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UVB light regulates expression of antioxidants and inflammatory mediators in human corneal epithelial cells

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Abstract

The cornea is highly sensitive to ultraviolet B (UVB) light-induced oxidative stress, a process that results in the production of inflammatory mediators which have been implicated in tissue injury. In the present studies, we characterized the inflammatory response of human corneal epithelial cells to UVB (2.5 - 25 mJ/cm²). UVB caused a dose-dependent increase in the generation of reactive oxygen species in the cells. This was associated with increases in mRNA expression of the antioxidants Cu,Zn superoxide dismutase (SOD), Mn-SOD, catalase and heme oxygenase-1 (HO-1), as well as the glutathione *S*-transferases (GST), GSTA1-2, GSTA3, GSTA4, GSTM1, mGST2. UVB also upregulated expression of the proinflammatory cytokines, IFN γ , IL-1 β , TGF β and TNF α , and enzymes important in prostaglandin (PG) biosynthesis including cyclooxygenase-2 (COX-2) and the PG synthases mPGES-2, PGDS, PGFS and thromboxane synthase, and in leukotriene biosynthesis including 5-lipoxygenase (5-LOX), and the epidermal and platelet forms of 12-LOX and 15-LOX-2. UVB was found to activate JNK and p38 MAP kinases in corneal epithelial cells; ERK1/2 MAP kinase is constitutively active, but its activity was increased following UVB treatment. Inhibition of p38 blocked UVB-induced expression of TNF α , COX-2, PGDS and 15-LOX-2, while JNK inhibition suppressed TNF α and HO-1. These data indicate that UVB modulates corneal epithelial cell expression of antioxidants and proinflammatory mediators by distinct mechanisms. Alterations in expression of these mediators are likely to be important in regulating inflammation and protecting the cornea from UVB-induced oxidative stress.

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Keywords

ultraviolet light; ocular; oxidative stress; prostaglandins; leukotrienes

1. Introduction

The higher energy wavelengths of ultraviolet light from sunlight including UVB (290-320 nm) are known to cause oxidative stress and ocular injury [1, 2]. The corneal epithelium serves as a protective barrier, absorbing UVB light and minimizing damage to the interior eye including the lens and retina. However, in excessive amounts, UVB light can injure the cornea, a process that causes aberrant epithelial cell growth and differentiation, as well as cell death via necrosis and apoptosis [3, 4]. This can result in corneal opacities or photokeratitis [5-7]. UVB-induced corneal injury is thought to be initiated by oxidative stress due to the localized generation of reactive oxygen species (ROS) by corneal epithelial cells [8, 9]. ROS, including superoxide anion, hydrogen peroxide and hydroxyl radicals, can damage DNA, resulting in mutations [10]. ROS can also oxidize proteins and lipids, leading to the generation of highly toxic electrophilic species including malondialdehyde and 4-hydroxynonenal, which can initiate inappropriate or altered cellular signal transduction pathways and contribute to toxicity [10].

A critical step in limiting oxidative damage involves upregulation of antioxidants that remove or detoxify ROS [11]. UVB light has been shown to upregulate the antioxidant enzymes, glutathione peroxidase, heme oxygenase-1 (HO-1) and the glutathione *S*-transferases GSTA1A-2 and GSTM1 in mouse keratinocytes [12] and glutathione peroxidase, GSTA4 and GSTP1 in human and rat skin [13, 14]. In a rabbit eye model, reduced corneal expression of antioxidants including catalase, glutathione peroxidase and superoxide dismutase (SOD) was observed following UVB light treatment, a process thought to accelerate oxidative stress and tissue injury [15]. UVB-induced oxidative stress in the skin is also associated with the initiation of an inflammatory response characterized by the generation of cytokines, growth factors and lipid-derived eicosanoids [16]. Previous studies in mouse and human keratinocytes and corneal epithelial cells showed that exposure to UVB light stimulated production of proinflammatory cytokines and growth factors including tumor necrosis factor- α (TNF- α), interleukin (IL)-1 β , tumor growth factor β (TGF β) and interferon- γ (IFN γ) [7]. In skin keratinocytes, UVB light increased the release of PGE₂ and leukotriene B₄, a process associated with upregulation of cyclooxygenase-2 (COX-2) and prostaglandin and leukotriene synthases, enzymes mediating the generation of prostaglandins and leukotrienes [17-19].

