Protective effects of (-)-epigallocatechin gallate on blue light-induced damage in retinoblastoma Y79 cells by activating estrogen receptor pathway

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Abstract: Light-induced photoreceptor cell death can be caused by a variety of cellular mechanisms that involve oxidative stress. Therefore, the eye depends on the presence of antioxidants to protect the retina from light-induced damage. Visible light is generated by the sun as well as by a wide variety of artificial illumination sources such as light emitting diodes (LED). Excessive exposure to light would be damaging to the eye. The short-wavelength visible light between 430 nm to 500 nm (blue light) is especially associated with retina damage as evidenced by photoreceptor degeneration. Recent investigations demonstrated that estrogen receptors (ERs) have antioxidant and antiinflammatory effects on neuronal cells in brain. However, estrogen receptor (ER)-mediated effects of the (-)-epigallocatechin gallate (EGCG), extracted from green tea, have not been examined extensively in photoreceptors of the eyeball. EGCG were examined for the ability to elicit ERs and ER-mediated gene expression *in vitro*. Our studies were demonstrated that the cell degeneration of retinoblastoma Y79 cells was observed after blue light exposure. Apoptosis related proteins, p53 and caspase-3, increased the expression after blue light illumination. After EGCG treatment, increased ER proteins production and inhibited the blue light-induced retinoblastoma Y79 cells death were investigated. These results indicated the short-wavelength visible light, such as white LED exposure, leads to retinoblastoma Y79 damage. EGCG regulates the expression of neuroprotective proteins, ER, and modulates degeneration responses in human retinoblastoma Y79 cells.

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1. Introduction

Photodegeneration resulted in extensive cell death in the retina, mainly in the outer nuclear layer (ONL), where nuclei of the rod and cone photoreceptors were contained. Bright light and continuous exposure to moderately intense light interfere with the visual cycle and induced photoreceptor damage [1-2]. In addition, light exposure has a role in the progression of age-related macular degeneration (AMD) [3] as determined in a human population based study [4]. Photoreceptor cell loss is an irreversible injury and constriction of the visual field, leading to the loss of central vision. Light-induced photoreceptor cell death can be caused by different of intracellular mechanisms that involve oxidative stress, reactive oxygen species, and activation of caspase-relative proteins [5].

Besides UV light, the visible blue light can cause damage to the eye [6]. Short-wave length blue radiation is believed to cause retinal damage or to contribute to the development of age-related macular degeneration [7-8]. Animal experiments indicate photochemical damage of photoreceptor and retinal pigment epithelial cells after eye exposure to excessive levels of short-wave length visible blue light [9-10]. The high-energy photons generate reactive oxygen species, which are deleterious to DNA and to a variety of cellular organelles, predominantly the mitochondria [11-12]. Shorter wavelength light is the most hazardous component of the visible spectrum, and is known to generate reactive oxygen species in the retina [13-14].

AMD is the most common cause of lost central vision in elderly people [15]. Human public-health studies signify that women with early

menopause appear at hazard for worse AMD [16] and estrogen absence may contribute to the onset or severity of AMD in these patients [17]. These statistical studies have provided the evidences for a great clinical trial to evaluate the effectiveness in preventing the onset or progression of AMD [18]. Recent findings of an association between use of postmenopausal exogenous estrogen and a minor hazard of macular degeneration also suggest a role for estrogen in the pathogenesis of the disease [19].

Estrogens are steroid hormones long known for their profound effects on both male and female reproductive systems. Estrogens regulate growth, differentiation, and function of diverse tissues both within and outside the reproductive system. The effects of estrogens are mediated by specific nuclear receptors, which act as hormone-inducible transcription factors. Relatively recent findings demonstrate important roles of the steroid hormones in the cardiovascular system, in specific brain regions, in the liver, and in the maintenance of bone tissue. Despite the wide range of tissues influenced by estrogens and the broad transcriptional regulatory properties of the ligand-activated ERs, the literature contains limited evidence of these receptors in normal ocular tissues [20-22].

