New Perspectives on the Potential Role of Water Channels in Skin Pyroptosis

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1. Introduction

Water channels are integral membrane proteins essential for maintaining cellular homeostasis. They are broadly classified into two major subtypes: classical aquaporins, which are selectively permeable to water, and aquaglyceroporins, which facilitate the transport of water, glycerol as well as hydrogen peroxide [1]. Beyond their established role in skin hydration, aquaporins (AQPs) have garnered increasing attention for their emerging functions in the regulation of skin inflammatory processes [2,3]. Skin inflammation underpins a wide spectrum of skin aging conditions. Pyroptosis, a gasdermin-mediated inflammatory cell death [4], is characterized by pore formation, cell swelling, membrane rupture, and the release of intracellular inflammatory factors, such as interleukins, into the extracellular matrix [5].

Our previous study linked keratinocyte pyroptosis to metaflammation as a novel key driver in skin aging. Our findings indicate that, beyond the modulation of caspase-1-dependent pyroptosis, caspase-1-independent pathways may also be implicated in skin inflammation, these alterations can be mitigated through the application of a skin protectant agent. All prior observations were conducted using LPS-stimulated keratinocytes and focused on gasdermin D (GSDMD)-mediated pyroptosis. Recent evidence suggests that another pore-forming protein, gasdermin E (GSDME), may also contribute to pyroptotic processes [6–8]. While aquaporins are well-known for their roles in hydration and wound healing, their involvement in pyroptosis remains poorly understood. This study explores the potential relationship between water channel proteins and distinct forms of pyroptosis in the context of skin inflammation, along with their prospective applications in cosmetology.

2. Materials and Methods

Preparation for the Natural Skin Protectant

By employing advanced AI-driven screening technology, the flower of *Opuntia ficus-indica*, a cactus species cultivated in the Mediterranean region and traditionally utilized in Berber ethnobotany for food and cosmetic purposes, was identified for further investigation. The Cactus Flower Extract (CFE) was obtained from floral by-products of the food industry, sourced from

certified organic plantations. The *in vitro* tests are conducted with the pure active matter of CFE at various concentrations in the culture medium.

Immunofluorescence Staining and Quantification of Biomarkers

Human primary keratinocytes were seeded at 15,000 cells per well in 96-well plates and cultured in a medium at 37°C with 5% CO_2. Cells were treated with various CFE concentrations (0.01; 0.03%) for 24 hours. After treatment, keratinocytes were fixed, blocked, and incubated with primary antibodies targeting Aquaporin 1 (AQP1), Aquaporin 3 (AQP3), or Filaggrin. Following washes, cells were incubated with fluorophore-conjugated secondary antibodies, and nuclei were stained with DAPI. Fluorescent images were captured using an EVOS M5000 microscope and analyzed with ImageJ software. Biomarker fluorescence intensity was quantified, normalized to the control (set at 100%), and expressed as mean ± standard deviation. Statistical analysis was performed using GraphPad software with an unpaired t-test and Welch's correction (95% confidence interval).

Human Keratinocyte Culture and Quantification of Target Proteins

Human primary keratinocytes (NHEKs) were obtained from a 49-year-old donor. NHEKs have been cultivated in monolayers until reaching confluency. Cells were pre-incubated during 24 hours in absence (control) or in the presence of increasing concentrations (0.01; 0.02; 0.03%) of our test compound. At the end of the pre-incubation period, cells were either exposed or not to UVB radiation (20 mJ/cm²) and then incubated again for 24 hours. CFE was solubilized at 1% (w/v) in the incubation medium. The solution obtained was then diluted directly in incubation medium in order to reach the different concentrations described above. After incubation, the culture medium was collected and stored at -20°C for further analysis. The monolayer cells were lysed to quantify GSDMD and GSDME levels in cell lysates using a sensitive and specific ELISA kit. The incubation medium was used to measure interleukin-1 alpha (IL-1α) and interleukin-1 beta (IL-1β) levels following the same experimental protocol.

