Effect of ultraviolet A exposure on transport of compatible organic osmolytes in human lens epithelial cells

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ABSTRACT. Compatible organic osmolytes, such as betaine, myoinositol, and taurine, are involved in antioxidant defense, protein stabilization, and stress responses. This osmolyte strategy requires the expression of specific osmolyte transporters such as betaine (BGT-1), myoinositol (SMIT), and taurine (TAUT). In contrast to the kidney, keratinocytes, and neural cells, few studies have examined osmolytes in human lens epithelial cells (HLECs). We examined the expression of mRNA specific for BGT-1, SMIT, and TAUT in HLECs. In comparison to normoosmotic (305 mOsM) controls, there was a 3-5-fold time-dependent reaction of *BGT-1*, *SMIT*, and *TAUT* mRNA levels in HLECs exposed to hyperosmotic stress (405 mOsM). Maximal responses were obtained for *BGT-1*, *SMIT*, and *TAUT* mRNA expression after 3, 24 and 9 h of hyperosmotic exposure, respectively. This expression was correlated with increased osmolyte uptake. In contrast, hypoosmotic (205 mOsM) stimulation led to a significant efflux of osmolytes. Exposure to ultraviolet A (340-400 nm) radiation

significantly stimulated osmolyte uptake. Increased osmolyte uptake was associated with upregulation of mRNA steady-state levels for osmolyte transporters in irradiated cells. These results demonstrate that ultraviolet A radiation leads to the accumulation of compatible organic osmolytes in HLECs as hyperosmotic pressure, which can maintain cellular environmental homeostasis.

Key words: Human lens epithelial cells; Organic osmolytes; Ultraviolet A

INTRODUCTION

Long-term exposure to ultraviolet radiation can cause cataract occurrence and development. Ultraviolet A (UVA, 320-400 nm) is a risk factor of cataract mainly because of lens absorption (Yam and Kwok, 2014). The mechanism of ultraviolet radiation in accelerating cataract development is caused by the generation of oxygen radicals, DNA fracture, and degeneration of lens protein kinase activation (Beshtawi et al., 2013). However, the effect of ultraviolet radiation on compatible organic osmolytes inside of the crystalline body is not well understood.

Compatible organic osmolytes, such as betaine, myoinositol, and taurine, are compounds that specifically accumulate in cells in response to hyperosmotic stress and are rapidly released from cells after hypoosmotic exposure (Kwon and Handler, 1995; Haussinger, 1996). These osmolytes are also involved in antioxidant defense, protein stabilization, and stress responses. Hypertonia can cause the acceleration of translation and synthesis of compatible organic osmolyte transporters, including BGT1, SMIT, and TAUT, which transport betaine, myoinositol, and taurine, respectively. A variety of tissues and organs for the osmolyte transport systems widely exist in the human body, including in the kidney, liver, and skin (Zhang et al., 1996; Burg et al., 1997; Warskulat et al., 1997a,b,c; Weik et al., 1998). However, there have been few reports regarding osmolyte transport systems in the human crystalline lens.

UV was previously found to increase intracellular reactive oxygen species, activation of protein kinase C, and p38 (Sarasin, 1999; Cheng et al., 2002), with reaction conditions similar to the hyperosmotic stress reaction. The final target of hypertonia and ultraviolet radiation is DNA, which leads to DNA fracture in cells (Heck et al., 2003). Numerous studies have found that high osmotic pressure can lead to the accumulation of compatible organic osmolytes inside cells, while UVA can cause a response that is similar to the hyperosmotic reaction (Thompson, 1988; Berry et al., 1994). Therefore, we examined whether UVA can cause similar accumulation of compatible organic osmolytes inside human lens epithelial cells (HLECs) as hyperosmotic pressure.

HLECs were found to maintain cellular environmental homeostasis. Furthermore, the taurine transport system is an important component of compatible organic osmolytes and resists DNA fracture caused by UVA in HLECs.

MATERIAL AND METHODS

Cells

HLECs were obtained from ScienCell Research Laboratories (Carlsbad, CA, USA)

and cultured in Dulbecco's modified Eagle medium containing 10% fetal bovine serum, 50 U/mL penicillin, and 200 mg/mL streptomycin, and stored at 37°C under an atmosphere of 5% CO_2 in air. Osmolarity changes were achieved by varying the NaCl concentration in the medium, as osmotic regulation of mRNA steady state levels for TAUT, BGT-1, and SMIT and osmotic effects on osmolyte transport are independent of Na^+ and Cl^- activity in all cell types examined, such as in kidney and liver cells as well as in human monocytes and macrophages.