At present, little is known about the mechanisms by which corneal epithelial cells respond to UVB light-induced oxidative stress and this represents the focus of the present studies. We found that human corneal epithelial cells were highly responsive to UVB light, which effectively upregulated mRNA expression of proinflammatory cytokines, growth factors and enzymes important in mediating prostaglandin and leukotriene biosynthesis, as well as antioxidants. Moreover, these responses were regulated by mitogen activated protein kinases. Coordinate regulation of the production of inflammatory mediators is likely important in mediating UVB light induced corneal inflammation and tissue injury. Alterations in antioxidants may be a key adaptive mechanism of controlling oxidative stress and protecting against ROS-induced cytotoxicity following exposure to UVB light.

2. Materials and Methods

2.1. Chemicals and reagents

Rabbit polyclonal antibodies to p38, phospho-p38, JNK, phospho-JNK, ERK 1/2, and phospho-ERK 1/2 were purchased from Cell Signaling Technology (Beverly, MA), and horseradish peroxidase-labeled goat anti-rabbit secondary antibodies and detergent-compatible protein assay reagents from Bio-Rad Laboratories (Hercules, CA). M-MLV Reverse Transcriptase was from Promega (Madison, WI) and the Western Lightning enhanced chemiluminescence kit (ECL) from Perkin Elmer Life Sciences, Inc. (Boston, MA). SYBR Green Master Mix and other PCR reagents were from Applied Biosystems (Foster City, CA). SP600125 was purchased from Calbiochem (La Jolla, CA). Cell culture reagents, 2',7'-dichlorofluorescein-diacetate (DCFH-DA) and keratinocyte growth medium with supplement kits were purchased from Invitrogen Corp (Carlsbad, CA). Tri reagent, SB203580, protease inhibitor cocktail and all other chemicals were Sigma (St. Louis, MO) unless otherwise indicated.

2.2. Cells and UVB light treatments

The human corneal epithelial (HCE) cell line was kindly provided by Kaoru Araki-Sasaki, Kinki Central Hospital (Hyogo, Japan) and grown in keratinocyte serum-free medium (K-SFM), supplemented with fetal bovine serum (5%), gentamicin (0.05 %), epidermal growth factor (0.05 µg/ml) and bovine pituitary extract (0.05 mg/ml). The cells were originally isolated from donor corneas and immortalized using a recombinant SV40-adenovirus [20]. HCE cells were exposed to UVB light using a bank of 2 Westinghouse FS40BL light bulbs, calibrated with an International Light IL-1700 UV-radiometer as previously described [12, 21]. Briefly, cells (2×10^5) were seeded into 6 well culture dishes and allowed to grow 3-4 days until approximately 90% confluent. Cells were then exposed to UVB (2.5 - 25 mJ/cm²) in phosphate-buffered saline (PBS); PBS was then removed and the cells were refed with medium. The times of exposure to UVB light were 30 sec, 1 min, 2 min and 5 min. In control experiments, cells were mock irradiated for 5 min. In some experiments, the p38 MAP kinase inhibitor, SB203580 (10 µM), or the JNK kinase inhibitor, SP600125 (20 µM), was added to the medium and the cells preincubated at 37°C for 3 h prior to UVB treatment. Cell viability following UVB treatment was assessed by measuring cell growth as determined by protein content of the cultures. Low doses of UVB light (2.5-10 mJ/cm²) had no effects of cell growth. At 25 mJ/cm², we found a 15% inhibition of cell growth.