Statistical analyses were performed as follows: the significance level between the "Control - UV" and "Control + UV" conditions was assessed using Student's t-test (*: $p < 0.05$; **: $p < 0.01$; **: $p < 0.001$). The significance level between the "Control + UV" condition and the test compound-treated groups was assessed using a one-way analysis of variance (One-way ANOVA), followed by a Holm-Sidak test (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).

3. Results

Stimulation of Aquaporins 1 and 3 Production in NHEKs

As previously mentioned, AQPs facilitate the selective transport of water and glycerol between the extracellular matrix and the cytoplasm. In this study, we first evaluated cellular hydration capacity by measuring the production of AQP1 and AQP3 in NHEKs (Figure 1).

Under the experimental conditions, CFE at 0.01% and 0.03% significantly ($p < 0.05$) enhanced the intracellular levels of AQP1 in a dose-dependent manner. Regarding AQP3, CFE at 0.01% and 0.03% significantly increased its intracellular levels ($p < 0.05$ and $p < 0.01$, respectively) (Figure 2).
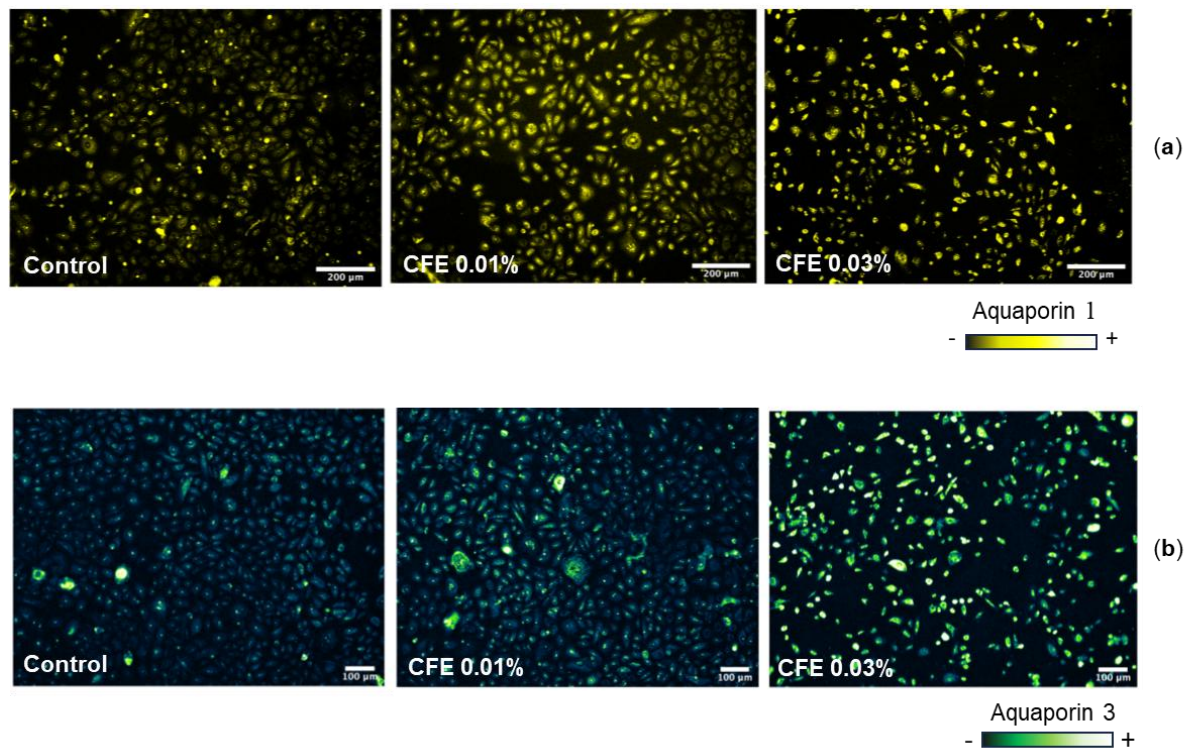
Figure 1. *In situ* visualization of aquaporin (AQPs) levels in NHEKs by epifluorescence microscopy: (a) The specific signal of aquaporin 1 (AQP1) labelling is shown in yellow. *10x Objective - Scale bar, 200 μm*; (b) The specific signal of aquaporin 3 (AQP3) labelling in a colour range (low levels in dark, high levels in bright colours). *10x Objective - Scale bar, 100 μm*.
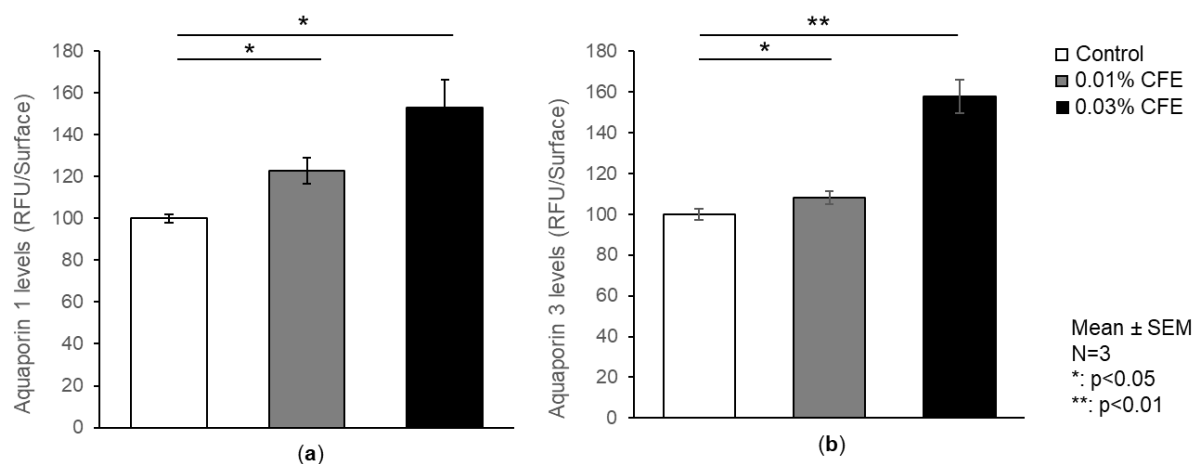

