Potential anti-inflammatory treatments for chronic wounds

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Abstract

Skin is the primary protective barrier against physical, chemical, thermal and infectious threats in the environment. Maintaining the integrity of skin is a matter of survival. The collaborative term – wound healing – describes the complex, yet well-organised, network of biological processes that enables tissue injuries to resolve. The many stages include haemostasis, inflammation, matrix synthesis, angiogenesis, re-epithelialisation, contraction and remodelling; often simplified to inflammation, tissue proliferation and maturation. Advances in our understanding of the influence of immune cells, and growth factors and cytokines at each stage of wound healing have led to the development of potential treatments. Here we review the biology of chronic wound healing and discuss the potential of epicatechin gallate and activated protein C to promote wound healing in a clinical setting.

Keywords: wound healing, leg ulcer, activated protein C, epicatechin gallate.

Introduction

Pressure, venous and diabetic leg ulcers are the most common type of chronic wounds affecting the Australian population. The prevalence of chronic leg ulcers in the community is 0.11%, with 24% of ulcerations persisting for more than one year[1]. As a result, 45% of ulcer patients suffer immobility problems, with many being house-bound. Despite wound healing techniques dating back to the Edwin Smith papyrus in 1700 BC, treatments for chronic wounds have largely fallen short. While there has been a movement from 'dry dressings' to 'growth factors' and 'silver treatment' these treatments still struggle to obtain and/or maintain wound closure, and cost the Australian Government an excessive amount in wound management costs[2]. More comprehensive anti-inflammatory treatments that promote angiogenesis and re-epithelialisation may be more advantageous. In this review we discuss the biology of chronic wound healing and potential new treatments in wound management, epicatechin gallate (ECG) and activated protein C (APC).

Biology of chronic wound healing

Foremost in treating chronic wounds is to understand their pathobiology. The interplay of immune cells, growth factors and cytokines during the various stages of wound healing is key to the success of wound resolution. Injury to blood vessels leads to the extravasation of blood constituents and the formation of a clot. As one of the first cell types activated after injury, platelets secrete a number of growth factors such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and cytokines including transforming growth factor-β (TGF-β). In addition, disruption of the epidermal barrier promotes secretion of interleukin (IL)-1 and tumour necrosis factor-α (TNF-α) by keratinocytes. These chemical signals attract inflammatory cells to the site of injury. Neutrophils and monocytes are recruited to the site of injury where they clear the wound site of contaminating bacteria and debris. After debridement, macrophages release inflammatory cytokines including IL-1, IL-6, EGF, PDGF, TGF-β, fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) to stimulate the formation of granulation tissue and neovascularisation by fibroblasts and endothelial cells respectively. In chronic wounds, a number of factors contribute to impaired healing, including:

1. Prolonged inflammation

In normal wound healing, neutrophils are cleared from the wound after 72 hours by macrophages. Exudates collected by lavage from the surface of ulcers have shown that 95% of inflammatory cells are neutrophils[3]. Macrophages are infrequent and inactive, and $CD4^+$ T-lymphocytes are evident at the wound edge[4]. The persistence of inflammatory cells in the wound space contributes to elevated levels of cytokines, including IL-1α, IL-1β, IL-6 and TNF-α[5], which promote the inflammatory environment and stimulate the production of tissue-degrading proteinases[6].

2. Delayed granulation tissue formation

Prior to wound closure the wound space must be filled by granulation tissue – a combination of extracellular matrix (ECM) proteins including fibronectin, vitronectin and subsequently type I collagen. In chronic wounds, these ECM proteins are increasingly degraded by elevated levels of inflammatory cell-derived proteinases, including urokinase, cathepsin-G, neutrophil-derived elastase, and

matrix metalloproteinases (MMP)-1, MMP-2, MMP-8, MMP-9[7-9]. These proteinases also degrade cytokines and growth factors required by fibroblasts for the deposition of ECM proteins (PDGF, TGF-β and FGF)[5,10]. At the same time, there is a relative decrease in proteinase inhibitors such as α1-antitrypsin, α2-macroglobulin and tissue inhibitor of metalloproteinases-1 due to inactivation[11,12]. These inhibitors normally act to regulate the activity of proteinases in chronic wound fluid. The resultant imbalance of proteinases and inhibitors, and reduction in fibroblast growth factors, leads to a delay in granulation tissue deposition, and subsequent new blood vessel formation (or neovascularisation) and re-epithelialisation of the wound.

