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Full Length Research Paper

Green tea polyphenol protecting human retinal pigment epithelial cells from ultraviolet B (UVB)-induced injuries *in vitro*

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Green tea polyphenol (GTP) is water-soluble medical additives which possess particular significance as free radical scavengers or antioxidants in biological systems. The present study investigated the protective effect of green tea polyphenols against ultraviolet B (UVB)–induced damage to human retinal pigment epithelial (RPE) cells. Microstructure of RPE cells was examined by transmission electron microscopy and the expression of c-fos was examined in messenger ribonucleic acid (mRNA) as well as protein level by using real-time polymerase chain reaction (PCR) and western blot assay. The results indicated that UVB irradiation-induced injuries in RPE cells were markedly suppressed by GTP. The mechanism of GTP protected RPE cells from UVB damage might be related to signal pathway regulation and DNA restoration, suggesting GTP as a potential candidate for further development and aslo a chemoprotective material for prevention of UVB exposure induced eye diseases.

Key words: Green tea polyphenol, ultraviolet B (UVB) irradiation, C-fos.

INTRODUCTION

RPE cells play an essential role in retinal function, it was clear that RPE cells involved in process and transport of vitamin A (Bok, 1990), and degeneration of RPE cells may lead to retinal diseases such as age-related macular degeneration (AMD) (Liang et al., 2000; Dunaief et al., 2002). Previous studies indicated that ultraviolet (UV) involved in the pathogenesis of AMD. UV irradiation can induce the production of reactive oxygen species (ROS) and deoxyribonucleic acid (DNA) mutation (Afaq, 2011), causing the deterioration of RPE cells (Liang et al., 2003), which might contribute to the pathogenesis of AMD. Although the exact cause of RPE cell degeneration is not clear, it was postulated that the sensitivity of RPE cells to UV light could be an important factor (Noell et al., 1966; Young, 1988; Szaflik et al., 2009; Xu et al., 2010). GTP is a kind of natural antioxidant extracted from green tea which could attenuate cell damage against UV irradiation (Yusuf et al., 2007; Wu et al., 2009; Katiyar et al., 2010; Xu et al., 2010). It was also proved that GTP could protect against damage induced by UV irradiation *in vivo* (Kelly et al., 2001; Lu, 2002; Jane et al., 2003; Vayalil et al., 2004).

The anti-irradiation effect of GTP results from the mechanism of electron transfer from catechins to radical sites on the DNA (Anderson et al., 2001; Malhomme de la Roche et al., 2010). C-fos gene belongs to the group of immediate-early inducible genes (Hollander et al., 1989; Dosch et al., 1996). It is believed that c-fos gene is involved in the regulation of DNA repair and the expression of c-fos could be induced by many types of genotoxic stress and implicated in maintaining genomic stability and cell survival (Christmann et al., 2006). It was confirmed that c-fos played a decisive role in cellular defense against a broad spectrum of induced DNA

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Abbreviations: GTP, Green tea polyphenol; **UVB,** ultraviolet B; **RPE,** retinal pigment epithelial; **AMD,** age-related macular degeneration; **FBS,** fetal bovine serum; **GAPDH,** glyceraldehyde-3-phosphate dehydrogenase; **CREB,** cAMP response element binding protein; **ROS,** reactive oxygen species; **CPD,** cyclobutane pyrimidine dimer; **EGCG,** (-)-epigallocatechin-3-gallate.

Table 1. Composition of green tea polyphenols (mg/g).

GC	EGC	C	EC	EGCG	GCG	ECG	CG	Total catechins
44.38	85.47	14.09	41.13	344.73	42.49	103.37	8.80	684.47

C, (+)-catechin; CG, (+)-catechin-3-gallate; EC, (+)-epicatechin; ECG, (+)-epicatechin-3-gallate; EGC,(+)-epigallocatechin; EGCG, (+)-epigallocatechin-3-gallate; GC, (+)-gallocatechin; GCG, (+)-gallocatec-gin-3-gallate.

damages (Haas et al., 1995; Christmann et al., 2006). Many studies on the GTP protective effects against UV have focused on the biochemical properties; however few reports regarding the effects of GTP on c-fos expression in UVB-induced injuries in RPE cell have been available. In present study, the expression of c-fos in both mRNA and protein levels were determined in RPE cells in order to investigate the protective effects of GTP on UVB-stressed RPE cells.