UV irradiation

The medium was replaced with phosphate-buffered saline, and cells were exposed at a distance of 30 cm to UVA radiation from a UVA irradiation device (MUA-165, Beijing Huabin Photoelectric Instrument Co., Beijing, China) emitting over a range of 340-400 nm with a maximum of approximately 360 nm. Dosimetry was conducted using a Waldmann UV meter type II (Herbert Waldmann GmbH & Co., Villingen-Schwenningen, Germany) with a spectral sensitivity matching the main emission spectrum of approximately 350-400 nm. For sham-irradiation, cells were not exposed to UVA radiation, but were otherwise treated identically.

Real-time polymerase chain reaction (PCR)

Total RNA from HLECs was isolated using a total RNA extraction kit (Omega Bio-Tek, Norcross, GA, USA) and reverse-transcribed using a first-strand cDNA synthesis kit (D6110A; TAKARA, Shiga, Japan). Gene expression levels were measured by real-time SYBR Green PCR with the Gene Amp 5700 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) according to manufacturer instructions. The primers used for PCR were 5'-AAAGCTGTGACAATGATGCCG-3' and 5'-CCAGTCCAAGCAAGAGAAGCA-3' for *TAUT*, 5'-AACAGTGCCACCAGCTTTGTG-3' and 5'-ACTCGGCCACTTCAGAAATGG-3' for *BGT-1*, 5'-TCAACCACATCATTCCCAACG-3' and 5'-TGCTGTCTTCAGATTTCCCGT-3' for *SMIT*, and 5'-AAGATGG-TCAAGGTCGCAAGC-3' and 5'-GGTCCTTTTCACCAGCA AGCT-3' for hypoxanthineguanine-phosphoribosyl-transferase as an internal standard. PCR conditions were: 1 cycle at 50°C for 2 min and 95°C for 10 min, followed by 40 cycles at 95°C for 15 s, and 60°C for 1 min. The sample volume was 25 μL. After PCR, the specificity of the amplified DNA was assessed by recording the dissociation curve to distinguish the fluorescence peak corresponding to the amplicon from the peak due to primer-dimer formation.

Uptake of betaine, myoinositol, and taurine

Uptake of organic osmolytes was measured as described previously (Wu and Zhang, 2014). Briefly, HLECs were incubated for 16 h in serum-free culture medium at the indicated osmolarity. Thereafter, the medium was replaced with an identical volume of medium containing100 mM [^{14}C]betaine (48.1 Ci/mmol), [^{3}H]taurine (24 Ci/mmol), or [^{3}H]myoinositol (22.3 Ci/mmol, all purchased from Shanghai Company, Shanghai, China), and cells were incubated for the indicated periods of time. Cultures were rinsed 3 times with ice-cold stop solution containing 10 mM Tris-HEPES, pH 7.4, 300 mM mannitol, and 300 mM NaCl. Cells were air-dried and lysed, and aliquots were subjected to liquid scintillation counting and protein determination (Bio-Rad Protein Assay, Bio-Rad, Hercules, CA, USA).

Efflux of betaine, myoinositol, and taurine

HLECs were loaded with 10 mM [^{14}C]betaine, [^{3}H]taurine, or [^{3}H]myoinositol in serum-free culture medium for 16 h. Cells were rinsed twice with phosphate-buffered saline. After UVA irradiation, cells were incubated in betaine-, myoinositol-, and taurine-free norm-osmotic (305 mOsM) or hypoosmotic (205 mOsM) culture medium for the indicated periods of time. Medium and cell lysates were collected and radioactivity was measured by scintil-lation counting. Efflux of [^{14}C]betaine, [^{3}H]myoinositol, and [^{3}H]taurine into the supernatant was expressed as the percentage of total radioactivity in cell lysates plus supernatants.

Statistical analysis

Statistical analysis was performed using the 2-tailed Student t-test. P values < 0.05 were considered to be significant.

