Chronic wound research: an integrated approach

Wallace HJ & Stacey MC

Abstract

For 20 years a significant research component has been integrated into the clinical services provided for patients with chronic leg wounds at Fremantle Hospital. The development of a team of clinicians, nurses and scientists has enabled a broad range of clinical and laboratory wound research to be undertaken on human subjects, which is only possible in a small number of centres worldwide. The key areas of research focus have been chronic wound epidemiology, clinical investigations, exercise and venous disease, pathophysiology of venous leg ulceration and clinical trials. Research on the pathophysiology of venous leg ulceration has been facilitated by the development of a human model of non-healing and healing venous ulcers. More recently, a genetic approach has been used to identify key molecules in venous ulcer pathogenesis through the investigation of gene polymorphisms as risk factors for the development of venous leg ulceration.

Genetic studies and randomised control trials for chronic wound therapies require large numbers of subjects to provide adequate statistical power. Additional funding and leadership is required to increase the number of centres across Australia with the capacity to participate in multi-centre chronic wound studies, and to facilitate effective collaborations between hospital- and community-based wound care providers, research institutions and industry.

Introduction

A specialised clinic was set up in Fremantle Hospital in July 1988 [1] with the aim of performing a thorough clinical and laboratory assessment to establish the cause of leg ulceration in every patient, and therefore treating patients according to the cause of ulceration. Fremantle Hospital is a 450-bed acute-care teaching hospital within the South Metropolitan Area Health Service of Western Australia. From the outset, a significant research component was integrated into the clinical and diagnostic services provided by the out-patient leg ulcer clinic and vascular laboratory. Research is principally conducted within the Vascular Research Laboratory and the University of Western Australia Combined University Departments Research Laboratory, both located on the Fremantle Hospital site.

Hilary J Wallace BVSc BVMS (Hons) PhD
Research Fellow
University of Western Australia
School of Surgery, Fremantle Hospital, WA

Michael C Stacey MBBS DS FRACS
Professor of Surgery
University of Western Australia
School of Surgery, Fremantle Hospital, WA

The establishment of an integrated research team of clinicians, nurses and laboratory scientists has meant this is only one of a small number of centres worldwide that is capable of undertaking both clinical and laboratory research on human subjects with chronic wounds. This, along with the successful development of a model for non-healing and healing chronic wounds in human subjects, has encouraged companies and research groups in Australia, Europe and the USA to seek to collaborate on clinical research and research into the basic biochemical abnormalities that are present in non-healing chronic wounds. By illustrating the scope of research activities that have been conducted in chronic wounds at Fremantle Hospital, we aim to demonstrate the benefits of working in a team that brings together clinical and scientific expertise. Areas in high need of new research have been identified where appropriate.

Epidemiology

A key early research activity was to define the scope of the problem of chronic leg wounds in Western Australia (WA) [2,3]. A metropolitan population of 238,000 in Perth, WA, was screened for chronic ulceration of the leg, giving a point prevalence of 0.11%. Patients with a chronic leg ulcer and a venous abnormality comprised 57% of all patients with a chronic leg ulcer. There was an increasing prevalence with age – 90% of patients with venous ulcers were 60 years or older.

While this early study is still frequently cited, including at the recent 2008 Congress of World Union of Wound Healing Societies in Toronto, it is important to conduct new epidemiological studies in Australia and determine the prevalence of leg ulceration now that the proportion of elderly people in the population has increased. Such studies also have the opportunity to assess the impact of interventions that may influence ulcer prevalence. For example, has the use of hormone replacement therapy, which peaked at 21% of women ≥50 yrs in Australia in 2001 [4], had an influence on the current prevalence of venous leg ulcers in women [5]? Fresh epidemiological data is essential to underpin health economic analyses to determine both the cost of leg ulceration to the Australian healthcare system, and the size of the wound care market.

Clinical investigations

Validation studies have been undertaken on tests used to evaluate venous disease, in particular plethysmography for the detection of venous reflux [6]. More recently, high frequency ultrasound has been investigated as a tool to quantify oedema in venous disease. The test for oedema measures the thickness of the dermis, which increases in proportion to the extent of oedema [7, 8]. The quantification of oedema using high-frequency ultrasound has the potential to be a useful test to monitor the effectiveness of compression therapy, and in the diagnosis of the post-thrombotic syndrome [9].

Exercise and chronic venous disease

Important studies on calf muscle function in chronic venous disease have been conducted in the Vascular Research Laboratory. An initial study showed that patients with chronic venous disease have a significant impairment of calf muscle function compared with healthy control subjects [10]. A follow-up study found that poor calf muscle pump function in patients with chronic venous ulceration can be significantly improved by physical exercise [11]. New studies are required to evaluate whether programmes to improve muscle strength may be of benefit in both healing and preventing the recurrence of chronic venous ulcers.

Pathophysiology of venous leg ulceration: *in vivo* model

A continuous research strand at Fremantle Hospital has been the identification of basic biochemical abnormalities in non-healing chronic wounds. The objectives are two-fold – to identify molecules for use as surrogate markers of ulcer healing or non-healing, and to identify new treatment targets.

With the absence of suitable animal models of chronic venous ulceration, an *in vivo* standardised model of 'non-healing' and 'healing' ulcers has been developed to reduce the variability between wounds due to their variation in healing status (Figure 1). 'Non-healing' ulcers are those that do not reduce in size in 3 months, despite out-patient compression therapy. These patients are admitted to hospital for 2 weeks of strict bed rest prior to skin grafting. The reduction in venous hypertension due to leg elevation in bed induces observable signs of healing in the majority of cases, with the formation of granulation tissue and reduction in wound surface area. Samples (wound fluid and biopsies) are taken on admission to hospital (non-healing) and after 2 weeks (healing) (Figure 2).

