptosis was found in 40% and 70% of the cells, respectively (Fig. 2A). Similarly, cells exposed to 1, 2.5 and 10 µ*M* hypericin plus UV-A exhibited a concentration-dependent increase in apoptosis. Pretreatment of cells with lutein or *N*-acetyl cysteine did not protect from hypericin-induced apoptosis on UV-A or visible light exposure (Fig. 2). Exposure of HLE B-3 cells to visible light (Fig. 1D) or UV-A (4 J/cm^2) alone had no effect on apoptosis or necrosis (Fig. 2C,D).

The results of this study demonstrate that hypericin, a component of SJW, in combination with low levels of UV-A or visible light can damage human lens epithelial cells. The endogenous antioxidant lutein (30–32) and the glutathione mimic *N*-acetyl cysteine (21) appear to provide little or no protection. Harris *et al.* (17) have also shown that hypericin can cause phototoxic damage to human retinal

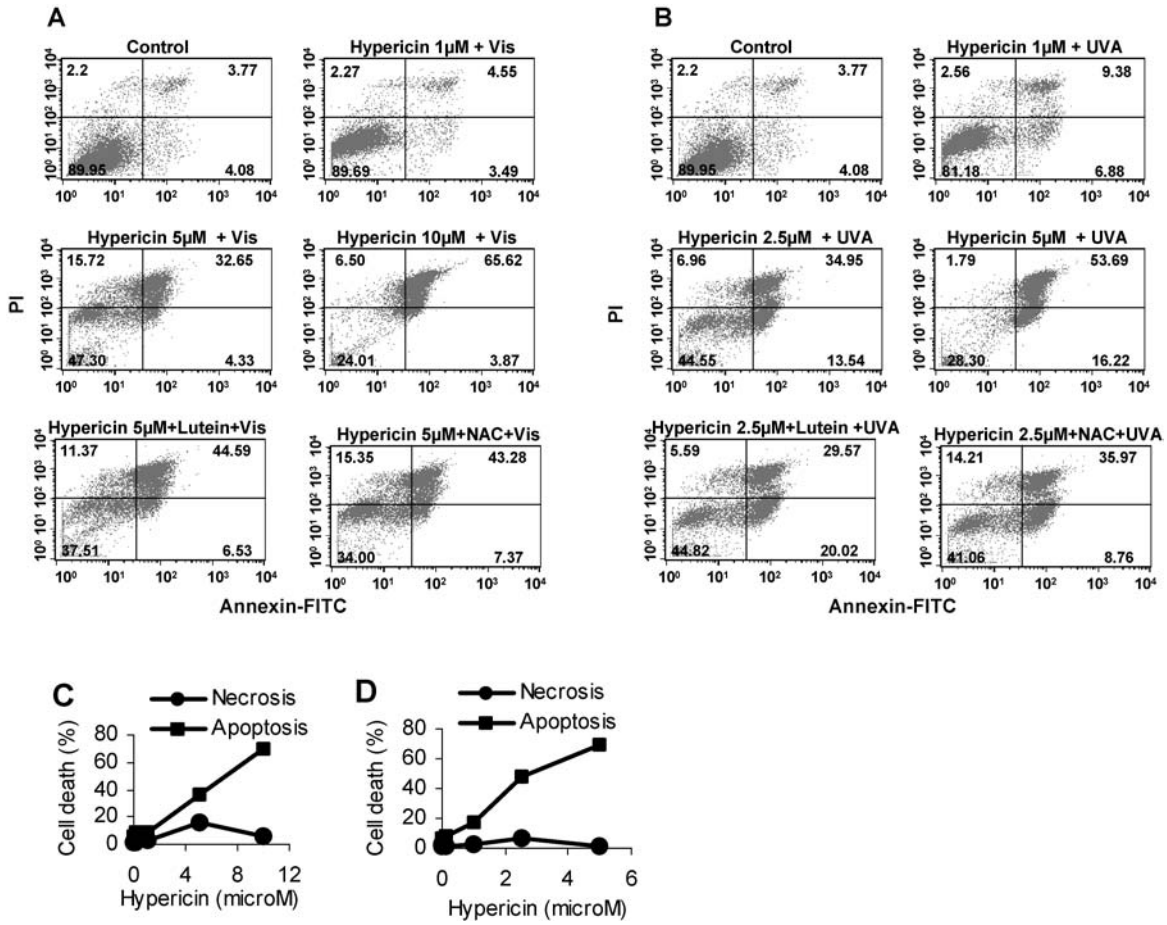

Figure 2. Hypericin-induced apoptotic and necrotic cell death on visible (A) and UV-A (B) radiation. Cells were seeded in plastic petri dishes (100 cm^2) and pretreated with hypericin of different concentrations and then exposed to visible light (0.9 J/cm^2) (A) or UV-A (4 J/cm^2) (B). For lutein or *N*-acetyl cysteine protection assay, cells were preincubated with lutein (20 μ*M*) or *N*-acetyl cysteine (1 m*M*). After treatment, supernatant was replaced by fresh medium and cells were cultured in the incubator for 15 h. After trypsinization, cells were collected, washed with cold PBS once and stained with Annexin V–PI (TACS kit) according to the manufacturer's instruction. Flow cytometry was used to determine the normal viable, early-apoptotic, late-apoptotic or necrotic cells. (C) Visible light–induced hypericin dose-dependent apoptosis. (D) UV-A radiation–induced hypericin dose-dependent apoptosis.

pigment epithelial cells at these concentrations. Taken together, these findings are particularly disturbing because they argue that UV-A and UV-B sunglasses will not protect critical components of the eye against damage by hypericin and visible light.

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