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3. Delayed neovascularisation and re-epithelialisation

Akin to fibroblasts, endothelial cells and keratinocytes are dependent on growth factors and cytokines for neovascularisation and re-epithelialisation of the wound, respectively. Therefore, the above-mentioned proteinase-mediated degradation of growth factors and cytokines (PDGF, TGF-α, IGF-I, FGF and EGF) in chronic wounds contributes to delayed wound healing[5,10].

An important premise in chronic wound therapy is to tailor treatment to the aetiology of the wound whenever possible. According to factors that contribute to impaired healing, the ideal treatment would control inflammation to enable progression through to the proliferative stages; granulation tissue formation, neovascularisation and re-epithelialisation. Deficiency of neutrophils and macrophages using anti-neutrophil serum and *PU.1* null mice has shown that the removal of inflammatory cells from a wound is crucial for accelerated wound healing and reduced scar formation[13,14]. Two agents that have potential to meet these criteria are ECG and APC.

Epicatechin gallate (ECG)

Catechins are a family of polyphenolic flavonoids found in green tea extracts. The three major isoforms, epigallocatechin gallate (EGCG), ECG and epicatechin, have anti-oxidant, -proliferative, -tumour and -inflammatory properties[15]. While *in vitro* studies assessing the mechanisms of ECGs actions are not clearly understood, *in vivo* studies have demonstrated a potential role for ECG in chronic wound healing.

Anti-inflammatory

Catechins decrease inflammatory cell infiltration[16] and production of pro-inflammatory cytokines IL-1β, IL-6 and TNF-α, while enhancing the production of the anti-inflammatory cytokine, IL-10 by human inflammatory cells[17,18]. The suppression of inflammatory cytokines by catechins is mediated by reduced nuclear factor *kappa* B (NF-kB) activation[18].

Granulation tissue deposition, neovascularisation and re-epithelialisation

ECG inhibits MMP-9 and MMP-2 activity[19], and collagen degradation by binding to collagen and preventing tissue-degrading enzymes binding[20]. Subcutaneous injection of ECG at the wound margin enhances collagen deposition and maturation, increases vascular endothelial growth factor and accelerates angiogenesis[21]. ECG also increases the activity of enzymes, inducible nitric oxide synthase and cyclooxygenase-2, required for the re-epithelialisation of

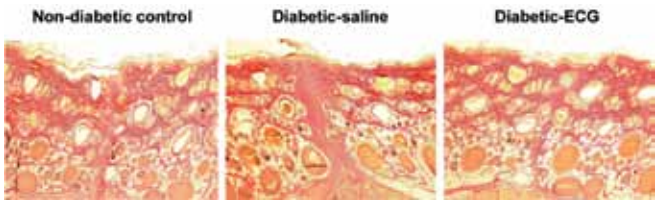

Figure 1. Representative micrographs of wound healing and scar formation of incisional wounds in non-diabetic, and diabetic mice treated with saline or ECG at 14 days. Subcutaneous injections of saline or ECG were given daily for seven days. Magnification 3X.

wounds[21]. In the full-thickness wound healing rat model, ECG increases granulation tissue formation, neovascularisation and re-epithelialisation of wounds.

Wound healing

In a murine model of type II diabetes, ECG accelerated wound healing[22]. Figure 1 shows the quality of wound healing and scar formation at 14 days in non-diabetic control, and diabetic wounds treated with saline or 0.8 mg/ml ECG daily for seven days. Diabetic wounds treated with saline demonstrated a wide, clearly visible wound tract with high proportions of disorientated immature (pink) collagen fibres indicating poor wound healing. By contrast, non-diabetic and diabetic wounds treated with ECG exhibited near complete wound contracture and a high proportion of mature (red) collagen fibres orientated parallel to the epidermis, comparable to the surrounding skin. These results indicate that ECG may improve the healing of diabetic ulcers. Similar results of improved cellular organisation of granulation tissue, neovascularisation, and re-epithelialisation in another murine model of type II diabetes have been demonstrated using EGCG[23].