Green tea has been shown to have antiinflammatory and antioxidant properties on different types of cells [23-24]. The green tea extracts contain (-)-epigallocatechin gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin gallate (ECG), (-)-epicatechin (EC), and catechin. Among these constituents, EGCG is the most abundant and the most active component in green tea. Transcription factors play a crucial role in regulating gene expression by interacting with epigenetic modulators. In breast cells, EGCG can affect chromatin modifications as well as the binding alteration of a transcription repressor complex, Rb/p130-E2F4/5-HDAC1-DNMT1-SUV39H1, to the ERs promoter, resulting in ER reactivation [25]. HDAC/DNMT1 involves a series of gene silencing through recruiting transcriptional repressors to the gene promoter. Moreover, histone methyltransferase, SUV39H1, is another important epigenetic factor for transcriptional silencing. A recent study has shown that a multimolecular complex, pRb2/p130-E2F4/5-HDAC1-DNMT1-SUV39H1, binding to the ER promoter, is associated with ER transcriptional repression [26].

Studies demonstrate that activation of ER enhance the viability of neuronal cells exposed to a variety of toxic conditions. The protective effects of activation of ER in models of acute neuronal stress and neurodegeneration suggest that these female sex hormones might represent an effective approach for the treatment and prevention of neurological disorders. Nevertheless, the exact role and underlying molecular mechanisms of ERs in photoreceptor protective roles are largely unknown. In this study, we propose that EGCG can protect retinoblastoma Y79 from blue light-induced cell death through its antioxidative effect. We investigated the protective effects of EGCG against blue light-induced retinoblastoma Y79 cell death and the possible mechanisms involved in retinoblastoma Y79 cell survival.

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2. Material and Methods

Human cell line culture

Human Retinoblastoma Y79 cells, revealed with photoreceptor-specific nuclear receptor localized expression in retinal photoreceptor cells, were obtained from the American Type Culture Collection (Manassas, VA). Media and fetal bovine serum (FBS) were purchased from Gibco-BRL (Rockville, MD). Y79 was cultured in RPMI (Gibco-BRL) 1640, supplemented with 10% heat-inactivated fetal calf serum, 0.1% ciprofloxacin, 2 mM L-glutamine, 1 mM sodium pyruvate, and 4.5% dextrose. Another control neuro2A was propagated in DMEM (Gibco-BRL), supplemented with 7.5% heat-inactivated FBS, 7.5% heat-inactivated horse serum. Cultured cell lines were grown at 37°C in a 5% CO2-humidified incubator.

Blue light exposure on cultured cells

Spectrum distribution from LED lamps and white floursent lamps (China electric MFC, Taiwan) was analyzed using an UV-visible spectrophotometer (Ultrospec 3000, Pharmacia Biotech, Cambridge, UK) from 300 nm to 800 nm. Peak wavelengths of the LED lighting are 440 nm and 470 nm within the blue light wavelengths. To create a constant level of illumination, we placed some cells into the same cell culture incubator, with identical ambient conditions to normal cultures except that light was housed 15 cm directly above culture plates to insure that all cultures received the same lighting levels. Cultured cells were exposed to diffused light from LED lamps ranged from 10 minutes. The core temperatures were measured and were found not to increase by more than 1°C during illumination. Cells were further incubated for 12 to 24 hours under normal conditions (culture medium, 37°C, 5% CO2, 95% air) before analysis.

EGCG treatment on cultured cells

EGCG was purchased from Sigma Chemical Co. (St. Louis, MO). Human retinoblastoma Y79 cells were treated with various concentrations of EGCG for 3 days. The medium with EGCG was replaced every 24 h for the duration of the

experiment. Control cells received equal amounts of DMSO (Sigma) in the medium. For the combination study, cells were treated with an optimal concentration of EGCG based on our following results and ER antagonist (ICI 182,780) (Sigma) alone or together for a total 3 days as the common recommended doses of these compounds.