2.3. Western blotting

Cell lysates were prepared from control and UVB light treated cells using SDS-lysis buffer (10 mM Tris-base and 1% SDS, pH 7.6 supplemented with a protease inhibitor cocktail consisting of 4-(2-aminoethyl)benzenesulfonyl fluoride, aprotinin, bestatin hydrochloride, N-(trans-epoxysuccinyl)-L-leucine 4-guanidinobutylamide, EDTA and leupeptin) as previously described [12]. Protein lysates (20 µg) were separated on 10% SDS-polyacrylamide gels and then transferred to nitrocellulose. After incubation in blocking buffer (5% dry milk Tris-buffered saline with 0.1% Tween 20), the membranes were incubated overnight at 4°C with primary antibodies followed by horseradish peroxidase-conjugated secondary antibodies for 1 hr at room temperature. Protein expression was visualized using ECL reagents.

2.4. Measurement of ROS production

Intracellular hydrogen peroxide production was measured using DCFH-DA and flow cytometry as previously described [22]. Briefly, the cells were incubated with DCFH-DA (5 µM) in PBS for 15 min, washed with PBS, exposed to UVB light (2.5-25 mJ/cm²), and then

immediately analyzed on a Beckman Coulter Cytomics FC 500 flow cytometer (Miami, FL). Control cells were mock irradiated. In preliminary experiments we found similar DCF fluorescence in cells mock irradiated for 30 sec, 1 min, 2 min and 5 min. Therefore, we used a 5 min mock irradiation for control experiments. Data were analyzed using Beckman Counter CXP software.

2.5. Real-time PCR

RNA was isolated from the cells using Tri Reagent (Sigma) following the manufacturers protocol. RNA was converted to cDNA using M-MLV reverse transcriptase. The cDNA was diluted 1:10 in RNase-DNase-free water for PCR analysis. For each gene, a standard curve was generated from serial dilutions of cDNA. All values were normalized to β -actin ($n = 3$). The control was assigned a value of one and changes in gene expression calculated relative to this control. Real-time PCR was performed on an ABI Prism 7900 Sequence Detection System (Life Technologies, Carlsbad, CA) using 96-well optical reaction plates. SYBR-Green was used for detection of the fluorescent signal and the standard curve method was used for relative quantitative analysis. The primer sequences for the genes were generated using Primer Express software (Life Technologies) and the oligonucleotides were synthesized by Integrated DNA Technologies, Inc. (Coralville, IA). The forward and reverse (5'-3') primer sequences used are listed in Table 1.

2.6. Statistical analysis

Data were evaluated using the Student's *t*-test and differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. UVB light-induced oxidative stress in HCE cells

In initial studies, we determined if UVB light causes oxidative stress in corneal epithelial cells by quantifying its ability to generate intracellular hydrogen peroxide. Using the hydroperoxy-sensitive probe DCFH-DA in conjunction with flow cytometry, we found that HCE cells generated low basal levels of hydrogen peroxide (Fig. 1). UVB (2.5 - 25 mJ/cm²) treatment resulted in a dose-dependent increase in hydrogen peroxide production; a 20-30-fold increase in hydrogen peroxide production was evident after exposure to the maximal UVB dose tested.

3.2. UVB light alters mRNA expression of antioxidant enzymes

We next examined the effects of UVB light on expression of primary antioxidant enzymes. UVB light treatment of HCE cells resulted in increased mRNA expression of Cu,Zn-SOD, Mn-SOD, catalase and HO-1 (Fig. 2). For each of these genes, maximal effects were observed at 5-10 mJ/cm². At the highest dose of UVB (25 mJ/cm²), mRNA expression was reduced to near basal levels. The glutathione *S*-transferases (GST) consists of a class of secondary intracellular antioxidant enzymes that function to eliminate oxidized macromolecules and xenobiotics via glutathione conjugation and include both cytosolic (GSTA, GSTM and GSTP families) and microsomal (mGST1, mGST2 and mGST3) enzymes [23-26]. Significant increases in mRNA expression of the GSTA family including GSTA1-2, GSTA3 and GSTA4, as well as GSTM1 and mGST2 were noted, in HCE cells following UVB light treatment (Fig. 2). In contrast, a 2-fold decrease in mGST3 expression was evident after exposure of the cells to 25 mJ/cm² UVB light. No major changes were observed in mRNA expression for GSTP1 or mGST1 at any dose of UVB tested.

