Does Blue Light Restore Human Epidermal Barrier Function via Activation of Opsin During Cutaneous Wound Healing?

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Background and Objective: Visible light has beneficial effects on cutaneous wound healing, but the role of potential photoreceptors in human skin is unknown. In addition, inconsistency in the parameters of blue and red light-based therapies for skin conditions makes interpretation difficult. Red light can activate cytochrome c oxidase and has been proposed as a wound healing therapy. UV-blue light can activate Opsin 1-SW, Opsin 2, Opsin 3, Opsin 4, and Opsin 5 receptors, triggering biological responses, but their role in human skin physiology is unclear.

Materials and Methods: Localization of Opsins was analyzed *in situ* in human skin derived from face and abdomen by immunohistochemistry. An *ex vivo* human skin wound healing model was established and expression of Opsins confirmed by immunohistochemistry. The rate of wound closure was quantitated after irradiation with blue and red light and mRNA was extracted from the regenerating epithelial tongue by laser micro-dissection to detect changes in *Opsin 3* (*OPN3)* expression. Retention of the expression of Opsins in primary cultures of human epidermal keratinocytes and dermal fibroblasts was confirmed by qRT-PCR and immunocytochemistry. Modulation of metabolic activity by visible light was studied. Furthermore, migration in a scratch-wound assay, DNA synthesis and differentiation of epidermal keratinocytes was established following irradiation with blue light. A role for OPN3 in keratinocytes was investigated by gene silencing.

Results: Opsin receptors (OPN1-SW, 3 and 5) were similarly localized in the epidermis of human facial and abdominal skin *in situ*. Corresponding expression was confirmed in the regenerating epithelial tongue of *ex vivo* wounds after 2 days in culture, and irradiation with blue light stimulated wound closure, with a corresponding increase in *OPN3* expression. Expression of Opsins was retained in primary cultures of epidermal keratinocytes and dermal fibroblasts. Both blue and red light stimulated the metabolic activity of cultured keratinocytes. Low levels of blue light reduced DNA synthesis and stimulated differentiation of keratinocytes. While low levels of blue light did not alter keratinocyte migration in a scratch wound assay, higher levels inhibited migration. Gene silencing of *OPN3* in keratinocytes was effective (87% reduction). The rate of DNA synthesis in OPN3 knockdown keratinocytes did not change following irradiation with blue light, however, the level of differentiation was decreased.

Conclusions: Opsins are expressed in the epidermis and dermis of human skin and in the newly regenerating epidermis following wounding. An increase in OPN3 expression in the epithelial tongue may be a potential mechanism for the stimulation of wound closure by blue light. Since keratinocytes and fibroblasts retain their expression of Opsins in culture, they provide a good model to investigate the mechanism of blue light in wound healing responses. Knockdown of OPN3 led to a reduction in early differentiation of keratinocytes following irradiation with blue light, suggesting OPN3 is required for restoration of the barrier function. Understanding the function and relationship of different photoreceptors and their response to specific light parameters will lead to the development of reliable light-based therapies for

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