Figure 2. Quantification of aquaporin (AQPs) levels in NHEKs: (a) The levels of aquaporin 1 (AQP1); (b) The levels of aquaporin 3 (AQP3). *Results are expressed as RFU/surface. Each experimental group are expressed as relative values (% vs Control). The SEM bars are represented in black lines on each histogram. The statistical analysis was performed using GraphPad software with an unpaired t-test and Welch's correction (95% confidence interval).*

Stimulation of Filaggrin Levels in NHEKs

To assess skin barrier integrity, filaggrin levels in NHEKs were measured (Figure 3). Under our experimental conditions, treatment with CFE at concentrations of 0.01% and 0.03% resulted in a statistically ($p<0.05$ and $p<0.001$, respectively) significant upregulation of filaggrin production in NHEKs (Figure 4). These findings suggest that this novel natural compound may contribute to strengthening the skin barrier.
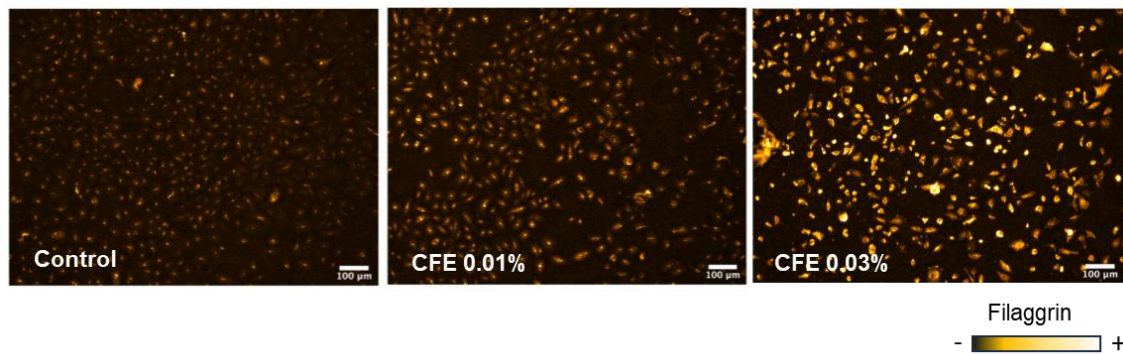