3.3. UVB light alters mRNA expression of enzymes generating eicosanoids

Arachidonic acid, released from the phospholipid membrane by phospholipase A₂, is oxidized by cyclooxygenase (COX) and lipoxygenase (LOX) enzymes to generate prostaglandins (PG) and leukotrienes, respectively [19]. UVB light treatment of HCE cells resulted in a dose-related increase in expression of mRNA for COX-2, microsomal PGE₂ synthase (mPGES-2), PGD₂ synthase (PGDS), PGF₂ synthase (PGFS), and thromboxane synthase (TXAS) (Fig. 3). Maximal increases in expression of these genes were observed at 10 mJ/cm² UVB. In contrast, at the highest dose of UVB tested, a 2-fold decrease in mPGES-1 and PGI₂ synthase (PGIS) expression was evident (Fig. 3). UVB light had no effect on COX-1 mRNA expression.

We next determined if UVB light altered expression of the LOX family of enzymes. Increases in mRNA expression for 15-LOX-2, LTC₄ synthase, 12-LOX, 5-LOX, FLAP and LTA₄ hydrolase were observed in HCE cells after UVB treatment (Fig. 3). While increases in expression of LTC₄ synthase, 5-LOX, FLAP and LTA₄ hydrolase were evident after exposure to 5-10 mJ/cm² UVB, 15-LOX-2 and epidermal type 12-LOX increased continuously over the dose range tested (2.5 to 25 mJ/cm²) (Fig. 3). Cytokine expression was also analyzed as a marker of UVB-induced inflammation. UVB caused a dose-dependent increase in IFN γ , TNF α , TGF β and IL-1 β mRNA expression with maximal effects at 10-25 mJ/cm² (Fig. 4).

3.4. Role of MAP kinase signaling in regulating expression of antioxidants and inflammatory proteins

In our next series of studies, we analyzed the role of MAP kinases in UVB-induced alterations in gene expression. UVB treatment (10-25 mJ/cm²) resulted in a dose-dependent activation of JNK and p38 MAP kinase expression in HCE cells, as measured by increases in the phosphorylated forms of the enzymes (Fig. 5). In contrast, relatively small increases in activated ERK1/2 were detected at doses of (2.5-15 mJ/cm² UVB (Fig. 5). HCE cells also constitutively expressed activated phosphorylated ERK1/2 kinases, but not JNK or p38 kinase. To determine if JNK and p38 MAP kinases mediated UVB-induced changes in mRNA expression in HCE cells, we used specific enzyme inhibitors. Treatment of the cells with the p38 MAP kinase inhibitor, SB203580, markedly suppressed UVB-induced expression of COX-2, 15-LOX-2 and TNF α while expression of PGDS and IFN- γ was decreased. In contrast, inhibition of JNK kinase using SP600125 markedly suppressed expression of HO-1 and TNF α and decreased PGDS, 15-LOX-2 and IFN- γ (Fig. 6).

4. Discussion

ROS are key mediators of many of the cytotoxic effects of UVB light in epithelial cells [27, 28]. ROS generated by UVB can trigger oxidative stress including lipid peroxidation, protein oxidation and DNA damage, as well as cytokine and growth factor signaling leading to changes in gene expression [29, 30]. Consistent with previous studies in mouse and human keratinocytes [12, 31], we found that UVB light readily stimulates the formation of intracellular ROS in human corneal epithelial cells, as measured by the production of hydrogen peroxide. These findings are in accord with reports that UVB alters expression of markers of oxidative stress in human and rabbit corneal epithelial cells, and in rabbit corneal tissue [32-34].

