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Celecoxib inhibits early cutaneous wound healing

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ABSTRACT

Background: Cyclooxygenase-2 (COX-2) is an inducible enzyme that is rapidly upregulated in response to injury, resulting in the production of prostaglandin E2 (PGE_2), a primary mediator of inflammation and wound healing. The selective COX-2 inhibitor, celecoxib, is was used to treat pain and inflammation. When used to treat injuries, we postulated that loss of PGE_2 activity by COX-2 inhibition would have detrimental effects on wound healing. Our objective was to study the effect of selective COX-2 inhibition with celecoxib on cutaneous wound healing.

Materials and methods: C57BL/6J mice with uniform full-thickness wounds (1 cm^2) to their dorsum were fed diet with or without celecoxib (1500 ppm). Wound closure analysis measured wound contraction, reepithelialization, and open wound as a percentage of the initial wound area, and was quantified by planimetry. Wounds were excised *en bloc* at day 7 to examine cellular proliferation, angiogenesis, cytokine production, and extracellular matrix (ECM) formation.

Results: Celecoxib-induced reduction in wound PGE_2 levels was documented by enzyme-linked immunosorbent assay on day 7 after wounding. Wound contraction and reepithelialization were significantly reduced by celecoxib treatment, resulting in a 20% greater open wound area at day 7 ($P < 0.05$). In response to celecoxib treatment, immunohistochemistry analysis showed epithelial cell proliferation, angiogenesis, and ECM components including collagen and myofibroblasts were significantly decreased.

Conclusions: Wound healing is significantly delayed by celecoxib treatment. These data indicate that COX-2 and its downstream product PGE_2 modulate the activity of multiple essential functions of the inflammatory stroma, including epithelial proliferation, angiogenesis, and ECM production. As a result, reepithelialization and wound closure are delayed by celecoxib treatment. These findings have potential clinical implications in postoperative wound management.

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1. Introduction

Wound healing is a complex process during which multiple epithelial and stromal tissue elements interact to reconstitute an injured organ. At the onset of epithelial injury, the activation of multiple signaling pathways initiates a cascade of events that reestablish tissue architecture and barrier function. The process of tissue regeneration and repair after epithelial injury can be divided into three sequential phases [1]. The inflammatory phase begins immediately after injury with activation of the clotting cascade to seal vascular leakage. By 6 h after injury, fibrin, platelets, neutrophils, and erythrocytes have attached to the wound. Neutrophils and macrophages are recruited to remove debris and microorganisms, and also release inflammatory cytokines and mediators such as interleukin (IL)-1β, tumor necrosis factor-alpha, and prostaglandins that attract mesenchymal cells to the injury site. The proliferative phase of wound healing begins 12–24 h after injury and is characterized by mesenchymal cell activation, proliferation, and matrix deposition. During this phase, granulation tissue composed of diverse stromal elements covers the epithelial defect. This granulation tissue is produced by activation of angiogenesis, recruitment, and activation of tissue-resident and bone marrow-derived mesenchymal stromal cells, $CD34^+$ hematopoietic stem cells, and proliferation of fibroblasts that lay down a transient collagen 1-rich extracellular matrix (ECM). Also during the proliferative phase, epithelial stem cells undergo symmetrical division and migrate from the wound edges along collagen fibrils. Later in this phase, wound contraction occurs in response to differentiation of hematopoietic stem cells-derived $CD34^+$ fibrocytes cells into myofibroblasts [2,3]. The proliferative phase of wound healing ends when the normal epithelial layer is reestablished, and symmetrical epithelial stem cell division ceases. During the resolution phase of wound healing, stromal remodeling occurs, with apoptosis or differentiation of mesenchymal cells returning the stroma to a basal activation state. This final healing phase can persist for many months.

Tissue prostaglandins regulate key cellular functions relevant to injury repair, including angiogenesis, vascular permeability, and cellular proliferation [4]. Cyclooxygenase-2 (COX-2) is an inducible enzyme that is rapidly upregulated in response to injury, resulting in the production of prostaglandin E2 (PGE_2), a primary mediator of inflammation in epithelial tissues [5]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are inhibitors of Cox commonly used for relief of pain and inflammation after injury. Although many studies have investigated the role of NSAIDs on fracture and tendon healing, a limited number have examined the effect of NSAIDs on epithelial wound healing [6–9]. We studied the effect of the selective COX-2 inhibitor, celecoxib, on cutaneous wound healing in a murine model, with the hypothesis that celecoxib treatment would produce deficits in wound healing.

2. Methods and methods

2.1. Animal and wounding model

Female 6-wk-old C57BL/6J (B6) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). All mice were fed AIN-76A high fat diet (5% fat) (Research Diets, Inc, New Brunswick, NJ) *ad libitum* starting 2 wk before wounding and continuing for the duration of the experiment. Four days before wounding, half of the mice ($n = 21$) had celecoxib (1500 ppm, LC Laboratories, Woburn, MA) added to their diet, whereas the other half ($n = 21$) remained untreated. The day before surgery, dorsal hair was shaved and depilated. Full-thickness cutaneous wounds of uniform dimensions (1 cm^2) were created on the dorsal surface. Wounds were covered with a semiocclusive polyurethane Tegaderm (3M, St Paul, MN) dressing that was changed on days 2 and 4. On day 7, animals were euthanized, and the wound area with a small amount of surrounding epidermis and underlying tissue was excised *en bloc*. Wound tissues were divided into two parts as follows: half of the wound was fixed in 10% buffered formalin (Fisher, Waltham, MA) for 24 h then kept in 70% ethanol at 4°C until paraffin embedding and ½ was frozen in liquid N_2 and stored at −80°C before preparing protein lysates.

2.2. Wound closure analysis

Wounds analysis was performed on B6 (Fig. 1A) and B6-celecoxib (Fig. 1B) mice on all mice at day 0, and after each dressing change (days 2 and 4), and at the time of tissue harvest (day 7). Digital photographs captured at the end of the experiment were compared with the initial images for each mouse by an independent blinded observer. Wound closure analysis measured contraction and open wound as a percentage of the original wound area and was quantified by planimetric analysis using ImageJ software (NIH, Bethesda, MD) [10]. Wound reepithelialization was calculated by measuring the epithelial tongue length and reported as a percentage of the entire wound. The epithelial tongue was defined by the area of epithelialization extending from the edge of the first hair follicle closest to the wound to the tip of the epithelial edge over the wound using ImageJ software (Fig. 1C).

2.3. Histochemical staining and immunohistochemistry

Central wound cross sections were stained for hematoxylin and eosin using standard procedures. Serial sections (5 μm) of preserved wound specimens were deparaffinized in xylene and graded ethanol series. Antigen retrieval was performed in pH 6.0 citrate buffer (Millipore, Billerica, MA) by heating for 2 min in a decloaking chamber (Biocare Medical, Concord, CA) except in the case of CD31. CD31 antigen retrieval was performed using ×1 proteinase K solution prepared from a ×20 proteinase K stock. ×20 proteinase K solution was made by solublizing proteinase K in one part TE buffer (50 mM Tris Base, 1 mM EDTA, 0.5% Triton X-100 [bioWORLD, Dublin, OH],