Activated protein C (APC)

Activated protein C (APC) is an anticoagulant with anti-inflammatory properties. The PC zymogen is activated to APC by serine proteases after which it can engage the anti-inflammatory receptors endothelial protein C receptor (EPCR) and protease-activated receptor (PAR)-1[24,25]. Acting via these receptors, APC has demonstrated a number of wound healing actions.

Anti-inflammatory

In vitro and *in vivo* studies have revealed that APC has strong anti-inflammatory properties associated with a reduction in inflammatory cells and inflammatory cytokines. For example, APC suppresses neutrophil migration by binding to the β1 and β3 integrin adhesion molecules[26], and inhibits the activation of NF-kB in various cell types including keratinocytes[27]. The NF-kB pathway is important for the production of inflammatory cytokines, including TNF-α and cell adhesion molecules associated with inflammation. Specific treatment of monocytes with APC decreases the production of the pro-inflammatory cytokines TNF-α, IL-1β, IL-6, and IL-8[28,29], and increases anti-inflammatory cytokine IL-10 production[30].

Granulation tissue deposition, neovascularisation and re-epithelialisation

Treatment of cultured human endothelial cells or keratinocytes with APC inhibits apoptosis and promotes cell proliferation[31,32], migration and survival[33], necessary for neovascularisation and re-epithelialisation of the wound. Studies using the chick chorioallantoic membrane assay have shown that APC not only stimulates angiogenesis but causes epithelial cells to grow across the top of the gelatin sponge, mimicking keratinocyte re-epithelialisation in *in vivo* wound healing[34]. APC differentially regulates proteinase activity by suppressing MMP-9 activity[35], a collagen-degrading MMP associated with inflammatory conditions, yet increases anti-inflammatory MMP-2 activity in keratinocytes[34,36], endothelial cells[36] and dermal fibroblasts[37].

Wound healing

The extensive *in vitro* research on APC and its potential mechanisms for wound healing led to its application in animal models, and subsequently pilot human studies[34,38]. Rodent models of wound healing showed that a single topical application of 20 μg APC reduced neutrophil infiltration, increased neovascularisation and significantly reduced the wound size of full-thickness punch biopsy wounds by day 4, with no adverse effects[34]. In an open pilot study of four patients with lower leg ulcers of varied cause, topical application of APC once a week for four weeks stimulated the formation of granulation tissue and re-epithelialisation with no adverse effects or safety issues reported[38].

Figure 2 shows an example of an APC-treated patient whose deep chronic ulcer on his heel had been present for four years with no response to standard wound treatment. Striking features of healing following APC application in this example

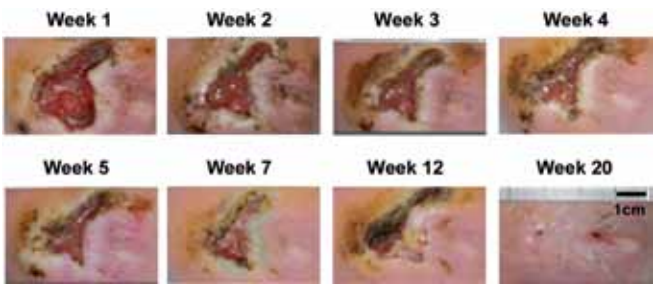

Figure 2. Progress of a patient with a four-year-old wound on the base of his heel. Topical APC treatment was given weekly for six weeks. Scale bar 1 cm.

were rapid improvement after a single treatment (week 2), and the formation of refractile translucent matrix (weeks 4–7) and granulation tissue to fill in the wound from its base. These results provide evidence that APC has the potential to improve healing in chronic wounds. Similar results were obtained in a study investigating topical APC treatment in conjunction with topical negative pressure, to treat long-standing orthopaedic wounds[39].

Future directions

Ideal chronic wound treatments would inhibit excessive inflammation, whilst stimulating keratinocyte and endothelial cell proliferation, migration and survival. Both ECG and APC appear to have the ability to stimulate keratinocyte and endothelial cell growth whilst inhibiting inflammation. The therapeutic potential of ECG or APC, either alone or combined, as a topical treatment for chronic wounds requires confirmation in double blind, placebo-controlled randomised clinical trials.

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