We also found that UVB upregulates expression of both primary and secondary antioxidant enzymes, which most likely reflects an adaptive cytoprotective response to oxidative stress [35, 36]. Thus, UVB increased mRNA expression of Mn-SOD and Cu,Zn-SOD, HO-1 and catalase. Mn-SOD is a mitochondrial-associated enzyme, while Cu,Zn-SOD is cytosolic. Coordinate regulation of these antioxidants, as well as catalase, is presumably key in

protecting different intracellular compartments in corneal epithelial cells from UVB-induced increases in formation of superoxide anion and hydrogen peroxide, respectively [37]. We have previously shown that mRNA expression for catalase and Cu,Zn-SOD are not affected by UVB in mouse keratinocytes, while Mn-SOD is suppressed [12]. Differences in expression of these enzymes in cornea and skin may be due to unique characteristics of epithelial cells in different tissues. Increased expression of these enzymes in the cornea may be required to protect against ROS-induced damage to the lens and/or retina at higher fluences of UVB [9, 15]. HO-1, the rate-limiting enzyme in the catabolism of the prooxidant heme, functions as an antioxidant/antiinflammatory protein and is induced in many cells by oxidative stress [38]. Our findings of increased mRNA expression for HO-1 in corneal epithelial cells is consistent with earlier studies showing that UVB induces HO-1 mRNA and protein in keratinocytes [12]. In the rabbit cornea, HO-1 has been shown to attenuate corneal inflammation and accelerate wound healing after injury. Increased expression of HO-1 in human corneal epithelial cells following UVB treatment may be important not only in limiting oxidative stress and photokeratitis, but also in mitigating UVB-induced tissue injury [39].

The GST enzymes are secondary antioxidants that conjugate reduced glutathione to electrophilic compounds, decreasing their potential for inducing cytotoxicity [23]. Preferred substrates have been identified for different classes of GSTs. For example, GSTP1 and the GSTA family of enzymes are known to detoxify lipid peroxidation products, a reaction important in breaking radical-forming chain reactions, while GSTM1 protects against protein oxidation [23-25]. Total GST activity has been reported to increase in the skin following UVB treatment [23, 40, 41]. UVB also increases mRNA expression of GSTA1-2 and GSTA4 in primary cultures of undifferentiated and differentiated mouse keratinocytes [12]. Similarly, the present studies demonstrate UVB upregulated mRNA's for these GSTs, as well as for GSTA3 and GSTM1 in corneal epithelial cells. Microsomal GSTs consist of a class of enzymes that function to protect against microsomal lipid peroxidation. UVB exposure resulted in increased expression of mGST2 which may play a role in mitigating UVB-induced oxidative stress in the microsomal compartment of the corneal cells. Consistent with earlier findings in mouse keratinocytes [12], we found that mGST3 decreased following UVB treatment of corneal epithelial cells. mGST3 has been reported to mediate glutathione conjugation to leukotriene A₄, a reaction important in generating proinflammatory leukotrienes (see further below) [24]. In both corneal epithelial cells and keratinocytes, decreases in mGST3 may limit production of proinflammatory lipid mediators and contribute to the resolution of inflammation following UVB treatment.

As highly active arachidonic acid-derived mediators, prostaglandins are known to regulate inflammation and evidence suggests that they may contribute to UVB-induced tissue injury [42]. For example, in human skin and rabbit eyes, toxic doses of UVB light stimulate production of PGE₂, PGF_{2α} and PGD₂ [18, 43]. Moreover, in both of these tissues, non-specific inhibitors of COX-2 reduce prostaglandin biosynthesis and prevent tissue injury. Hence, treatment of human skin with diclofenac, which inhibits UVB light-induced erythema, also reduces dermal levels of PGE₂, while treatment of rabbits with indomethacin, which reduces UVB light-induced corneal injury, suppresses ocular levels of PGE₂ and PGF_{2α} [6, 44]. Our studies demonstrate that UVB light induces mRNA for enzymes that generate prostaglandin biosynthesis in corneal epithelial cells including COX-2, mPGES-2, PGDS and PGFS and are consistent with earlier studies on the effects of UVB on rabbit ocular tissue [18, 43]. Previous studies have shown that both rat and rabbit corneas have the capacity to synthesize thromboxanes [45, 46]. Our data show that UVB light can upregulate mRNA expression of thromboxane synthase. Taken together, these data suggest that arachidonic acid-derived thromboxanes as well as prostaglandins may be important mediators of UVB light toxicity in the cornea.