MATERIALS AND METHODS

The RPEcell line (no. D407) was purchased from the Center of Experiment Animals of Sun Yat-sen University (Guangzhou, China). Use of the no.D407 RPE cell line in the test was verbally consented by the Center of Animal Experiments of Sun Yat-sen University. The RPE cells were seeded in 50 mL flasks containing 5 mL RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 100U penicillin-streptomycin and were cultured at 37°C in humidified atmosphere of 5% CO_2. When the cells were 70% confluent (70% of the dish bottom surface area covered by cell monolayer), they were washed with 0.5% (vol/vol) trypsin/ ethylenediaminetetraac-etate (EDTA) and collected with a 1-mL pipette tip for further use. GTP used in the study was supplied by CinoTea Company Limited. (Hangzhou, China); its composition is listed in Table 1. The GTP was dissolved in RPMI-1640 medium without FBS supplement as stock solutions at concentrations 0, 70 and 140 mg/L before use. FBS was purchased from Life Technologies Inc (Gaithersburg, MD). Reagent (TRIzol) and penicillin-streptomycin were purchased from Invitrogen Corp. (Carlsbad, CA). MTT [3,(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide], trypsin, and EDTA were purchased from Amresco Inc. (Solon, OH). The other reagents used were purchased from TaKaRa Biotechnology (Dalian) Company Limited (Dalian, China).

GTP and UVB Treatment

RPE cells were seeded in 10 cm glass petri dishes (10^6 cells each dish) with 5 mL RPMI-1640 medium supplemented with 10% FBS and incubated as described until they were 70% cell confluent. For GTP pretreatment, the medium solution was drained and the cells were washed by phosphate buffer (PBS). After the PBS was drained, the RPE cells were incubated in GTP solution at 37°C for 2 h and washed with PBS. FBS-free RPMI-1640 medium was added to each sample and irradiated by 100 μw/cm^2 UVB for 2 h with UVB light tubes (Spectronics Corp,Westbury, NY). For the GTP post-treatment, the medium solution was drained and the cells were washed by PBS. The FBS-free medium was added to each sample and irradiated by 100 μw/cm^2 UVB for 2 h. Then each sample was washed by PBS and samples were incubated in GTP solution at 37°C for 2 h.

Transmission electron microscopy

The cells were washed with PBS and centrifuged at 2000 g for 5 min. The cell pellet specimen was prefixed with 1 mL 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0) at 4°C overnight, washed three times with 0.1 M PBS, finally fixed in 1% OsO_4 for 2 h, and again washed three times with 0.1M phosphate buffer. The fixed cell specimen was dehydrated in an increasing graded series of ethanol solutions (50, 70, 80, 90, 95 and 100%) in which each dehydration step lasted for 15 min. The dehydrated specimen was drenched sequentially in acetone for 20 min and embedded in Epon-812. Sections of 70- to 90-nm thickness were made on an ultra-microtome (OM U2; Reichert-Jung Company Heidelberg, Germany) and were stained using uranyl acetate and alkaline lead citrate for 15 min. Sections were examined and photographed with a transmission electron microscope (JEM-1230; JEOL Limited Akishima, Tokyo, Japan) (Du et al., 2008).

Real-time PCR analysis of C-fos gene expression

Samples were washed with PBS. Total ribonucleic acid (RNA) was extracted using reagent (TRIzol; Invitrogen) according to the manufacturer's protocol. Extracted RNA samples were checked by 1.5% agarose gel electrophoresis. RT-PCR was carried out using an RT-PCR kit (PrimeScript; TaKaRa). RT reaction was carried out in a 20 μL reaction system, including 4 μL 5×buffer (PrimeScript; TaKaRa), 1μL enzyme mix (PrimeScript RT Enzyme Mix I; TaKaRa), 1μL oligo dT primer (50 μM), 1μL random mers (100 μM), and 13 μL total RNA at 37°C for 5 min and then 85°C for 5 s. Quantitative PCR was performed (iCycle iQ; Bio-Rad Laboratories, Hercules,CA) using the RT reaction solution and primers for generating the c-fos gene (forward, 5'- ATTTGACTGGAGGTCTGCCTGAG-3'; reverse and 5'-CACCCCGTTTCCCCAATGACTTAG-3'). The glyceraldehyde-3-phosphate dehydrogenase gene (GAPDH; forward, 5'-GGGGAGCCAAAAGGGTCATCATCT-3'; reverse and 5'-GACGCCTGCTTCACCACCTTCTTG-3') was used as an endogenous control. The PCR reaction system consisted of 12.5 μL premix reagent (2×) (SYBR Premix Ex Taq; TaKaRa), 0.5 μL forward primer (10 μM), 0.5 μl reverse primer (10 μM), 9.5 μL ddH2O and 2 μl RT reaction solution. The reaction was carried out at 94°C for 3 min followed by 40 cycles of 94°C for 30 s and 65°C for 60 s. Samples were tested in triplicate and the mean values were presented.

