The Effect of K^+ on Caspase Activity of Corneal Epithelial Cells Exposed to UVB

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Introduction

We have published several reports showing that apoptosis of human corneal limbal epithelial (HCLE) cells can be inhibited if loss of intracellular K^+ in response to exposure to UVB radiation is prevented.[5,10,12,13] The mechanism by which the relatively high concentration of intracellular K^+ inhibits apoptosis in these cells has not been investigated.

Apoptosis usually begins with the activation of K^+ channels and the loss of intracellular K^+.[2,3,8,15,16] The loss of K^+ appears to cause activation of caspases, specifically caspases -9, -8, and -3, which serve an essential role in the execution of apoptosis. Caspases -8 and -9 are apoptosis initiators, while caspase-3 is an apoptosis effector enzyme.[7]

These caspases are activated in response to UVB radiation. Caspase-3 activity was reported to increase when human myeloblastic cells were exposed to UVB[1]. UVC also increases caspase-3 and caspase-8 activity in Jurkat T-cells.[14] UVB radiation increased caspase-3 activity in a human corneal epithelial cell line,[9] as well as in keratinocytes.[4] Finally, we have shown that HCLE cells exposed to UVB show increased caspase-9, caspase-8, and caspase-3 activity.[5,10]

Objectives

It has been suggested in previous studies that elevated intracellular [K^+] prevents activation of apoptotic pathways.[6,11] Exposure of HCLE cells to 150 mJ/cm^2 UVB activates K^+ channels, resulting in efflux of intracellular K^+.[10,13] After UVB exposure, incubating HCLE cells in medium with elevated extracellular [K^+] (25-100 mM) as compared to the usual [K^+] of 5 mM in culture medium, or with the K^+ channel blocker, Ba^{2+}, prevents UVB-induced loss of K^+ from the cells,[5,13] which in turn significantly decreases the activation of caspase-3, capsase-8, and caspase-9 by UVB.[5,10]

The purpose of the present study was to further investigate the effect of K^+ on the activity of caspase-9, caspase-8, and caspase-3 after UVB exposure. Our hypothesis was that addition of K^+ to the reaction medium would inhibit caspase activity in lysates of HCLE cells that had been exposed to UVB.

Methods

HCLE cells were grown to confluence in 6-well plates in Keratinocyte Serum-Free Media (KSFM, Life Technologies, Grand Island, NY). The cells were exposed to 150 mJ/cm^2 UVB, incubated in KSFM for 6 hours, collected and lysed as previously described.[10] Protein in the cell lysates was measured by the Bio-Rad assay (Berkeley, California). Caspase activity (relative fluorescence units / mg protein) was measured using fluorometric caspase-9, caspase-8, and caspase-3 assay kits (Biovision, Milpitas, California). It is important to note that all solutions in the caspase kits contained no K^+.
A saturated KCl solution (4.4 M in water) was prepared, and 24.1 µL of this solution was added to 12.0 µL of deionized water and 463.9 µL of reaction buffer. This resulted in a K^+ concentration of 210 mM. To initiate the caspase assay, a 50 µL aliquot of this solution and 5 µL of DEVD-AFC substrate were added to 50 µL of cell lysate, achieving a final K^+ concentration of 100 mM. The samples were then incubated at 37^o for two hours and caspase activity was measured using a fluorescence microplate reader.
Caspase-3 activity in the presence of increased osmolarity of the reaction buffer using sucrose rather than potassium was also measured. A saturated sucrose solution was prepared by adding 10 g sucrose to 5 mL of deionized water. A 24.1 µL aliquot of the sucrose solution was added to the reaction buffer as described above. This achieved a final sucrose concentration of about 133 mM and the same final osmolarity in the assay system, measured with a vapor pressure osmometer (Wescor, Logan, UT), as when KCl was added to the buffer. UVB-induced caspase-3 activity was measured as described above.

Results

Effect of K^+ on Caspase-9 Activity

UVB-induced caspase-9 activity significantly decreased, from 244.1 RFU/mg protein in the reaction buffer without K^+ to 133.3 RFU/mg protein in buffer containing 100 mM K^+.

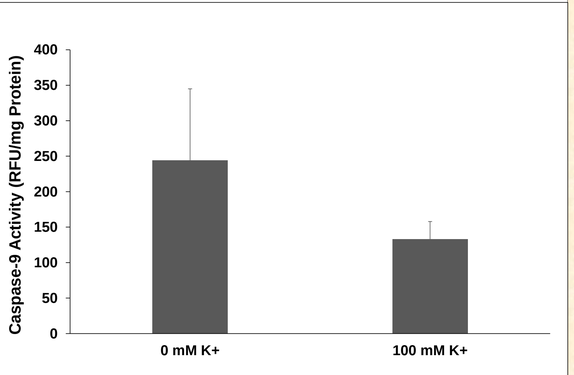

Figure 1. Effect of high [K^+] on UVB-activated caspase-9 activity after exposure of HCLE cells to 150 mJ/cm^2 UVB. Incubation of cell lysates with 100 mM K^+ caused a significant decrease in UVB-induced activity of caspase-9 (mean ± SD, t-test, n=12, $p \leq 0.05$).

