Apoptosis of Corneal Epithelial Cells Caused by Ultraviolet B-induced Loss of K^+ is Inhibited by Ba^{2+}

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ABSTRACT UVB exposure at ambient outdoor levels triggers rapid K^+ loss and apoptosis in human corneal limbal epithelial (HCLE) cells cultured in medium containing 5.5 mM K^+, but considerably less apoptosis occurs when the medium contains the high K^+ concentration that is present in tears (25 mM). Since Ba^{2+} blocks several K^+ channels, we tested whether Ba^{2+}-sensitive K^+ channels are responsible for some or all of the UVB-activated K^+ loss and subsequent activation of the caspase cascade and apoptosis. Corneal epithelial cells in culture were exposed to UVB at 80 or 150 mJ/cm^2. Patch-clamp recording was used to measure UVB-induced K^+ currents. Caspase-activity and TUNEL assays were performed on HCLE cells exposed to UVB followed by incubation in the presence or absence of Ba^{2+}. K^+ currents were activated in HCLE cells following UVB-exposure. These currents were reversibly blocked by 5 mM Ba^{2+}. When HCLE cells were incubated with 5 mM Ba^{2+} after exposure to UVB, activation of caspases-9, -8, and -3 and DNA fragmentation were significantly decreased. The data confirm that UVB-induced K^+ current activation and loss of intracellular K^+ leads to activation of the caspase cascade and apoptosis. Extracellular Ba^{2+} inhibits UVB-induced apoptosis by preventing loss of intracellular K^+ when K^+ channels are activated. Ba^{2+} therefore has effects similar to elevated extracellular K^+ in protecting HCLE cells from UVB-induced apoptosis. This supports our overall hypothesis that elevated K^+ in tears contributes to protection of the corneal epithelium from adverse effects of ambient outdoor UVB.

KEY WORDS apoptosis, caspase, corneal epithelium, K^+ channel, potassium, tears, ultraviolet

I. INTRODUCTION

Ultraviolet (**UV**) radiation at wavelengths in the UVB (peak 302 nm) or UVC (peak 254 nm) ranges causes cells to go into apoptosis. Upon exposure to UV, a common initiating factor for apoptosis appears to be activation of K^+ channels in the cell membrane and loss of intracellular K^+, which moves out of the cell down its concentration gradient.[1-5] UV-induced activation of apoptotic signaling pathways then leads to activation of initiator and effector caspases, DNA fragmentation, formation of apoptosomes and finally, cell death.[6,7]

The cornea protects the lens and retina from damage by absorbing a majority of ambient UVB radiation.[8-10] Although the corneal epithelium in vivo is exposed to outdoor UVB at levels that can cause apoptosis, it appears to be relatively resistant to damage by ambient UVB. In contrast, we have previously reported that exposure of human corneal limbal epithelial (**HCLE**) cells to UVB at 80-200 mJ/cm^2 (a range relevant to ambient, outdoor exposure) in culture medium containing 5.5 mM K^+ causes immediate activation of K^+ channels, loss of 50% of intracellular K^+ within 10 minutes after exposure[11,12] and activation of initiator caspases-9 and -8 and the effector caspase-3. This demonstrates that corneal epithelial cells are, in fact, susceptible to damage by ambient levels of UVB. Based on this observation, the question arises, why are corneal epithelial cells acutely damaged by UVB in vitro but relatively protected from damage in vivo by ambient outdoor UVB?

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OUTLINE

The K^+ concentration ($[K^+]$) of tear fluid is 20-25 mM, a relatively high concentration compared to the $[K^+]$ in most other extracellular fluids.[13-15] We have previously suggested that the high $[K^+]$ in tears may protect the corneal epithelium from UVB-induced apoptosis.[11] High extracellular $[K^+]$ in tear fluid reduces the concentration gradient between the inside and outside of the epithelial cells. This may attenuate loss of intracellular $[K^+]$ when K^+ channels are activated by ambient UVB, in turn inhibiting the activation of apoptotic mechanisms in the corneal epithelium. In previous studies using patch-clamp recording and measurement of intracellular $[K^+]$ by ion chromatography, we reported that UVB-induced loss of K^+ from human corneal limbal epithelial (HCLE) cells is inhibited by incubation in medium with increased concentrations of K^+ (25-100 mM).[11,12] Incubation of HCLE cells in elevated levels of extracellular K^+, including the 25 mM concentration found in tears, also inhibited UVB-induced caspase activation and DNA fragmentation.[11,16,17] These observations support our overall hypothesis that the high concentration of K^+ in tear fluid may contribute to protection of the in vivo corneal epithelium from the harmful effects of ambient UVB by reducing the electrochemical gradient for loss of intracellular K^+ in response to UVB.