Western blot

Samples of 25 mg protein extract were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) and electroblotted onto polyvinylidene fluoride (PVDF) membranes. Polyclonal c-fos antibodies (sc-52; Santa Cruz Biotechnology) were diluted 1:500 in 5% non-fat dry milk, 0.2% Tween/PBS and incubated with PVDF membranes overnight at 4°C. The PVDF membranes were wash with Tris Buffer Saline Tween20 (TBST) buffer for three times the next day. Glyceraldehyde-3-phosphate

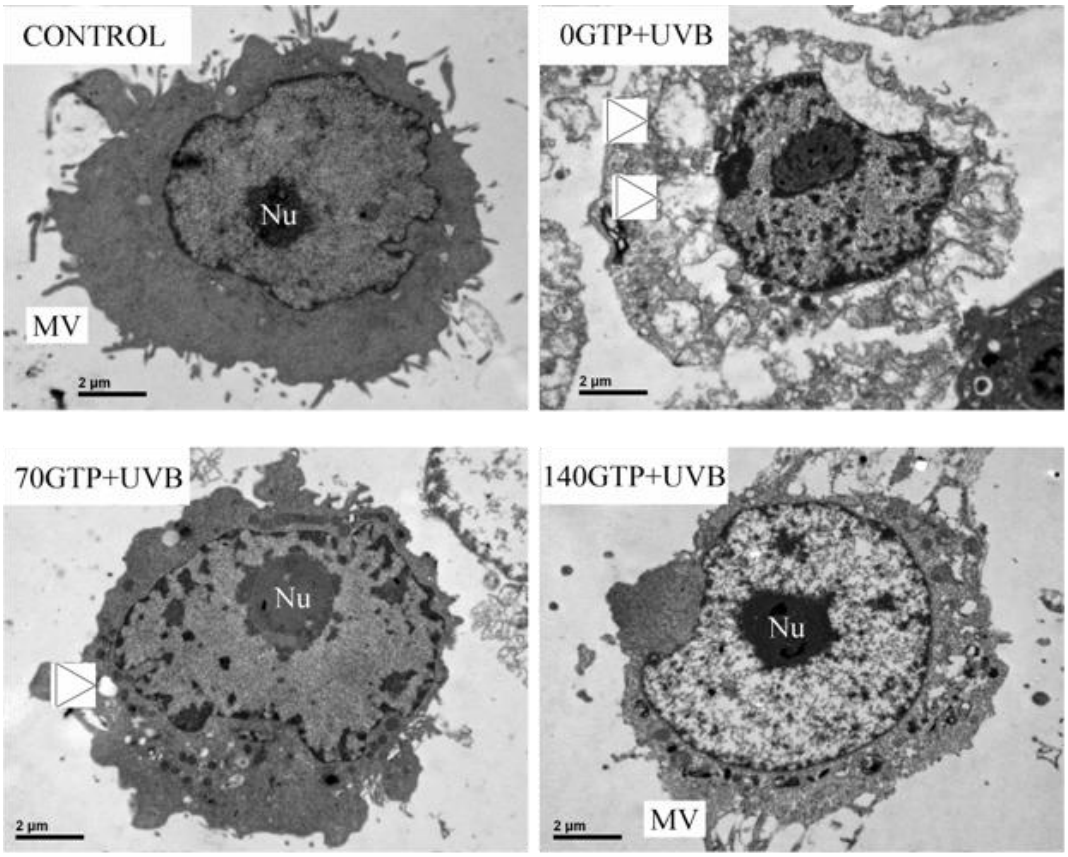

Figure 1. Changes in microstructure of UVB-irradiated RPE cells. The numbers before GTP indicate each GTP dose (mg/L). GTP+UVB, GTP pretreatment; Nu, nucleolus; MV, cell membrane microvilli. White triangle: vesicular structure.