Leukotrienes and their metabolites are pleiotropic autotoxins that regulate inflammation and wound healing in the cornea [47, 48]. We found that UVB light treatment of corneal epithelial cells increased expression of mRNA for 5-LOX and its activator protein FLAP, an important pathway for generating 5-hydroperoxyeicosatetraenoic acid and its dehydration product LTA₄ from arachidonic acid. LTC₄ synthase is key for the generation of the cysteinyl leukotrienes (LTC₄, LTD₄ and LTE₄) while LTA₄ hydrolase mediates the conversion of LTA₄ to LTB₄. We found that UVB light also upregulates LTC₄ synthase, an enzyme important in mediating LTC₄ biosynthesis. Earlier work has shown that corneal inflammation is associated with increased production of LTB₄ [49]. Both LTB₄ and the cysteinyl leukotrienes are proinflammatory and function by binding to specific G protein coupled receptors on target cells. The actions of the leukotrienes in inflammation are multifactorial. For example, both LTB₄ and the cysteinyl leukotrienes stimulate chemokine production and endothelial cell-leukocyte adhesion, while LTB₄ is a potent neutrophil chemoattractant [48]. Active neutrophil infiltration into the cornea is known to be associated with infection and injury and increased production of LTB₄ may be an important mechanism mediating this process.

UVB light was also found to increase corneal epithelial mRNA expression for the platelet and epithelial forms of 12-LOX, as well as 15-LOX-2, enzymes mediating the generation of anti-inflammatory and protective lipid mediators, including the lipoxins and the docosahexaenoic acid-derived protectins and resolvins [50, 51]. Recent studies have shown that lipid mediators such as lipoxin A₄ and the neuroprotectin D1 are generated in the cornea where they inhibit chemokine formation and promote corneal wound healing [52, 53]. The importance of these enzymes in corneal epithelial function is also evident by the findings that mice deficient in 12/15-LOX synthesize reduced amounts of lipoxin A₄, and exhibit defects in corneal reepithelialization and neutrophil recruitment following wounding [52]. The precise mechanisms by which lipoxin A₄ and neuroprotectin D1 promote wound healing in the cornea is not known and may involve enhanced epithelial cell proliferation and/or stimulation of pro-survival repair pathways which lead to inhibition of apoptosis [52, 54].

It is well recognized that MAP kinase signaling cascades regulate the action of many growth factors and inflammatory mediators [55]. Earlier studies demonstrated that UVB is a potent activator of MAP kinase signaling, a process thought to be due to direct or indirect activation of growth factor and/or cytokine receptors and subsequent down-stream signaling, referred to as "the UV response" [56, 57]. Our laboratory and others have shown that UVB is a potent inducer of MAP kinase signaling in keratinocytes [12, 21]. These enzymes control expression of genes important in regulating cell growth and differentiation as well as oxidative stress and inflammation [56]. The present studies show that corneal epithelial cells are also highly responsive to UVB-induced MAP kinase signaling, specifically JNK and p38 MAP kinases. These enzymes regulate UVB-induced mRNA for enzymes that generate both eicosanoids and inflammatory mediators, although their expression is regulated by distinct mechanisms. Thus, while both COX-2 and 15-LOX-2 expression are controlled by p38 MAP kinase, JNK regulates HO-1 and IFN γ and both kinases regulate TNF α . Generally similar results have been reported in UVB treated mouse and human skin and/or keratinocytes [21, 58-62].

In summary, our data demonstrate that corneal epithelial cells are highly sensitive to UVB light-induced oxidative stress. This is associated with increased mRNA expression for antioxidants, as well as proinflammatory mediators including cytokines and enzymes that generate prostaglandins and leukotrienes. UVB light also upregulates expression of enzymes that can generate mediators that downregulate inflammation and promote wound healing including lipoxin A₄ and neuroprotectin D1. Coordinate regulation of these mediators in the

cornea is likely important in initiating, as well as controlling the resolution of inflammation and the repair of damaged cells and tissues. Whether or not the genes induced by UVB in the cornea contribute to its biological actions will depend on levels of expression of functional proteins and on production of both pro- and anti-inflammatory mediators. Further studies are needed to more clearly define the mediators produced by the corneal epithelial cells and their roles in modulating the responses of the cornea to UVB light.

