Biochemical Markers Associated With Acute Vocal Fold Wound Healing: A Rabbit Model

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Summary: This study seeks to determine the ability of enzyme-linked immunosorbent assays (ELISAs) of vocal fold secretions to detect and describe the acute tissue response to injury in a rabbit vocal fold model. Vocal fold secretions were collected before the induction of a unilateral surgical injury to the vocal fold and at 6 timepoints after injury (1, 5, 7, 10, 14, and 21 days). Secretions were then subjected to ELISAs to assess concentrations of interleukin-1 beta (IL-1β) and prostaglandin-E2 (PGE-2). The results indicate that ELISAs may be useful in documenting fluctuations in these markers associated with the wound healing process in the rabbit model. The temporal expression of both IL-1β and PGE-2 was consistent with their proposed roles in the wound healing cascade in other systems, pointing to the potential that surface secretions may be at least partial indicators of wound healing events within the tissue.

Key Words: Larynx—Vocal folds—Injury—Secretions—Cytokines—Wound healing.

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INTRODUCTION

Wound healing in the skin requires a highly organized series of events including hemostasis, inflammation, re-epithelialization, cell proliferation, matrix deposition, angiogenesis, and wound contraction. A general timeline for such events and key mediators of each stage have been described in the skin and result in the resolution of epithelial defects as well as the reconstitution of functional tissue. The long-term sequelae of wound healing in the vocal folds, specifically, the formation of vocal fold scar and the resultant alteration in biomechanical function, have been described.[1,2] However, the *acute* response to vocal fold injury has not yet been investigated. Insight into the sequence of events associated with the acute response to injury in the

vocal folds might yield important information about critical periods for intervention as well as prognostic indicators for outcomes of treatment. The current study is a component of a long-term research program in our laboratory addressing the acute wound healing response in the vocal folds, as well as wound healing itself.

A description of the sequence of wound healing events in the skin serves as a foundation for the current study. Initially, after acute injury, the inflammatory response orchestrates the cascade of events associated with wound healing, and it ensures immune competence after injury.[3] The inflammatory response is initiated by the extravasation of blood constituents from damaged vessels. Chemoattractants, cytokines, and growth factors are released that recruit neutrophils and monocytes to the injury site and directly stimulate keratinocytes and fibroblasts to initiate the repair process by assuming the repair phenotype.[4] Macrophages continue to clean and debride the wound, in addition to releasing numerous cytokines key to fibroblast chemotaxis and proliferation. Those cytokines include platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), and transforming growth factor-beta (TGF-β). In addition, macrophage-derived cytokines such as interleukin-4 (IL-4) are responsible for tissue formation (ie, collagen production in fibroblasts).[5]

The re-establishment of a functional epithelium at the site of the wound is a key aspect of wound healing. Clinically, a dermal wound is re-epithelialized when a water-impermeable seal is present over the wound.[4] Keratinocyte migration into the site of injury from the periphery is stimulated by epidermal growth factor and fibroblast growth factor released by both inflammatory cells and tissue fibroblasts. This process typically begins 24 to 48 hours after injury.[6–8] Wound contraction begins 4 to 5 days after injury.[9] Contraction is characterized by the centripetal movement of the wound edge toward the center of the wound. Both myofibroblasts and fibroblasts are thought to be responsible for this process.[10,11] Scar remodeling is the final stage of wound healing. The accumulation of collagen becomes stable at approximately 21 days after injury.[9,12] However, remodeling, involving the synthesis and degradation of collagen, may continue for several months, and it contributes to the gradual regain of tissue tensile strength.

As noted, the sequence of events is relatively well documented in the skin, and little is known about acute wound healing activities in the vocal folds. Because the vocal fold has a unique subepithelial structure and highly specialized biomechanical functions, the study of the processes associated with acute wound healing in the vocal folds is warranted. This issue is addressed in this present study.

Before the introduction of the experimental questions, background information regarding the methods used in the current investigation is relevant. Previous investigation has suggested that analyses of biochemical markers associated with wound healing extracted from secretions collected from the vocal fold surface may serve as a noninvasive means to document the wound healing process in human vocal fold injury.[13–15] In short, dramatic shifts in pro-inflammatory markers such as interleukin-1β (IL-1β), tumor necrosis factor-alpha, and matrix metalloproteinase-8 were noted in a single adult subject after a 1-hour period of intense vocal loading that induced prominent vocal fold edema and dysphonia.[15] In another study, differential profiles of both IL-1β and PGE-2 were noted in surface secretions of patients with various lesions of the vocal folds.[14] The current study seeks to build on the previous ones by investigating the time-dependent expression of key biochemical markers in vocal fold secretions acutely after vocal fold injury, and to assess the extent to which such expression might reflect processes within the tissue. To that end, two biochemical markers were selected based on their known temporal regulation in the wound healing response in other tissues: interleukin-1β (IL-1β) and prostaglandin-E2 (PGE-2).

Interleukin-1β is a prototypical pro-inflammatory cytokine. It is the secreted isoform of the IL-1 family. It is produced by macrophages, monocytes, dendritic cells, and tissue epithelial cells as an *acute* response to injury and an initiator of the inflammatory process.[16,17] PGE-2 is involved in several aspects of the wound healing cascade and is produced by inflammatory cells, epithelial cells, and fibroblasts.[18–20] PGE-2 is a key inflammatory mediator, but it is also involved in later processes of wound healing, including inhibition of fibroblast migration,