dehydrogenase (GAPDH) antibodies (sc-166545; Santa Cruz Biotechnology) and goat anti-rabbit IgG-HRP (sc-2032; Santa Cruz Biotechnology) were diluted 1:5000 and incubated with PVDF membranes for 1 h at RT. Then the protein–antibody complexes were visualized by ECL (Amersham). Densitometer analysis was done using the quantity one software and density values were normalized to the non-treated control in pretreatment and post-treatment groups, respectively, which were assigned a value of 1.0.

Statistical analysis

The real-time PCR experiments were repeated three times. Data were processed with the Statistical Analysis System (SAS Institute, Cary, NC) for a one-way analysis of variance and Duncan's multiple range tests to determine whether significant difference ($P<0.05$) existed between mean values.

RESULTS

Cytoprotective effects of GTP on UVB irradiation in RPE cells

To investigate the cytoprotective effects of GTP against UVB-irradiation, we conducted the transmission electron microscopy experiments to evaluated microstructure of RPE cells which were pretreated with GTP. Before UVB irradiation, the intact cytoplasm of the RPE cell was evenly distributed and the nucleolus was located at the center of the cell. Outer cell membrane microvilli were observed (Figure 1, control). After irradiation with UVB at a dose of 100 mJ/cm^2 for 2 h, the cells were markedly damaged with microvilli shedding, nucleolus degeneration and formation of vesicular structures in cytoplasm (Figure 1; 0GTP+UVB). When the cells pretreated with GTP, the vesicular structures were smaller, even a part of microvilli were seen at the 140 mg/L GTP dose. (Figure 1, 70GTP+UVB and 140GTP+UVB), suggesting that GTP attenuated UVB-induced RPE cell degeneration.

Effect of GTP on expression of c-fos gene in RPE cells irradiated by UVB

The expression level of the c-fos gene in RPE cells was increased by UVB irradiation and both GTP pretreatment and GTP post-treatment could regulate c-fos expression in mRNA level (Figure 2). In this study, the expressionn of

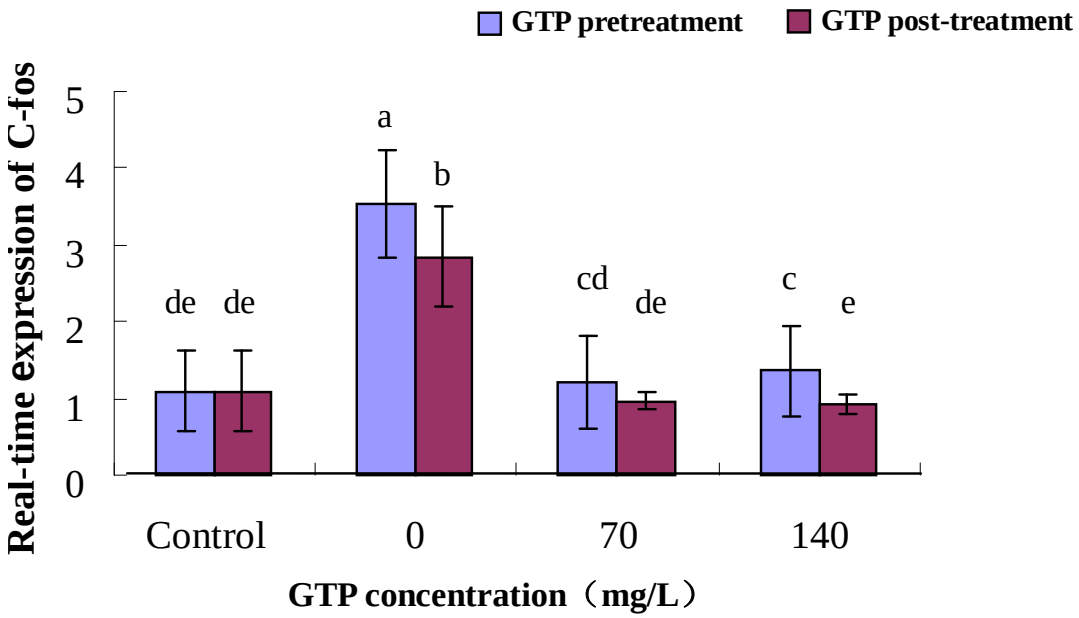

Figure 2. Expression of c-fos gene in RPE cells. Relative expression data were presented after normalization against GAPDH. Mean values with different letter are significant different (P<0.05) between groups.