Figure 3. *In situ* visualization of fiaggrin levels in NHEKs by epifluorescence microscopy. *10x Objective - Scale bar, 100 µm*.
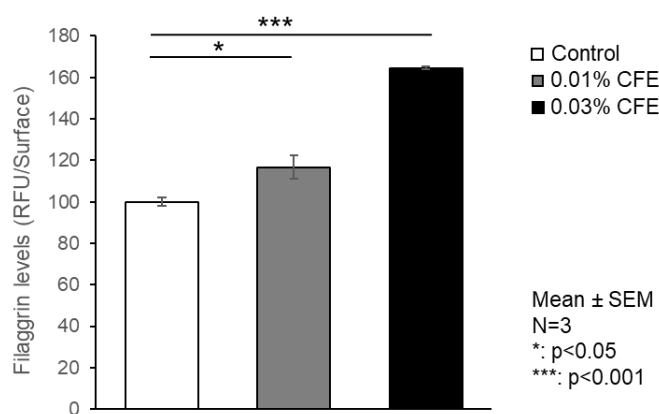

Figure 4. Quantification of filaggrin levels in NHEKs. *Results are expressed as RFU/surface. Each experimental group are expressed as relative values (% vs Control). The SEM bars are represented in black lines on each histogram. The statistical analysis was performed using GraphPad software with an unpaired t-test and Welch's correction (95% confidence interval).*

Effects on Pyroptosis-Related Gasdermin Production

Gasdermins are key protein effectors in pyroptosis. In this study, we investigated the impacts of UVB exposure and CFE on gasdermins involved in both canonical and non-canonical pyroptosis pathways. As shown in the image below (Figure 5), UVB irradiation significantly increased the protein levels of GSDMD and GSDME in NHEKs. CFE at concentrations of 0.01%, 0.02% and 0.03% significantly attenuated the UVB-induced increase in GSDMD. Furthermore, CFE at 0.02% and 0.03% also significantly reduced the UVB-induced GSDME increase. These findings suggest that UV exposure may trigger keratinocyte pyroptosis, CFE exerts potent inhibitory effects on different pathways of UVB-induced pyroptosis in NHEKs.
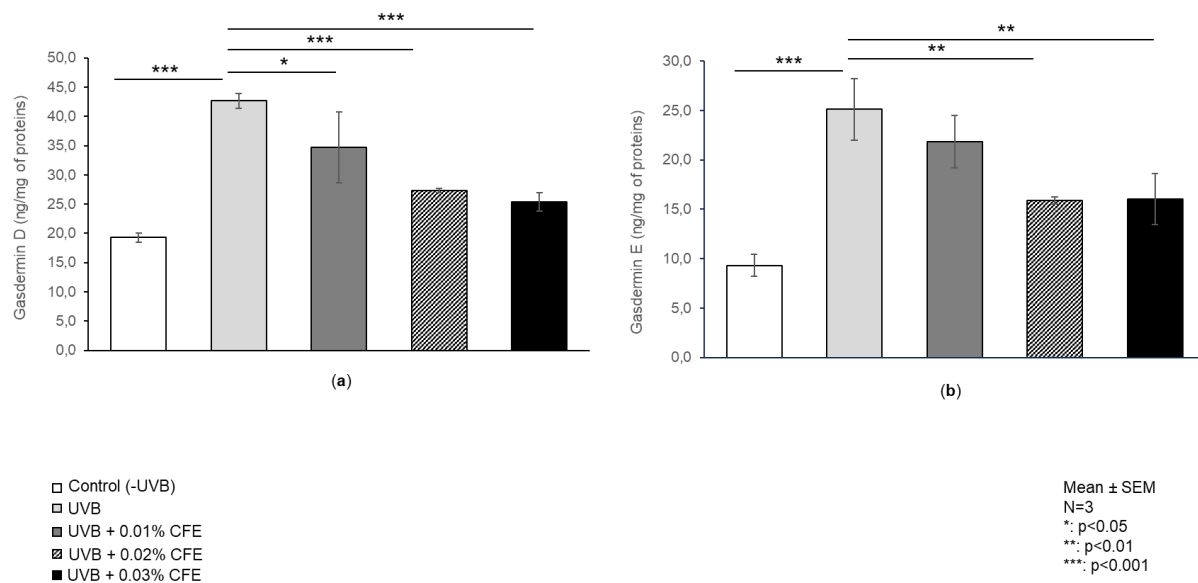
Figure 5. Effects of UVB and CFE on pytoptosis in NHEKs: (a) Gasdermin D (GSDMD) protein level measurement in NHEKs; (b) Gasermin E (GSDME) protein level measurement in NHEKs. *Results are expressed as ng of GSDMD or GSDME per mg of total proteins measured from cell monolayer lysate. The SEM bars are represented in black lines on each histogram. The statistical analysis was performed using T-test.*