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Abbreviations

COX-1	Cyclooxygenase-1
COX-2	cyclooxygenase-2
FLAP	5-lipoxygenase activating protein
GST	glutathione <i>S</i> -transferase
mGST	microsomal GST
HO-1	heme oxygenase-1
LTA₄ hydrolase	leukotriene A ₄ hydrolase
LTC₄ synthase	leukotriene C ₄ synthase
LOX	lipoxygenase
5-LOX	5-lipoxygenase
12-LOX-epi	12-lipoxygenase-epidermal type
12-LOX-plt	12-lipoxygenase platelet-type
IFN	interferon
IL	interleukin
PGE₂	prostaglandin E ₂
mPGES-1	microsomal PGE ₂ synthase-1
mPGES-2	microsomal PGE ₂ synthase-2
PGD₂	prostaglandin D ₂
PGDS	PGD ₂ synthase
PGF₂	prostaglandin F ₂
PGFS	PGF ₂ synthase
PGI₂	prostaglandin I ₂
PGIS	PGI ₂ synthase
PGH₂	prostaglandin H ₂
SOD	superoxide dismutase
TXA₂	thromboxane A ₂

TXAS	TXA ₂ synthase
TGFβ	tumor growth factor beta
TNFα	tumor necrosis factor alpha

Figure 1. UVB induces oxidative stress in human corneal epithelial cells

HCE cells were incubated with DCFH-DA (5 μ M) for 15 min. Cells were then rinsed with PBS, exposed to 2.5, 5, 10 or 25 mJ/cm² UVB light and analyzed by flow cytometry for DCF fluorescence. Control cells were treated with DCFH-DA and mock irradiated. The panel shows that UVB light increased DCF fluorescence intensity which is directly proportional to hydrogen peroxide production. Increases in fluorescence intensity are presented on a log scale.

Figure 2. Effects of UVB light on antioxidant enzyme gene expression

HCE cells were exposed to UVB (2.5 - 25 mJ/cm²) or control. After 24 hr, RNA was extracted and analyzed by real-time PCR. Data are presented as fold change in gene expression relative to controls. Each bar is the mean $\pm$ SE (n = 3). *Significantly different from control ($p < 0.05$).

Figure 3. Effects of UVB light on expression of the eicosanoid biosynthetic enzymes
HCE cells were exposed to UVB (2.5 - 25 mJ/cm²) or control. After 24 hr, RNA was extracted and analyzed by real-time PCR. Data are presented as fold change in gene expression relative to controls. Each bar is the mean $\pm$ SE (n = 3). *Significantly different from control ($p < 0.05$).

Figure 4. Effects of UVB light on cytokine expression

HCE cells were exposed to UVB (2.5-25 mJ/cm²) or control. After 24 h RNA was extracted and analyzed by real-time PCR. Data are presented as fold change in gene expression relative to controls. Each bar is the mean $\pm$ SE ($n = 3$). *Significantly different from control ($p < 0.05$).

Figure 5. Effects of UVB on MAP kinase expression in HCE cells

HCE cells were exposed to UVB (2.5-25 mJ/cm²) or control. After 15 min, cells were lysed and analyzed for total and phosphorylated p38, JNK, and ERK1/2 MAP kinases by Western blotting. One representative blot from three experiments is shown.

Figure 6. Effects of p38 and JNK MAP kinase inhibition on UVB-induced gene expression
Cells were incubated with SB203580 (10 μ M), a p38 MAP kinase inhibitor, or SP600125 (20 μ M), a JNK MAP kinase inhibitor, for 3 hr and then exposed to UVB (2.5 - 25 mJ/cm²) or control. After 24 h, RNA was extracted and analyzed by real-time PCR. Data are presented as fold change in gene expression relative to untreated controls. Differences were considered statistically significant at $p < 0.05$: ^ap38 kinase inhibitor treatment versus control, ^bJNK kinase inhibitor treatment versus control.