c-fos in UVB-exposed RPE cells was about 3 folds higher than that of control. Whereas the over expression of c-fos was inhibited greatly in both GTP pretreatment and post-treatment groups and this effect was marked in post-treatment groups, which might be related to the rapid decay of c-fos. C-fos is an immediate early-response gene which is constitutively expressed at low levels in many cell types (Melissa et al., 2002), however it would be induced by cellular oxidative stress and play a functional role in UVB-induced tumor promotion (Olson et al., 2010). This results indicated that the deregulation of c-fos expression in RPE cells could attribute to the UV-induced peroxide, suggesting that the protective effect of GTP against UVB damage to RPE cells might be related to its scavenge ability of oxidant.

Effect of GTP on expression of c-fos protein in RPE cells irradiated by UVB

The protein expression level of c-fos was determined and shown in Figure 3. In this study we analyzed c-fos protein levels by Western analysis and it was confirmed that a 100 μw/cm^2 UVB did increase c-fos protein by at least 2 folds compared with control after 2 h exposure. The treatment of RPE cells with GTP resulted in a level of c-fos that was substantially decreased to a normal level over UVB treatment alone. This result was in line with our finding for the GTP and UVB effects on c-fos mRNA level, suggesting that the c-fos protein level could rise or drop rapidly in a short term.

DISCUSSION

AMD is a commonly retina disease causing millions of dollars in health care costs annually. UV-induced damage is recognized as an important molecular trigger for the initiation of AMD and new treatment strategies are needed to prevent the development of AMD caused by prolonged exposure to UV light. Our aim in this study is to evaluate GTP as a potential chemopreventive agent in UVB-induced damage to RPE cells because it has been proved that GTP had a protective effect against UVB-induced damage to HaCaT cells. The results from our study also showed that GTP alleviated UVB-induced intracellular ultra structure degeneration, especially reducing the deformation of mitochondrion, implicating that GTP might alleviated the UVB-induced mitochondrion-mediated cell damage. We also analyzed c-fos mRNA and protein levels by real-time PCR and western blot. It was confirmed that UVB did increase expression of c-fos, which was similar to previous study (Olson et al., 2010). In addition the UVB-caused ROS may be a factor that plays a role in RPE cell damage because of its affecting on intracellular signal pathway.

The production of ROS may lead to activate downstream cAMP response element binding protein (CREB) kinases to phosphorylate CREB, then the phosphorylated CREB could activate the transcription of the c-fos genes and the synthesis of c-fos protein (Tang et al., 2001; MelissaBowden 2002; Olson et al., 2010), which was confirmed in our experiment. The c-fos protein would drive the synthesis of transcription factor activator

