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Epidermal aquaporin-3 is increased in the cutaneous burn wound

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ABSTRACT

Introduction: Aquaporins (AQP) are a family of transmembrane proteins that transport water and small solutes such as glycerol across cell membranes. It is a mediator of transcellular water flow and plays an important role in maintaining intra/extracellular fluid homeostasis by facilitating water transport in response to changing osmotic gradients. In the skin, AQPs permit rapid, regulated, and selective water permeability and have been demonstrated to play a role in skin hydration, cell proliferation, migration, immunity, and wound healing. However, the expression of AQP-3 in the cutaneous burn wound has never been elucidated. We sought to assess the expression of AQP-3 in patients with burn wounds.

Methods: A fresh full thickness biopsy sample was taken from the center of the burn wound, the burn wound edge, and the graft donor site in 7 patients ($n = 21$), approximately 3–7 days post injury. Fixed, paraffin embedded sections were stained using AQP-3 specific antibody and examined by immunofluorescence. Fresh samples were processed to quantify AQP-3 protein expression with Western blot analysis.

Results: The central portion of the burn wound revealed destruction of the epidermis and dermis with no AQP-3 present. Along the burn wound edge where the epidermal architecture was disrupted, there was robust AQP-3 staining. Western blot analysis demonstrated deeper staining along the burn wound edge compared to unburned skin (control). Quantification of the protein shows a significant amount of AQP-3 expression along the burn wound edge (3.6 ± 0.34) compared to unburned skin (2.1 ± 0.28, $N = 7$, $^{*}p < 0.05$). There is no AQP-3 expression in the burn wound center.

Conclusion: AQP-3 expression is increased in the burn wound following injury. While its role in wound healing has been defined, we report for the first time the effect of cutaneous burns on AQP-3 expression. Our data provides the first step in determining its functional role in burn wounds. We hypothesize that development of AQP3 targeted therapies may improve burn wound healing.

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1. Introduction

Aquaporins (AQP) are a family of transmembrane proteins that transport water and small solutes such as glycerol across cell membranes [1]. It is a mediator of transcellular water flow and plays an important role in maintaining intra/extracellular fluid homeostasis by facilitating water transport in response to changing osmotic gradients [2]. AQPs are found in the major systems of the human body such as the nervous, renal, cardiovascular, respiratory, reproductive, digestive, musculoskeletal, and integumentary systems [2].

In the skin, AQPs permit rapid, regulated, and selective water permeability and have been demonstrated to play a role in skin hydration, cell proliferation, migration, immunity, and wound healing [3–5]. Overall, 6 skin AQPs have been identified. At the epidermal level, the most important and abundant of these proteins is AQP-3. It is a member of the aquaglyceroporin subfamily and is found primarily in the plasma membrane of keratinocytes and dermal fibroblasts [6,7]. AQP-3 has function in the transportation of water and glycerol, a natural moisturizing factor that keeps the skin hydrated, in the interstitial space and intracellularly [8].

In the epidermis, AQP3 is organized to create a highly selective permeability gradient (Fig. 1). For example, epidermal permeability progressively increases from the stratum corneum to the stratum basale. Not coincidentally, this difference in cell permeability is proportional to the concentration of AQP3 within each epidermal layer, with high concentrations being found in the stratum basale and absent in the stratum corneum and granulosum. In fact, it is this proportionate increase in membrane permeability to AQP3 expression that forms the biologic rationale for an AQP3 mediated water clamp or gate phenomenon, a regulatory mechanism by which water moves across cellular membranes dependent on the conformation of the protein channel in the open or closed position [9].

Wound healing in the skin is a multi-step process that involves several cell types, including epidermal keratinocytes, fibroblasts, endothelial cells, and peripheral nerve cells [10]. In addition to cellular involvement, a number of cell signaling molecules and proteins are involved in this process as well. AQP-3 is integral to wound healing by facilitating water and glycerol transport and therefore keratinocyte migration and proliferation respectively [3]. In fact, the absence of AQP3 regulated transport of water and glycerol has been shown to impair wound healing in animal models [11].

While the role of role of AQP-3 in cutaneous wound healing has been defined, its activity in the burn wound has not previously been determined. The aim of our study was to determine the effect of a cutaneous burn on AQP-3 expression within the wound.

2. Materials and methods

Following institutional review board approval (IRB), a total of 7 patients were selected for our study. Full thickness biopsy samples were taken from 3 locations on each patient; the burn wound center, edge, and graft donor sites for a total of 21 specimens (3 per patient) approximately 3–7 days post injury. The areas of burn to be biopsied were determined from physical examination by an experienced burn surgeon. The samples were immediately processed and stored in alloprotect reagent (Qiagen). For western blot analysis, skin samples were minced and processed (Navy Bullet Blender). Bicinchoninic acid protein assay was used to normalize the samples before deglycosylation with PNGase-F (Sigma) for 1 h. Samples were then loaded on to 10% acrylamide gels (Invitrogen) and transferred onto polyvinylidene fluoride membranes (Invitrogen) to detect AQP-3. For immunofluorescence, each skin sample was fixed in formalin and embedded in paraffin. Sections from each block were placed onto glass slides. The slides were then deparaffinized and rehydrated in various gradients of xylene, ethanol, and water. Antigen retrieval was performed with 10 mM citric acid buffer (Sigma). After blocking with 20% donkey serum (Invitrogen), the slides were incubated with anti-AQP-3 antibodies (Sigma) overnight and

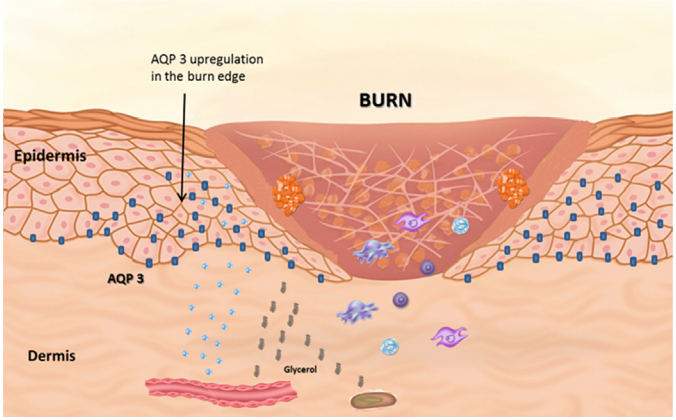

Fig. 1 – Aquaporin-3 distribution within the epidermis. Aquaporin-3 is bound to keratinocyte membranes in the layers of the epidermis extending from the stratum granulosum to stratum basale. Following a cutaneous burn, there is an increase in AQP-3 expression which will transport water and glycerol. This process plays a key role in the keratinocyte proliferation, hydration, and migration to promote wound healing.