Immunocytochemistry for cultured cells

Cultured cells were washed in PBS, fixed in methanol at 20℃ for 10 minutes, equilibrated in PBS, blocked for 30 minutes with 5% normal goat serum, incubated with the primary antibodies overnight, and washed extensively with PBS. Cells were than incubated for 1 hour with the secondary antibodies and again washed with PBS. Subsequently, cells were mounted and observed under a Leica TCS SP2 confocal spectral microscope.

Cell fractionation and Western Blot

Samples were rinsed once with ice-cold PBS and then lysed with PBS containing 1% Triton X-100, 0.1% SDS, 0.5% sodium deoxycholate, 1 μg/ml leupeptin, 10 μg/ml aprotinin and 1 mM phenylmethylsulfonyl fluoride on ice for 10 minute. After sonication using microprobe sonicator, crude extracts were subjected to centrifugation at 4 °C. The supernatants were collected as cell lysates. All protein concentrations were determined by a protein assay (Bio-Rad laboratories, Richmond, California). Aliquots (50 μg) of cell lysates or nuclear extracts were separated electrophoresed on 8 or 12% SDS-polyacrylamide gel and then transblotted onto the ImmobilonTM-P membrane (Millipore). After being blocked with 10% skim milk in Tween-20/PBS, blots were incubated with various primary antibodies and then incubated with HRP-conjugated secondary antibodies. The protein bands in the blots were detected using enhanced chemiluminescence kit (ECL; PerkinElmer Life Sciences, Inc. Boston).

Morphological evaluation

Cultured cells were fixed in methanol for 30 min at 4°C. Hematoxyline was applied to stain nuclei. Subsequently, culture cells were mounted and examined under a Zeiss Axiophot microscope (Carl Zeiss, Oberkochen, Germany).

Statistical Analysis

Data are presented as the mean ± SEM. Statistical comparisons were made using a one-way analysis of variance (ANOVA) followed by a Student's t test. The statically significance was assessed at $p < 0.05$.

3. Results

3.1 The spectral irradiance profile of LED source

The emission spectrum of UV/visible lighting ranging from 300 to 800 nm was recorded. The wavelength within the UV range was not detectable from LED source. The spectrum distribution of lighting began to increase at 300 nm and reached sustained maximum transmission at 450 nm. The chief peak of the spectrum distributes was about 0.35 mW/cm2 within the visible wavelength of the blue light. Spectral irradiance profile of LED source showed that the main optical radiation ranging from 430nm to 500 nm as our previous study [6].

3.2 Effects of Human Retinoblastoma Y79 cells damage induced by short-term blue light LED light exposure

No available studies had investigated on the cytotoxic effect of short-wave length blue light radiation on human retinoblastoma cells. To illustrate the relationship between short-wave length blue light radiation and cell death, the immunostaining of active caspase-3 was applied. Apoptosis-related protein, caspase-3, was determined at 30 min and then for 12 and 24 incubated time after blue light LED irradiation. As we expected, Human Y79 cells underwent apoptosis cell death after blue light LED exposure in our system. The increase percentage of caspase-3 positive cells after light exposure was observed (Fig. 1). After 12 and 24 hr, very few immunopositive cells with active caspase could be detected in the Y79 cells from control and visible-light exposure groups, whereas a number of neurons were labeled with active caspase-3 and their nuclei were in an eccentric position after blue light-exposed.

With a statistical analysis, the percentage of active caspase-3 immuno-positive neurons was significantly increased in the cultured neurons blue light irradiation compared with that from control and visible-light exposure groups. These results implied that the cell death was associated with short-wave length blue light radiation.