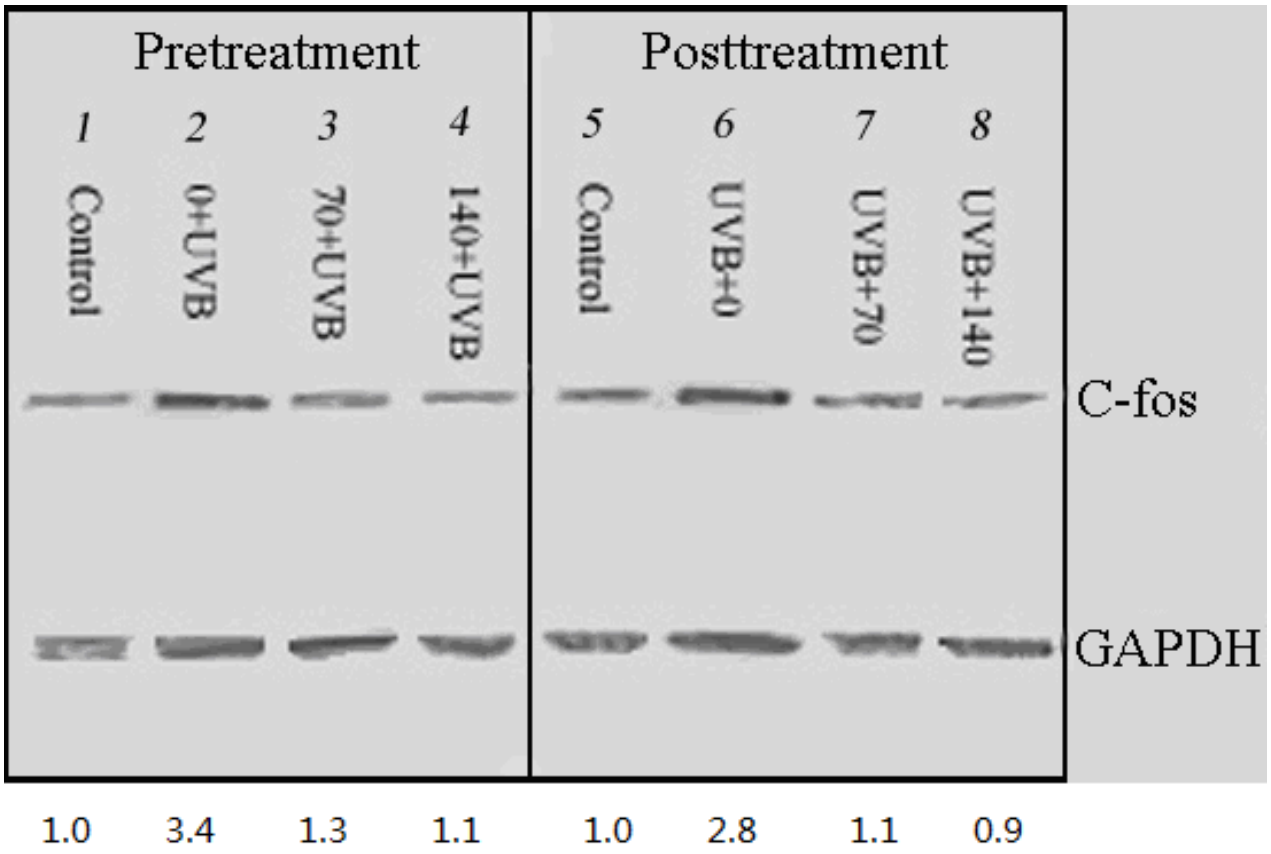

Figure 3. Expression of c-fos and GAPDH protein. The RPE cells were exposed to 100 mJ/cm^2 UVB for 2 h. Protein extracts were prepared and 25 mg were subjected to western blot analysis. For loading control, GAPDH was detected.

protein-1 (AP-1) (Cooper et al., 2003). And AP-1 protein, mostly controlling cell life and death through their ability to regulate the expression and function of cell cycle regulators such as Cyclin D1, p53, p21$^{cip1/waf1}$, p19^{ARF} and p16, would trigger the programmed cell death of RPE (Eitan et al., 2001). In our study, pretreatment or post-treatment with GTP on RPE cells could decrease c-fos expression to a normal level comparable with control, which was related to the ROS-scavenging capacity of GTP (Abe et al., 2000; Paul et al., 2005; Weinreb et al., 2009), suggesting that the AP-1 mediated cell necrosis or programmed death could be suppressed by GTP indirectly.

It was also revealed that GTP could repair UVB-induced DNA lesions. GTP could reduce the DNA cyclobutane pyrimidine dimers (CPDs), which was produced after UVB irradiation (Afaq, 2011). It was clear that pretreatment of human skin with GTP before UVB exposure could decreased the formation of UVB-induced CPDs (Katiyar et al., 2001). GTP and its major component (-)-epigallocatechin-3-gallate (EGCG) could reduce UVB-induced DNA damage in human cells through induction of DNA repair (Schwarz et al., 2008). And GTP could alleviate the damage to SKH-1 hairless mice caused by UVB due to induction of DNA repair (Mantena et al., 2005). In our previous experiment, the DNA single strand break caused by UVB was inhibited by the treatment of GTP (Xu et al., 2010). Taken together, the results of our research indicated that UVB induces c-fos activity to levels greater than that of non-treated cells, while the application of GTP could scavenge the ROS induced by UVB radiation, block the subsequent signal pathways as well as inhibit the cell necrosis or programmed death. It was concluded that GTP could be a potential treatment for UVB-induced AMD. While the dose used in our experiment was extremely higher than maximum amount of absorbing in human body, the further investigation on pharmacokinetics of GTP should be investigated in future.