Effect of K^+ on Caspase-8 Activity

UVB-induced caspase-8 activity significantly decreased, from 578.1 RFU/mg protein in the reaction buffer without K^+ to 424.7 RFU/mg protein in buffer containing 100 mM K^+.

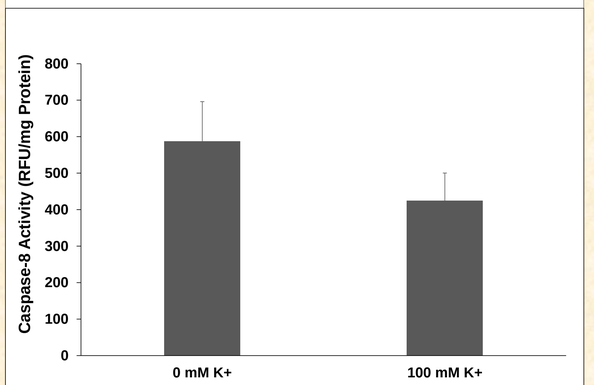

Figure 2. Effect of high [K^+] on UVB-activated caspase-8 activity after exposure of HCLE cells to 150 mJ/cm^2 UVB. Incubation of cell lysates with 100 mM K^+ caused a significant decrease in UVB-induced activity of caspase-8 (mean ± SD, t-test, n=12, $p \leq 0.05$).

Effect of K^+ on Caspase-3 Activity

UVB-induced caspase-3 activity significantly decreased, from 994.7 RFU/mg protein in the reaction buffer without K^+ to 862.4 RFU/mg protein in buffer containing 100 mM K^+.

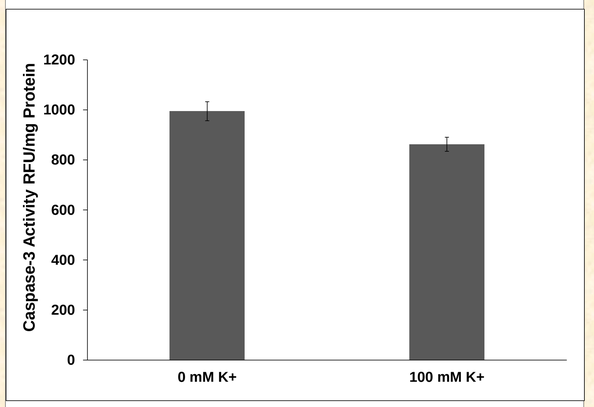

Figure 3. Effect of high [K^+] on UVB-activated caspase-3 activity after exposure of HCLE cells to 150 mJ/cm^2 UVB. Incubation of cell lysates with 100 mM K^+ caused a significant decrease in UVB-induced activity of caspase-3 (mean ± SD, t-test, n=12, $p \leq 0.05$).

Effect of Sucrose on Caspase Activity

It is possible, that the inhibition of the caspases by addition of KCl to the reaction buffer was caused by the increase in osmolarity of the solution. In order to test this, we measured caspase-3 activity in the presence of increased osmolarity of the reaction buffer using sucrose rather than potassium.

UVB-induced caspase-3 activity did not significantly change with the addition of sucrose to the reaction medium.

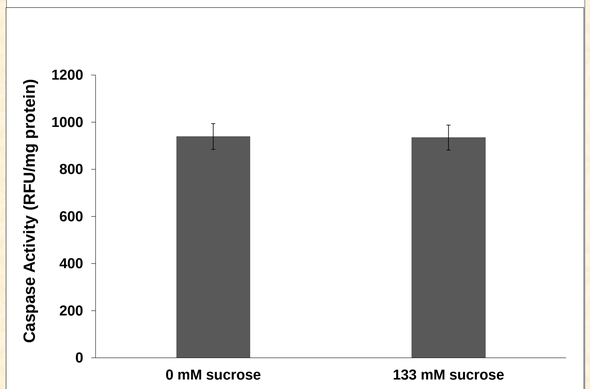

Figure 4. Raising the osmolarity of the reaction medium using sucrose rather than KCl had no effect on caspase-3 activity (mean ± SD, t-test, n=12, $p \leq 0.05$).

Conclusions

This study showed that Caspase-9 was inhibited by 45.4% in the presence of 100 mM K^+, while Caspase-8 was inhibited by 27.7% and caspase-3 was inhibited by 13.3%. This agrees with our original hypothesis that high K^+ in tears would reduce caspase activity.

This study supports our overall hypothesis that the relatively high [K^+] in tears reduces the electrochemical gradient for loss of intracellular K^+ in corneal epithelial cells in response to UVB.[5,10,12,13] This may contribute to protection of the corneal epithelium from ambient UVB by inhibiting apoptotic pathways that are normally suppressed by the relatively high intracellular K^+ concentration.

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