Table 1
Realtime PCR primer sequences

Gene	Forward (5' → 3')	Reverse (5' → 3')
β-actin	AAAGACCTGTACGCCAACAC	GTCATACTCCTGCTTGCTGAT
catalase	CGGAGATTCAACACTGCCAA	GAATGCCCGCACCTGAGTAA
COX-1	GTACCGCAAGAGGTTTGCA	ACGAGCTCCTGGAAGGAGG
COX-2	GCCTGATGATTGCCCCGACT	GCTGGCCCTCGCTTATGATCT
cPLA2	GATTGCATTCTGCACGTGATG	CCACCTGAACCCAATATGGC
FLAP	AAGTGGAGCACGAAAGCAGG	CGGTCCTCTGGAAGCTCCTC
GSTA1-2	TTGATGTTCCAGCAAGTGCC	CACCAGCTTCATCCCATCAAT
GSTA3	TTCTGCCCTTATGTCGACCTG	TGATCAAGGCAATCTTGGCAT
GSTA4	GCTCCACTATCCCAACGGAA	AAAACCCATCTCACGGACTCC
GSTM1	AGCAACGCCATCTTGCTGCTAC	CTCCCCACACAGGTTGTGC
GSTP1	CTATGGGAAGGACCAGCAGG	CCGTCAATCACCATGTCCAC
HO-1	GCTCAAAAAGATTGCCCAGA	GCGGTAGAGCTGCTTGAAC
IL-1β	CACTACAGCAAGGGCTTCAGG	GTCCATGGCCACAACAAC
IFNγ	GGCTTTTCAGCTCTGCATCG	TCCTGGCAGTAACAGCCAAGA
5-LOX	TCGAGTTCCCCTGCTACCG	TCAGGACAACCTCGACATCG
12-LOX-epi	ACCGACCTCTTGTCGGGAAC	CCCCACAATGGTCAGTGAGAT
12-LOX-plt	AGATGGAGCCCAATGGGAAG	GAGGCTGAATCTGGATGACCA
15-LOX-1	GAGATCACTGAAATCGGGCTG	GACAGGAAACCTCGGTCCT
15-LOX-2	TGATATTACCTGCTCCGCC	CAAACCTGCCCTGCACTGACA
LTA ₄ hydrolase	TGAAGTTTACCCGGCCCTTA	GGATTTGTCAAAGGCAGCA
LTC ₄ synthase	AGTACTTCCCGCTGTTCTCG	GAAAGAAGATGCCGGCGAC
mGST1	GCCCACCTGAATGACCTTGA	GAGGCCAATTCCAAGAAATGG
mGST2	GAACCTCGATCCTGCTGGCTG	CTTTGCTGACAGGCCGAGA
mGST3	CCACCCGCGTATAGCTTCTG	ACTCGTCCAACAATCCAGGC
mPGES-1	CACCGGAACGACATGGAGAC	GACGAAGCCCAGGAAAAGG
mPGES-2	GATGTACGTGGTGGCCATCA	CTCTTCTTCCGCAGCCTCAC
PGDS	CAAGGCTGGTGACTTTACGGA	AGTTAGCGACGGCAGGAATG
PGFS	CTCTGTACCACCTGGGAGGC	GGCCAATCCTGCATCCTTAC
PGIS	AGAGTATCCTTTGGCAAGCGG	GGAGAGTGGTCGTCTGCGAG
Cu,Zn-SOD	GTCGTAGTCTCCTGCAGCGTC	CTGGTTCCGAGGACTGCAA
Mn-SOD	TCTGGACAAACCTCAGCCCT	GCAACTCCCCTTTGGGTTCT
TGFβ	GAAGGGAGACAATCGCTTAGC	TGTAGACTCCTTCCCGGTTGAG
TNFα	CCTGCCCAATCCCTTATT	CCCTAAGCCCCCAATTCTCT
TXAS	CATCCATAATGGTCCCCTG	CGTCTCGGTTCTTATTGGGC