RESULTS

Expression of *BGT-1*, *SMIT*, and *TAUT* mRNA in HLECs

Under normoosmotic (305 mOsM) conditions, real-time reverse transcription-PCR analysis revealed that HLECs expressed mRNA specific for BGT-1, SMIT, and TAUT (0.2 ± 0.1, 3.9 ± 0.3, and 0.1 ± 0.2 mRNA copies/hypoxanthineguanine-phosphoribosyl-transferase mRNA copy). The levels of BGT-1, SMIT and TAUT mRNA were increased by 3-5-fold when HLECs were exposed to hyperosmotic stress (405 mOsM). Maximum *BGT-1*, *SMIT*, and *TAUT* mRNA expression was observed after 3, 24, and 9h of hyperosmotic exposure, respectively (Figure 1).
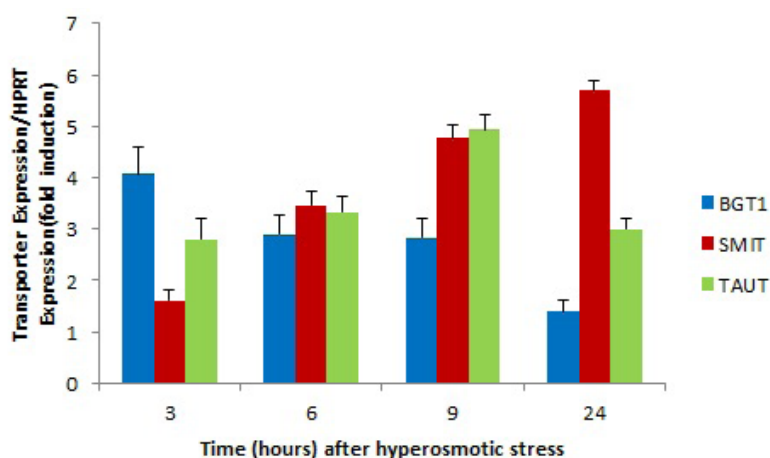

Figure 1. Reaction of *BGT-1*, *SMIT*, and *TAUT* mRNA in human lens epithelial cells (HLECs) after hyperosmotic exposure as detected by real-time reverse transcription-polymerase chain reaction. Hyperosmotic (405 mOsM) exposure of HLECs for the indicated time periods. Osmolarity was changed by adding NaCl. Results are given as the fold reaction based on *HPRT* (housekeeping) gene expression in stimulated *vs* unstimulated cells as a control. Gene expression under control (normoosmotic conditions in sham-irradiated cells) condition was measured at all indicated time points and arbitrarily set as 1. Data are from 3 independent experiments.

Organic osmolyte transport in HLECs

Measuring of the uptake of radiolabeled osmolytes indicated the functional significance of hyperosmotic stress-induced osmolyte transporter mRNA expression in HLECs. Under normoosmotic (305 mOsM) conditions, HLECs took up betaine, myoinositol, and taurine at low levels (Table 1). Taurine uptake was 3.6- and 2.9-fold higher than uptake of betaine and myoinositol over 2 h. Culture of HLECs in osmolyte-free, normoosmotic versus hyperosmotic media for 16 h and subsequent exposure for 2 h to media containing 100 mM betaine, myoinositol, or taurine led to a dramatic increase in osmolyte uptake in hyperosmotically stressed cells, with 2-, 1.7-, and 2.3-fold increases for betaine, myoinositol, and taurine, respectively. In addition, because of the moderate accumulation of osmolytes into cells, the 2-h uptake values do not reflect intracellular steady-state levels, but represent roughly initial uptake rates and thereby reflect transporter activity.

Table 1. Organic osmolyte transport in human lens epithelial cells.

Osmolyte Test conditions	Osmolyte uptake (nmol/mg protein)		
	Betaine	Myoinositol	Taurine
305 mOsM + sham-irradiation	1.1 ± 0.4	1.4 ± 0.1	4.0 ± 0.1
405 mOsM+sham-irradiation	2.3 ± 0.1*	2.4 ± 0.3*	9.0 ± 2.0*
305 mOsM+10 J/cm² UVA	1.3 ± 0.2	1.6 ± 0.4	5.4 ± 0.3
305 mOsM+30 J/cm² UVA	2.3 ± 0.3*	2.8 ± 0.2*	7.6 ± 0.1*

Cells were ultraviolet-irradiated or sham-irradiated as indicated and then incubated for 16 h in normoosmotic (305 mOsM)- or hyperosmotic medium (405 mOsM). Osmolarity was changed by adding 50 mM NaCl. Uptake of 100 mM taurine, betaine, or myoinositol was assessed for 2 h in medium with the same osmolarity. Data are reported as means ± standard error of the mean (N = 3). *Significantly different from the control condition (305 mOsM, sham-irradiated), P < 0.05.