3.3 Effects of EGCG on activating ER expression

To elucidate the effects of the green tea polyphenol, EGCG, on cellular viability and ER expression in human retinoblastoma Y79 cells, we initiated to determine the optimal dose that will induce ER transcriptional activation without causing toxicity to cells. We performed western-blot assays to detect the protein level of ER expression in human retinoblastoma Y79 cells (Fig. 2A). More strikingly, studies demonstrated that EGCG treatment can induce significant effects on cellular expression when chose to use the concentration of 25 μM EGCG (Fig. 2B). In summary, these results suggest that EGCGs

may play an important role in ER expression in retinoblastoma Y79 cells.

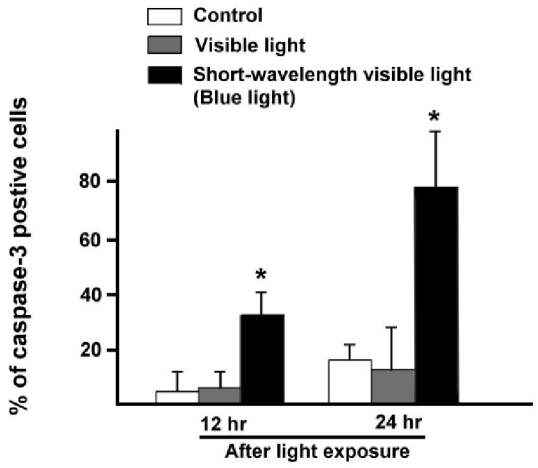

Fig. 1: Effects of Human Retinoblastoma Y79 cells damage induced by short-term blue light irradiation

Cultured cells were exposed to light for 10 minutes, and then for 12 to 24 hr incubated time. Human Retinoblastoma Y79 cells, revealed with photoreceptor-specific nuclear receptor localized expression in retinal photoreceptor cells, showed a significantly increased percentage of caspase-3 positive cells after light exposure with blue light, but not with visible light irradiation. Data are expressed as means (±S.D.); * indicate a value statistically different ($p < 0.05$) from the control.

3.4 Neuroprotection of ER expression in EGCG-treated human retinoblastoma cells

The morphological evaluation was performed to determine whether ER can inhibit apoptotic cell death induced by short-wavelength visible blue light. Few positive staining cells were noted in control cultured cells; whereas cultures treated with short-wavelength visible blue light h had large numbers of cells undergoing apoptosis. However, pretreatment of Y79 cells with 25μM EGCG for 3 day prior to light exposure led to a dramatic decrease in the numbers of apoptotic cells. Moreover, pretreatment with ER antagonist (ICI 182,780) prior to the addition of EGCG significantly block the neuroprotective effect of EGCG in this study (Fig. 3)

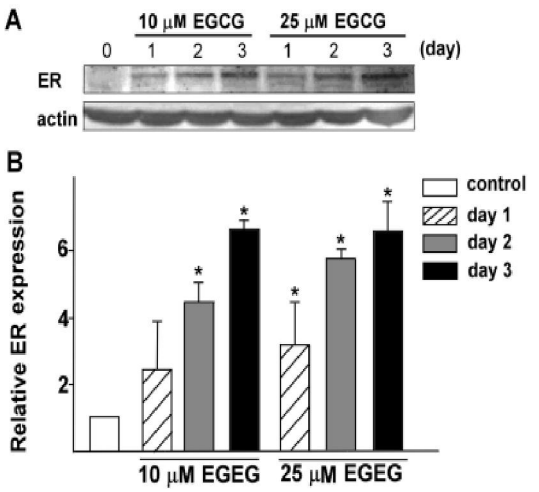

Fig. 2: EGCG induced ER proteins expression in human retinoblastoma Y79 cells

The retinoblastoma cells were treated either 10 μM or 25 μM EGCG for 1, 2, and 3 days. EGCG induced ER proteins expression in retinoblastoma cells (A). EGCG induced maximal ER expression at a concentration of 10 μM for 3 day treatment. β-Actin was as the loading control (B). Data are expressed as means (±S.D.); *