REFERENCES

Abe I, Kashiwagi K, Noguchi H (2000). Antioxidative galloyl esters as enzyme inhibitors of p-hydroxybenzoate hydroxylase. FEBS Lett., 483(2-3): 131-134.

Afaq F (2011). Natural agents: Cellular and molecular mechanisms of photoprotection. Arch. Biochem. Biophys., 508(2): 144-151.

Anderson RF, Fisher LJ, Hara Y, Harris T, Mak WB, Melton LD, Packer JE (2001). Green tea catechins partially protect DNA from ·OH

radical-induced strand breaks and base damage through fast chemical repair of DNA radicals. Carcinogenesis, 22(8): 1189-1193.
Bok D (1990). Processing and transport of retinoids by the retinal pigment epithelium. Eye, 4(2): 326-332.
Christmann M, Tomicic MT, Origer J, Aasland D, Kaina B (2006). c-Fos is required for excision repair of UV-light induced DNA lesions by triggering the re-synthesis of XPF. Nucleic Acids Res., 34(22): 6530-6539.
Cooper SJ, MacGowan J, Ranger-Moore J, Young MR, Colburn NH, Bowden GT (2003). Expression of Dominant Negative c-jun Inhibits Ultraviolet B-Induced Squamous Cell Carcinoma Number and Size in an SKH-1 Hairless Mouse Model11National Institutes of Health Grants CA27502 and the Cancer Research Foundation of America Fellowship Grant. Mol. Cancer Res., 1(11): 848-854.
Dosch J, Kaina B (1996). Induction of c-fos, c-jun, junB and junD mRNA and AP-1 by alkylating mutagens in cells deficient and proficient for the DNA repair protein O6-methylguanine-DNA methyltransferase (MGMT) and its relationship to cell death, mutation induction and chromosomal instability. Oncogene, 13(9): 1927-1935.
Du YY, Chen H, Zhong WL, Wu LY, Ye JH, Lin C, Zheng XQ, Lu JL, Liang YR (2008). Effect of temperature on the expression of genes related to the accumulation of chlorophylls and carotenoids in albino tea. Afr. J. Biotechnol., 7(12): 1881-1885.
Dunaief JL, Dentchev T, Ying GS, Milam AH (2002). The Role of Apoptosis in Age-Related Macular Degeneration. Arch. Ophthalmol., 120(11): 1435-1442.
Eitan S, Michael K (2001). AP-1 in cell proliferation and survival. Oncogene, 20: 2390-2400.
Haas S, Kaina B (1995). c-Fos is involved in the cellular defence against the genotoxic effect of UV radiation. Carcinogenesis, 16(5): 985-991.
Hollander MC, Fornace AJ (1989). Induction of fos RNA by DNA-damaging Agents. Cancer Res., 49(7): 1687-1692.
Jane VH, Balz F (2003). Tea Catechins and Polyphenols: Health Effects, Metabolism, and Antioxidant Functions. Crit. Rev. Food Sci. Nutr., 43(1): 89-143.
Katiyar SK, Afaq F, Perez A, Mukhtar H (2001). Green tea polyphenol (–)-epigallocatechin-3-gallate treatment of human skin inhibits ultraviolet radiation-induced oxidative stress. Carcinogenesis, 22(2): 287-294.
Katiyar SK, Vaid M, van Steeg H, Meeran SM (2010). Green Tea Polyphenols Prevent UV-Induced Immunosuppression by Rapid Repair of DNA Damage and Enhancement of Nucleotide Excision Repair Genes. Cancer Prev. Res., 3(2): 179-189.
Kelly MR, Geigerman CM, Loo G (2001). Epigallocatechin gallate protects U937 cells against nitric oxide-induced cell cycle arrest and apoptosis. J. Cell. Biochem., 81(4): 647-658.
Liang FQ, Godley BF (2003). Oxidative stress-induced mitochondrial DNA damage in human retinal pigment epithelial cells: a possible mechanism for RPE aging and age-related macular degeneration. Experimental Eye Res., 76(4): 397-403.
Liang Y, Jørgensen A, Kæstel CG, Wiencke AK, Lui GM, la Cour M, Röpke C, Nissen MH (2000). Bcl-2, Bax, and c-Fos expression correlates to RPE cell apoptosis induced by UV-light and daunorubicin. Curr. Eye Res., 20(1): 25-34.