Efflux of osmolytes after hypoosmotic exposure

HLECs were allowed to accumulate osmolytes (added at a concentration of 10 mM) for 16 h. Next, cells were washed twice and incubated in normoosmotic (305 mOsM) or hypoosmotic (205 mOsM) buffer for between 5 min and 1 h. This hypoosmotic stimulation induced a significant efflux of osmolytes (Table 2). The release of betaine in isoosmotic medium was only 31% after 1 h, whereas in hypoosmotic medium, this value was 36% after 5 min and 66% after 1 h. Efflux of myoinositol and taurine in isoosmotic medium was 15 and 26% after 1 h, but was 32 and 80% in hypoosmotic medium, respectively (Table 2).

Table 2. Osmolyte efflux from human lens epithelial cells following hypoosmotic exposure and ultraviolet A radiation.

Test conditions	Osmolyte efflux (% of total radioactivity)					
	Betaine		Myoinositol		Taurine	
	5 min	60 min	5 min	60 min	5 min	60 min
305 mOsM + sham-irradiation	17 ± 3	31 ± 1	13 ± 2	15 ± 2	16 ± 1	26 ± 4
205 mOsM + sham-irradiation	36 ± 4*	66 ± 6*	24 ± 3*	32 ± 3*	50 ± 3*	80 ± 6*
305 mOsM + 100 mJ/cm² UVA	22 ± 2	34 ± 3	16 ± 3	18 ± 3	19 ± 2	29 ± 3

Cells were allowed to accumulate betaine, myoinositol, or taurine (added at a concentration of 10 mM) for 16 h, washed twice, UVA- or sham-irradiated as indicated, and exposed to osmolyte-free normoosmotic (305 mOsM) or hypoosmotic (205 mOsM) Krebs-Henseleit buffer. Taurine, betaine, and myoinositol appearance in the supernatant was detected and expressed as the percentage of total radioactivity (contained in cells plus supernatant). Data are reported as the means ± standard error of the mean and are from 3 separate experiments. *Significantly different from the control condition (305 mOsM, sham-irradiated), P < 0.01.

UVA radiation induced alterations in *BGT-1*, *SMIT*, and *TAUT* mRNA levels and increased osmolyte uptake in HLECs

In this study, a UVA irradiation device was used, which primarily emitted in the long-wave UVA range 340-400 nm with an emission maximum of approximately 312 nm. UVA radiation (30 mJ/cm^2) significantly stimulated myoinositol and taurine uptake in HLECs; however, there was no significant difference after 10 mJ/cm^2 radiation (Table 1). In contrast to hypoosmotic exposure, UVA radiation did not affect osmolyte efflux from HLECs (Table 2). Modulation of osmolyte uptake and osmolyte transporter expression was observed after UVA irradiation. Accordingly, UVA radiation resulted in upregulation of all transporters tested (Figure 2). Expression of *BGT-1* mRNA was induced 4-, 4.5-, and 5-fold at 6, 12, and 24 h, respectively, after irradiation with 30 J/cm^2 UVA. *SMIT* mRNA expression increased by 3-fold at 10 and 30 J/cm^2 after 24 h. *TAUT* mRNA expression showed a concentration-dependent increase at both time points. Upregulation of osmolyte transporter mRNA expression was associated with significantly increased uptake of the respective osmolytes in UVA-irradiated HLECs (Table 1).
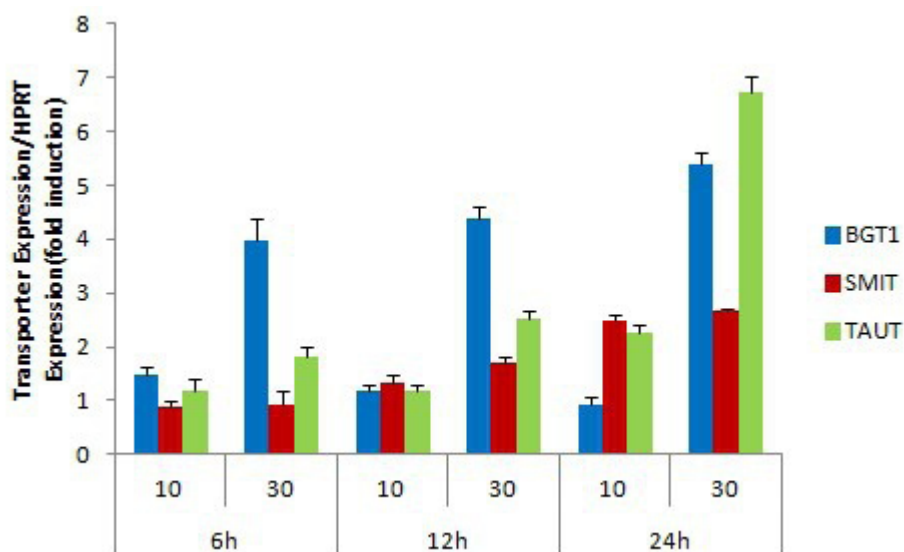

Figure 2. Reaction of *BGT-1, SMIT*, and *TAUT* mRNA in human lens epithelial cells (HLECs) after ultraviolet A (UVA) radiation as detected by real-time reverse transcription-polymerase chain reaction. HLECs were exposed to 2 doses of UVA radiation (10 or 30 J/cm^2) and detected at 6, 12, and 24 h after UVA treatment.