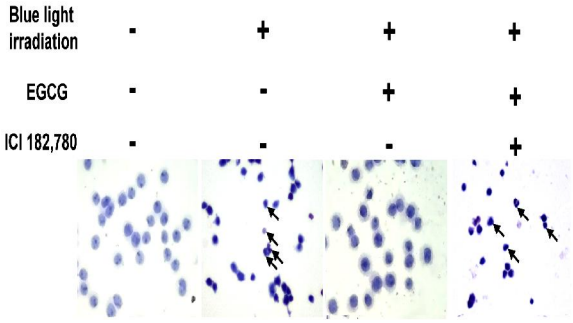

Fig. 3: EGCG modulate degeneration response in human retinoblastoma Y79 cells

The cytotoxic responses were measured by the morphological evaluation. Cultured Y79 cells were pretreated with 5 μM ER antagonist 30 min prior to 25 μM EGCG treatments. The cell number of apoptosis cells with chromatin condensation (arrows) was significantly increased after blue light irradiation or light irradiation combined with ER antagonist (ICI 182,780) treatment. Pretreatment of retinoblastoma Y79 cells with EGCG for 3 day prior to blue light exposure caused a significant decrease in the numbers of apoptotic cells

4. Discussion

In the present study, we demonstrated that EGCG has a neuroprotective effect in the retina which is mediated via the activating estrogen receptor pathway. It has been known that EGCG promotes

viability and survival of other primary neuronal culture systems, such as cortex, hippocampus, and hypothalamus [27-30]. EGCG is also known as a scavenger of ROS in extracellular environments and for its protective effect on many cell types against the damage of UV radiation in retinal pigment epithelium [31]. This is the first observation demonstrating that EGCG inhibits blue light-induced human retinoblastoma Y79 cell apoptosis, and activates ER expression.

As excessive light exposure is known to induce retinal dysfunction [1-2, 6], we investigated the effect of human retinoblastoma cells on light-induced cell degeneration in mice by Immunocytochemistry staining. Previous studies demonstrated that light exposure caused apoptosis in the retinal tissue [31] and that apoptosis was prevented by antioxidants such as ascorbic acid and dimethylthiourea [32-33]. Main effects of light-induced cell death have been reported to depend on the apoptotic pathway and also on ROS production [34]. In some tissues, the neuroprotection may be mediated by the estrogen receptor and relative protein synthesis, because estradiol can bind intracellular specific estrogen receptors, and the complex binds to specific sites on genomic DNA and control mRNA transcription. Definitely, estradiol was reported to provide neuroprotection mediated by estrogen receptors in cultured neurons including neurones from the ipothalamus, amygadala, cortex midbrain and hippocampus [35]. We observed that a competitive estrogen receptor antagonist, ICI 182,780, significantly attenuate the protection provided by ER. The absence of neuroprotective effects by ICI 182,780 completely impair the possibility that the neuroprotective functions that were mediated by estrogen receptors.

It is well identified that age is a risk factor for loss of visual function in many diseases, such as AMD [17]. Symptoms observed in menopausal women have been considered related to the abrupt decline in estrogen activity after the reproductive years. Several human public-health studies suggest potential participations of estrogen and its receptors in the homeostasis of the eye, but the mechanisms involved remain unclear. In our studies, these results indicated that EGCGs may play an important role in ER expression in retinoblastoma Y79 and ER expression modulates degeneration responses *in vitro*.

5. Conclusion

In summary, our study provides the first evidence that EGCG is a protective agent for human retinoblastoma Y79 cells after exposure to blue light radiation by activating ER expression pathway. Our results demonstrated that EGCG inhibits blue light-induced caspase-related proteins expression. Moreover, EGCG increases human retinoblastoma cell survival after exposure to visible blue light radiation. This suggests that EGCG is valuable in preventing retinal cells from short-wave length visible light-induced damage and may be appropriate to be further developed as a prophylactic health food for the prevention of retinal diseases. In addition, our results imply that green tea catechins can have a positive effect and can play an important role in the prevention of short-wave length visible light -induced visual disorders.

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