Effects on Pyroptosis-Related Interleukin Production

UVB radiation is a well-established environmental factor that accelerates skin aging by inducting skin chronic inflammatory responses [9]. In NHEKs, UVB markedly elevated the protein levels of the pro-inflammatory cytokines IL-1α and IL-1β, concomitant with upregulation of GSDMD and GSDME (Figure 6). Treatment with CFE effectively attenuated the UVB-induced increases in IL-1α and IL-1β in a dose-dependent manner (Figure 6), indicating its potential anti-photoinflammatory properties.
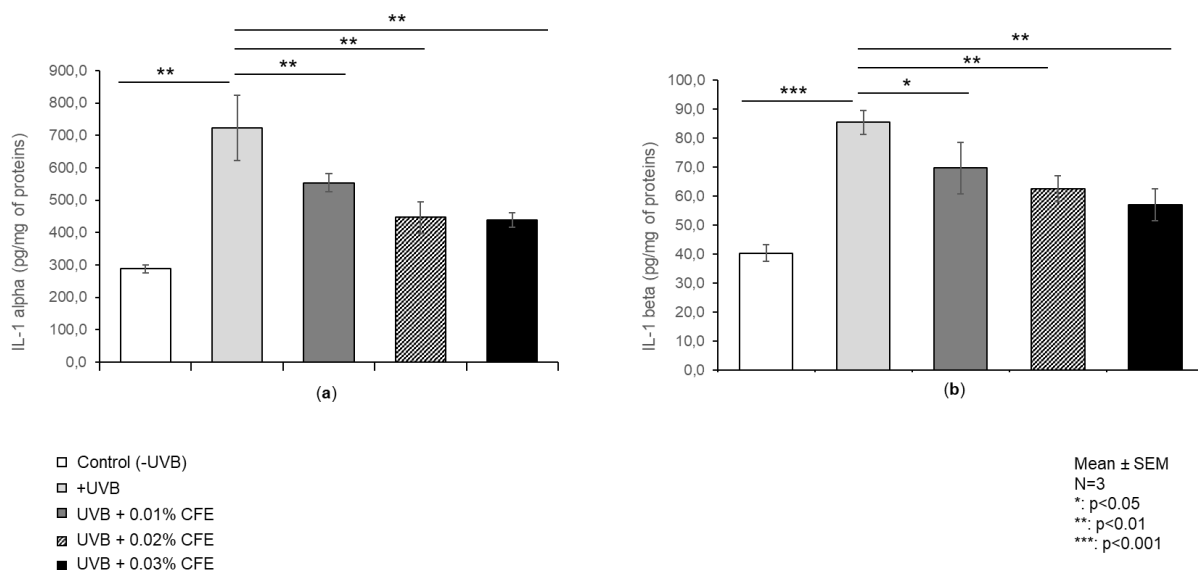
Figure 6. Effects of UVB and CFE on interleukin production in NHEKs: (a) IL-1α protein level measurement in NHEKs; (b) IL-1β protein level measurement in NHEKs. *Results are expressed as pg of IL-1α or IL-1β per mg of total proteins measured from cell monolayer lysate. The SEM bars are represented in black lines on each histogram. The statistical analysis was performed using T-test.*