If UVB-induced K^+ loss from cells causes apoptosis, then potassium channel blockers should also protect HCLE cells from UVB. We have previously reported that the $K_v3.4$ channel blocker, BDS-1, reduces UVB-induced K^+ currents in HCLE cells by 50-75% and also attenuates apoptosis.[11,17] BDS-1, however, blocks only one type of K^+ channel and the residual UVB-induced current in the presence of BDS-1 suggests that other channel types may be present in HCLE cells. Blocking of additional K^+ channels would be expected to cause a greater reduction in K^+ currents and increased inhibition of apoptosis. Many types of K^+ channels, both inward and outward rectifying, are blocked by Ba^{2+}.[18-20] Exposure of cells to Ba^{2+} should therefore stabilize intracellular $[K^+]$ in cells exposed to UVB. We previously observed, using ion chromatography, that Ba^{2+} at 0.1, 1 or 5 mM K^+ inhibits UVB-induced loss of intracellular K^+ ions after exposure to UVB in a dose-dependent manner.[12] This suggests that Ba^{2+} should also inhibit UVB-induced apoptosis, and, if so, this would support our hypothesis that inhibiting loss of potassium can protect corneal epithelial cells from damage by UVB.

The purpose of the present study was to expand and complete our studies of the effects of blocking K^+ channels on apoptosis of corneal epithelial cells exposed to UVB[12,17] by using Ba^{2+} to achieve a complete inhibition of K^+ currents. Patch-clamp recording was used to confirm that Ba^{2+} blocks outward rectifying UVB-induced K^+ current. To investigate effects on apoptotic pathways, activation of caspases-3, -8 and -9 and also TUNEL staining were measured in cells incubated with Ba^{2+} following exposure to UVB.

II. METHODS

A. Cell Line Preparation

HCLE cells were maintained as monolayers in 6-well plates in Keratinocyte-SFM (KSFM, Life Technologies, Grand Island, NY), as previously described.[11,21] HCLE cells have the ability to stratify in specific culture conditions.[21] We have shown that stratified and monolayer HCLE cells respond similarly to UVB exposure and incubation in medium with elevated $[K^+]_o$.[16] For convenience in timing of caspase and TUNEL assays, and dispersal of cells for patch-clamp recording, monolayers were used in the present study.

B. UVB Exposure

HCLE cells were exposed to UVB radiation (302 nm) using a UVM-57 lamp, (Ultraviolet Products, Upland, CA), as previously described.[11] The doses of UVB, 80 mJ/cm^2 for patch-clamp studies or 150 mJ/cm^2 for caspase and TUNEL experiments were chosen based on previous studies and are relevant to ambient outdoor exposure in less than 2 hr at midday in the summer at 42° north latitude.

C. Patch-clamp Recording

HCLE cells were removed from culture plates using TrypLE-Express and resuspended in a bath containing (in mM) 140 NaCl, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 HEPES, 10 glucose (pH 7.4). Pipettes were back-filled using bath solution with 0.25mg/ml amphotericin-B and standard amphotericin-B perforated patch techniques were employed to attain whole-cell voltage-clamp recordings. (Sigma, St. Louis, MO). Pipette solution was (in mM) 145 K-methanesulfonate, 2.5 $MgCl_2$, 2.5 $CaCl_2$, 5 HEPES (pH 7.3). Pipette resistances were 2-7 MΩ. Recordings began when membrane resistance exceeded 1GΩ and access resistance dropped below 20 MΩ. The holding potential of the cell was -80 mV and the recording protocol consisted of 250 ms duration voltage steps from -80 mV to +120 mV in 10 mV increments. Currents were recorded by an Axon Instruments Axopatch 200B (Molecular Devices, Sunnyvale, CA) and analyzed by accompanying software (Clampex/