Lu YP (2002). Topical applications of caffeine or (-)-epigallocatechin gallate (EGCG) inhibit carcinogenesis and selectively increase apoptosis in UVB-induced skin tumors in mice. Proc. Nat. Acad. Sci., 99(19): 12455-12460.
Malhomme de la Roche H, Seagrove S, Mehta A, Divekar P, Campbell S, Curnow A (2010). Using natural dietary sources of antioxidants to protect against ultraviolet and visible radiation-induced DNA damage: An investigation of human green tea ingestion. J. Photochem. Photobiol. B: Biol., 101(2): 169-173.
Mantena SK, Meeran SM, Elmets CA, Katiyar SK (2005). Orally Administered Green Tea Polyphenols Prevent Ultraviolet Radiation-Induced Skin Cancer in Mice through Activation of Cytotoxic T Cells and Inhibition of Angiogenesis in Tumors. J. Nutr., 135(12): 2871-2877.
Melissa G, Bowden GT (2002). Ultraviolet B (UVB) induction of the c-fos promoter is mediated by phospho-cAMP response element binding protein (CREB) binding to CRE and c-fos activator protein 1 site (FAP1) cis elements. Gene, 293: 169-179.
Noell WK, Walker VS, Kang BS, Berman S (1966). Retinal Damage by Light in Rats. Investigative Ophthalmol. Visual Sci., 5(5): 450-473.
Olson ER, Melton T, Dickinson SE, Dong Z, Alberts DS, Bowden GT (2010). Quercetin Potentiates UVB-Induced c-Fos Expression: Implications for Its Use as a Chemopreventive Agent. Cancer Prev., Res., 3(7): 876-884.
Paul B, Hayes CS, Kim A, Athar M, Gilmour SK (2005). Elevated polyamines lead to selective induction of apoptosis and inhibition of tumorigenesis by (−)-epigallocatechin-3-gallate (EGCG) in ODC/Ras transgenic mice. Carcinogenesis, 26(1): 119-124.
Schwarz A, Maeda A, Gan D, Mammone T, Matsui MS, Schwarz T (2008). Green Tea Phenol Extracts Reduce UVB-induced DNA Damage in Human Cells via Interleukin-12†. Photochem. Photobiol., 84(2): 350-355.
Szaflik JP, Janik-Papis K, Synowiec E, Ksiazek D, Zaras M, Wozniak K, Szaflik J, Blasiak J (2009). DNA damage and repair in age-related macular degeneration. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 669(1-2): 169-176.
Tang QB, Chen WX, Gonzales MS, Finch J, Inoue H, Bowden GT (2001). Role of cyclic AMP responsive element in the UVB induction of cyclooxygenase-2 transcription in human keratinocytes. Oncogene, 20(37): 5164-5172.
Vayalil PK, Mittal A, Hara Y, Elmets CA, Katiyar SK (2004). Green Tea Polyphenols Prevent Ultraviolet Light-Induced Oxidative Damage and Matrix Metalloproteinases Expression in Mouse Skin. J. Investig. Dermatol., 122(6): 1480-1487.
Weinreb O, Amit T, Mandel S, Youdim M (2009). Neuroprotective molecular mechanisms of (−)-epigallocatechin-3-gallate: a reflective outcome of its antioxidant, iron chelating and neuritogenic properties. Genes Nutr., 4(4): 283-296.
Wu LY, Zheng XQ, Lu JL, Liang YR (2009). Protective effect of green tea polyphenols against ultraviolet B-induced damage to HaCaT cells. Hum. Cell, 22(1): 18-24.
Xu JY, Wu LY, Zheng XQ, Lu JL, Wu MY, Liang YR (2010). Green Tea Polyphenols Attenuating Ultraviolet B-Induced Damage to Human Retinal Pigment Epithelial Cells In Vitro. Investigative Ophthalmology and Visual Sci., 51(12): 6665-6670.
Young RW (1988). Solar radiation and age-related macular degeneration. Surv. Ophthalmol., 32(4): 252-269.
Yusuf N, Irby C, Katiyar KS, Elmets CA (2007). Photoprotective effects of green tea polyphenols. Photodermatol. Photoimmunol. Photomed., 23: 48-56.