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Topical application of the bee hive protectant propolis is well tolerated and improves human diabetic foot ulcer healing in a prospective feasibility study

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ABSTRACT

Aims: Propolis is a naturally occurring anti-inflammatory bee derived protectant resin. We have previously reported that topically applied propolis reduces inflammation and improves cutaneous ulcer healing in diabetic rodents. The aim of this study was to determine if propolis shows efficacy in a pilot study of human diabetic foot ulcer (DFU) healing and if it is well tolerated.

Materials: Serial consenting subjects ($n = 24$) with DFU ≥4 week's duration had topical propolis applied at each clinic review for 6 weeks. Post-debridement wound fluid was analyzed for viable bacterial count and pro-inflammatory MMP-9 activity. Ulcer healing data were compared with a matched control cohort of $n = 84$ with comparable DFU treated recently at the same center.

Results: Ulcer area was reduced by a mean 41% in the propolis group compared with 16% in the control group at week 1 ($P < 0.001$), and by 63 vs. 44% at week 3, respectively ($P < 0.05$). In addition, 10 vs. 2% ($P < 0.001$), then 19 vs. 12% ($P < 0.05$) of propolis treated vs. control ulcers had fully healed by weeks 3 and 7, respectively. Post-debridement wound fluid active MMP-9 was significantly reduced, by 18.1 vs. 2.8% week 3 from baseline in propolis treated ulcers vs. controls ($P < 0.001$), as were bacterial counts ($P < 0.001$). No adverse effects from propolis were reported.

Conclusions: Topical propolis is a well-tolerated therapy for wound healing and this pilot in human DFU indicates for the first time that it may enhance wound closure in this setting when applied weekly. A multi-site randomized controlled of topical propolis now appears to be warranted in diabetic foot ulcers.

1. Introduction

Foot ulceration secondary to diabetes occurs in up to one quarter of people with diabetes (Bentley & Foster, 2007) and it is the commonest cause of lower limb amputation (Boulton, Vileikyte, Ragnarson-Tennvall, & Apelqvist, 2005). Diabetes increases the risk of lower extremity amputation by 10 to 20 times (Wrobel, Mayfield, & GE, 2005) and the estimated cost to the US healthcare system of diabetic foot ulceration and related amputations is more than $10.9 billion annually (Shearer, Scuffham, Gordois, Oglesby, & Tobian, 2003). Thus diabetic foot ulceration is a cause of significant morbidity and financial burden.

The delayed wound healing observed in diabetic foot disease is attributable to a variety of factors including peripheral arterial disease, peripheral neuropathy, foot deformity and secondary bacterial infection (Cavanagh, Lipsky, Bradbury, & Botek, 2005). Furthermore, the wound microenvironment in diabetes is abnormal and pathogenic factors lead to delayed ulcer closure, and suboptimal volume of granulation tissue formation with abnormal extra-cellular matrix (ECM) composition (Falanga, 2005; Pradhan et al., 2011). Specifically, it has been proposed that a persistent inflammatory infiltrate also associated with bacterial colonization in the wound contributes to delayed healing in diabetes (Falanga, 2005).

Propolis is a resinous bee-hive product consisting of plant materials that are initially collected on the hind legs of worker bees. The material is then masticated, salivary enzymes are added and mixed with wax to

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produce propolis (Bankova, De Castro, & Marcucci, 2000; Bufalo et al., 2013; Wagh, 2013a). Its most biologically active fractions are flavanoids and esters of caffeic acid (Banskota et al., 2002; Russo, Longo, & Vanella, 2002). Propolis has multiple properties that make it an attractive agent for treatment of diabetic foot ulcers, including being anti-inflammatory (Grunberger et al., 1988), anti-oxidant (Fonseca et al., 2011; Nagaoka et al., 2003; Talas et al., 2014) and anti-microbial (Gekker, Hu, Spivak, Lokensgard, & Peterson, 2005; Mirzoeva, Grishanin, & Calder, 1997) especially anti-bacterial (Astani et al., 2013; Scheller, Tustanowski, Kurylo, Paradowski, & Obuszko, 1977), in its actions. Furthermore, propolis component caffeic acid, has potent activity to inhibit the pro-inflammatory proteinase, matrix metalloproteinase-9 (MMP-9), and MMP-9 is known to be increased in diabetic foot ulcers (Jin et al., 2005; Ladwig et al., 2002; Liu et al., 2009).

We have previously published in a preclinical, diabetic rodent model of full thickness cutaneous wound healing, that a single application of topical propolis normalized ulcer closure rate and reduced persistent neutrophil infiltration and elastase activity (McLennan et al., 2008). In humans, propolis has been described as a useful topical treatment for ulcers (Wagh, 2013b). It is considered to have a low side-effect profile (Gallo et al., 2014; Rajpara et al., 2009; Sforcin & Bankova, 2011) and is approved in many countries for treatment of ulcers and abrasions, being sold over the counter in many parts of the world including in Australasia (Wagh, 2013b). However, despite the longevity and increasing popularity of use of propolis generally to treat many diseases, no systematic study has been reported in the use of propolis in humans with diabetic foot ulcers. The principal aim of the current work in diabetic foot ulcers was to determine if topically applied propolis on a recurrent basis is well tolerated and if it demonstrates promise as a wound healing agent. The potential benefit of propolis treatment in addition to antibiotic therapy was also investigated.