DISCUSSION

We found that betaine, myoinositol, and taurine were compatible organic osmolytes in HLECs. Our results showed that i) HLECs expressed mRNA specific for BGT-1, SMIT, and TAUT, and this expression was upregulated upon hyperosmotic exposure; ii) mRNA expression of osmolyte transporters was associated with uptake of the respective osmolytes, and osmolyte uptake was significantly increased in these cells under hyperosmotic stress; and iii)

incubation of HLECs in hypoosmotic medium led to rapid efflux of betaine, myoinositol, and taurine from cells.

Compatible organic osmolytes are compounds that specifically accumulate in cells in response to hyperosmotic stress. Organic osmolytes are non-perturbing solutes that do not interfere with protein function, even when present at high intracellular concentrations (Burg et al., 1997). Betaine, myoinositol, and taurine were previously identified as osmolytes in the brain, kidney, and liver and interfere with cell volume regulation and cell function (Haussinger, 1998). Compatible organic osmolytes are important not only for cell volume homeostasis but also for cell protection, such as against oxidative stress (Yancey et al., 1982; Huxtable, 1992). They can stabilize native protein structures and protein function (Welch and Brown 1996), and thus betaine and taurine can protect cells *in vitro* against various types of injury (Wettstein and Haussinger, 2000; Alfieri et al., 2002). Altered osmolyte content has been described for important diseases (vom Dahl et al., 2000; Hansen, 2001). Thus, it appears likely that similarly to the kidney, liver, and neural cells, HLECs possess an osmolyte strategy that may be important for maintaining cell volume homeostasis and reflect part of their stress response towards environmental noxae such as ultraviolet radiation.

A major source of oxidative stress is ultraviolet radiation, and cellular oxidative stress may result in cell hydration changes (Peus and Pittelkow, 2001). Because ultraviolet radiation and osmotic stimulation induce similar signaling events, the influence of UVA on osmolyte transport was investigated in HLECs in the present study. It should be noted that at wavelengths above 300 nm, the generation of oxidative stress becomes increasingly important because of the direct formation of DNA photoproducts (Kochevar, 1985; Stege et al., 2000), and the direct damage to lens homeostasis (Wu et al., 2011).

In this context, taurine was the most affected osmolyte under the stimulation conditions tested, and this osmolyte is therefore most likely to be related to UVA responses of HLECs. Increased osmolyte uptake after UVA irradiation in HLECs was associated with upregulation of osmolyte transporter mRNA expression. This indicates that the UVA-induced increase in osmolyte uptake most likely reflected an active process such as increased synthesis of transporter proteins rather than short-term activation of pre-existing transporters. Similarly, long-term induction of osmolyte transporter expression and osmolyte uptake has been reported for hyperosmotically stressed rat liver cells (Weik et al., 1998) and human monocytes and macrophages (Denkert et al., 1998) as well as ultraviolet-exposed keratinocytes (Thompson, 1998). It was recently shown that taurine improves epidermal barrier properties and may play a role in barrier homeostasis (Anderheggen et al., 2006). However, the mechanical effect of the UVA compromising the membrane followed by influx from a hyperosmolarity environment cannot be completely ruled out. Because taurine and betaine have antioxidative activities (Mehta et al., 2002; Patrick, 2002), the increased osmolyte uptake by UVA-irradiated HLECs may not only be important in cell volume homeostasis but also may be part of their defense strategy against UVA-induced disease such as cataract.

In conclusion, HLECs possess an osmolyte strategy with induction of osmolyte uptake by UVA. However, the influence of organic osmolytes is unknown. Thus, future *in vivo* studies should examine whether this osmolyte, through this or additional functions, is capable of protecting HLECs against ultraviolet radiation-induced biological effects. Studies employing TAUT knockout mice (Rockel et al., 2007) will be useful for testing taurine functions and their effects on the human lens.

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