4. Discussion

Water channels AQPs play a critical role in skin hydration and homeostasis, with growing evidence highlighting the potential adverse effects associated with their upregulation. Among them, AQP1 and AQP3 are the most extensively studied in the skin. AQP1 plays a critical role in osmotic water transport across cellular membranes and has been identified in various skin cell types [10,11]. Although primarily functioning as a water channel, AQP1 has also been shown to possess cation conductance capabilities [12]. AQPs-mediated water transport has been proposed to contribute to keratinocyte migration and proliferation. The transmembrane influx of water mediated by AQP1 or AQP3 is hypothesized to generate localized hydrostatic pressure, thereby facilitating membrane protrusion and cytoskeletal remodeling essential for cellular motility [13], an integral process during cutaneous wound healing.

In contrast, the transport of hydrogen peroxide through AQP3 is a pivotal regulatory step in the activation of NF-κB signaling pathways in keratinocytes and plays a contributory role in the development of psoriasis [14]. In parallel, upregulation of AQP3 has been correlated with a suppression of filaggrin expression [15], leading to barrier disruption and the development of atopic dermatitis [16]. Accordingly, it is of critical importance to maintain and enhance filaggrin expression in tandem with AQP3 upregulation to preserve epidermal homeostasis and barrier integrity.

Furthermore, excessive cellular hydration and swelling driven by AQPs may exacerbate cell pyroptosis [17,18], thereby intensifying inflammaging. Our previous study showed that LPS-induced GSDMD-mediated pyroptosis pathways in keratinocytes may be targeted during metaflammation-type skin aging. Recent evidence indicates that UVB radiation downregulates AQP3 expression in both epidermal keratinocytes [19] and dermal fibroblasts [20], it may additionally promote keratinocyte inflammation through the induction of pyroptosis [21]. While caspase-1 is conventionally recognized as a central executor of pyroptosis, emerging evidence has demonstrated that GSDME-dependent sublytic pyroptosis may facilitate the release of IL-1α under conditions of caspase-1 inhibition [22].

In this research, we found for the first time that, in addition to UVB-induced GSDMD-mediated pyroptosis, GSDME-associated pathways may also be modulated by natural skin protectant, concurrently influencing the balance of proinflammatory cytokine production, including IL-1α and IL-1β. These regulatory mechanisms exhibit an inverse correlation with the modulation of AQPs. Considering these findings, it is imperative to investigate strategies aimed at modulating AQP activity to preserve cutaneous hydration homeostasis while attenuating pyroptotic cell death. Collectively, this study underscores the potential involvement of specific water channels, particularly AQP1 and AQP3, in the regulation of various skin pyroptosis, offering new perspectives on their contribution to skin inflammation and photoaging.

5. Conclusion

Water channels play a critical role in maintaining skin hydration and homeostasis. However, accumulating evidence suggests the potential adverse effects associated with their upregulation. Excessive water influx mediated by AQPs can lead to cellular overhydration and swelling, potentially exacerbating keratinocyte pyroptosis and thereby contributing to skin inflammaging. In this study, we report for the first time that, in addition to UVB-induced GSDMD-mediated pyroptosis, GSDME-associated pathways may also be modulated, concurrently influencing the balance of proinflammatory cytokine production, including IL-1α and IL-1β. These regulatory mechanisms exhibit an inverse correlation with AQP modulation. Given these findings, it is essential to explore strategies aimed at modulating AQPs to maintain hydration homeostasis while mitigating pyroptotic damage. This study highlights the potential of targeting specific water channels, particularly AQP1 and AQP3, in the context of UV-induced skin pyroptosis, offering new perspectives on their contribution to skin inflammation and photoaging.

6. Acknowledgements

7. Conflict of Interest Statement

The authors declare no competing interests.

8. References

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