2. Research design and methods

This study was a prospective, externally (historic) controlled design. Patients with type 1 or type 2 diabetes attending the High Risk Foot Service (HRFS) at Royal Prince Alfred Hospital Sydney across the 2011 calendar year, and who fulfilled the study inclusion criteria, as described below, were invited to take part. The HRFS is a well-established multidisciplinary foot care service where we have previously reported outcome data related to wound biomarkers (Liu et al., 2009) and bacterial counts (Xu et al., 2007) in foot ulcer healing. In this study, $n = 24$ serial patients were recruited, while three other patients declined to take part. The protocol was approved by the ethics committee of Sydney South West Area Health Service, NSW, Australia, and informed consent was obtained from each enrolling patient.

A foot ulcer of 4 weeks' duration or more was deemed to be classified as a chronic ulcer, as adopted by the American Diabetes Association, (1999) and included in this study. For study inclusion, patients with a chronic foot ulcer needed to be at or above 18 years of age, with diabetes mellitus and able to give informed consent. All ulcers included in the study were classified by the established University of Texas grade and staging system, which predicts ulcer healing outcomes (American Diabetes Association, 1999). Ulcers were also described as 'neuropathic', 'neuro-ischaemic' or 'post-operative/pressure related', to help distinguish the type of ulcer category, which as described by others typically have different healing outcomes (Oyibo et al., 2001). Study exclusion criteria were: (i) patients with severe peripheral arterial disease (PAD) defined as ischemic pain at rest and/or ankle-brachial pressure index (ABPI) at or below 0.7, as these wounds were deemed unlikely to heal in the absence of revascularization (Stadelmann & Digenis, 1998); and/or (ii) foot ulcers with attendant severe infection, defined as those deemed by High Risk Foot Service medical staff to require intravenous antibiotics and/or hospital admission.

Propolis in aqueous liquid form sourced in Australia, (Honey Spring Variety, batch number 7232, Vastrade, Lidcombe NSW), was administered to cover the entire ulcer each time the patient attended from week 0 in the clinic for 6 weeks, or until the ulcer healed, whichever occurred first. A thin and even coating of propolis was painted onto the entire wound surface with a sterile cotton bud. The study personnel (FH) who applied the propolis was not involved in ongoing patient care, nor in determining ulcer area. The propolis was applied at the conclusion of each scheduled treatment, just prior to application of dressings, to minimize any potential bias from any change in routine care. The average time between visits was 10.5 days, with most individuals being seen weekly or fortnightly for standard care as is usual practice in the HRFS. This time frame of application was timed to be in keeping with the usual attendance times of patients to the Clinic, including the historic controls used in this study.

Each subject was followed up for a further 6 weeks after propolis treatment ceased, or until their wound healed, whichever occurred first. At each visit wound area was measured using acetate tracing and was scanned onto a PC all as previously described (Liu et al., 2009; Xu et al., 2007), and measured using Bersoft Image Measurement (BIM) analysis (Bersoft.com). Comparison with previous tracings enabled wound closure to be determined as a percentage of original wound area, per unit time.

In addition, on each occasion where an adequate volume of sample could be obtained ($n = 25$ μl), following ulcer debridement but prior to the application of propolis, 2×25 μl samples of wound fluid were obtained from study subjects using a calibrated sterile paperpoint tip, (Meta Biomed Co., Elmhurst, NY). The samples were mixed with 100 μl PBS and stored frozen at −80 °C for subsequent protein analysis. Samples used for bacterial count analysis were placed at 4 °C and the samples were then distributed within 2 hours onto blood agar plates.

To quantitate bacterial load, 10 μl of the post-debridement wound fluid supernatant was serially diluted (10^{-2} to 10^{-7}), then streaked onto blood agar plates, and incubated aerobically in 5% CO_2 at 37 °C for 24 hours. The number of colony-forming units (CFUs) on each plate was counted. Bacterial species were identified by standard microbiological techniques, including Gram stain, automated identification of isolates (Vitek2, Biomérieux) and susceptibility testing of *Staphylococcus aureus* isolates to determine methicillin-resistance. Previous studies in our laboratory have verified the reproducibility of sampling in a post-debridement wound fluid sample by this method (Xu et al., 2007). For matrix metalloproteinase determination, frozen wound fluids were thawed and analyzed for wound fluid MMP-2 and MMP-9, by zymography using established techniques (Liu et al., 2009).

As an external control, ulcer healing results were compared with the cohort of recently treated historical controls ($n = 84$) derived from high risk foot clinic patients with ulcers, subject to the same inclusion/exclusion criteria and receiving ongoing care in the same HRFS. Notably, the standard of care provided in the HRFS had not changed from the historic controls and the propolis treated series. All study subjects who would have qualified for the propolis study and who were treated in the same HRFS but were treated in recent years prior to study recruitment for the propolis active treatment, were included as controls. This historic control group of $n = 84$, was derived from across 2008 to 2010 calendar years. During those years the standardized approach to treating DFU in the clinic was the same as in 2011–2012 inclusive, and attendant senior medical, nursing and allied health staff were similar and in continuity across the 5 years. Treatment consisted of careful assessment of ulcer precipitating and predisposing factors with ulcer classification, followed by multidisciplinary management including pressure off-loading, debridement and dressings, and treatment of clinical infection, all as previously described in our Service (Xu et al., 2007) and following international