Inflammation and oxidative stress

Results from studies performed with this model suggest that healing may be impaired by inflammatory mediators rather than inhibited by a deficiency of growth factors in venous leg ulcers. General biochemical analyses suggest an inflammatory process (C-reactive protein) in the non-healing wound [12]. The

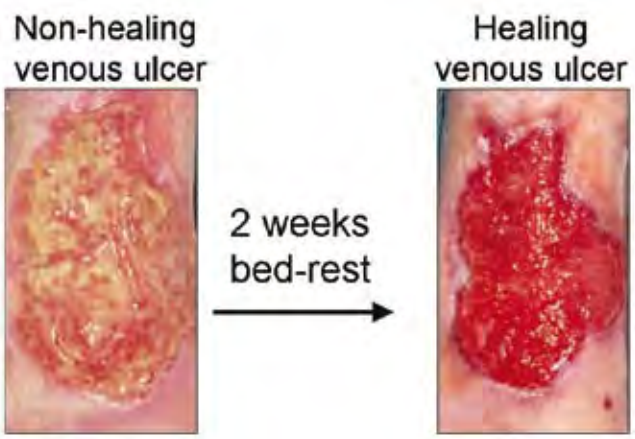

Figure 1. In vivo *human model.*

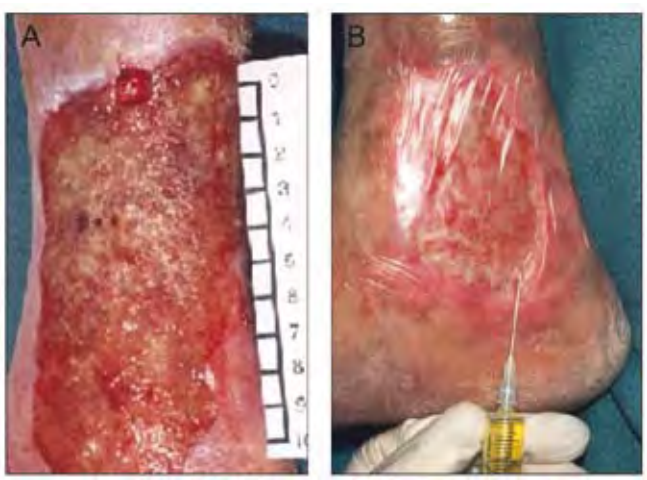

Figure 2. Ulcer biopsy and wound fluid collection.

pro-inflammatory cytokines interleukin-1, interleukin-6 and tumour necrosis factor-α (TNF-α) are found to be present in significantly higher concentrations in wound fluid from non-healing compared to healing leg ulcers [13, 14]. Interestingly, the levels of bioactive TNF-α are not significantly different [14]. There are detectable levels, but no significant change, in the levels of platelet derived growth factor, epidermal growth factor, basic fibroblast growth factor or transforming growth factor-ß as ulcers heal. Immunohistochemical analysis show up-regulation of the production of inflammatory cytokines and growth factors in keratinocytes of chronic leg ulcers (compared to normal skin) that is greater when ulcers are non-healing [15].

Iron (ferritin) levels are also elevated in wound fluid from non-healing ulcers compared to healing ulcers [16] and elevated levels of 8-isoprostane and antioxidants are measured in chronic wound fluid compared to acute wound fluid. These findings suggest an environment of oxidative stress exists in chronic wounds which may be related to iron deposition in the skin.

Matrix metalloproteinases (MMPs)

Inflammatory cytokines up-regulate the expression of matrix metalloproteinases (MMPs), enzymes that degrade extracellular matrix [17, 18]. Early studies confirmed that MMP (collagenase) activity is significantly elevated by 30-fold in chronic wounds compared to acute wounds [19]. In addition, using the *in vivo* model described above, levels of MMP activity decrease significantly with healing in venous leg ulcers [19]. Significantly higher MMP-1 and MMP-3 levels, and reduced TIMP-1 levels, are induced in dermal fibroblasts by chronic venous leg ulcer wound fluid compared to acute wound fluid [20]. These data support the hypothesis that excessive proteolytic activity, in particular an imbalance of MMPs and their natural inhibitors, the tissue inhibitors of MMPs (TIMPs), may be part of the pathogenesis of venous leg ulceration.

Gene polymorphisms as risk factors for venous leg ulcers

It is known that not all individuals with chronic venous insufficiency develop leg ulcers. Like many other diseases,

Table 1. Results of genotype analysis [21].

Gene (polymorphism *)	High-risk allele: Carrier frequency † (%) *Ulcer patients*	*Controls*	OR (95% CI) Significance	Adjusted OR Δ (95% CI) Significance
TNFA (-308 G/**A**)	43.1	22.6	2.59 (1.64-4.08) p=0.00003	2.48 (1.54-3.97) p=0.000155
BAT1 (-/**C** in intron 10)	28.8	16.3	2.08 (1.23-3.52) p=0.0055	2.00 (1.16-3.44) p=0.012
MMP-3 (-1171 **5A**/6A)	73.5	72.4	1.00 (0.62-1.60) NS	1.11 (0.68-1.80) NS
MMP-2 (–1306 **C**/T)	93.2	92.9	1.08 (0.46-2.51) NS	1.17 (0.47-2.92) NS
PAI1 (–675 **4G**/5G)	82.3	79.5	1.20 (0.71-2.02) NS	1.11 (0.64-1.92) NS
IL-1RN (variable number of tandem repeats in intron 2. **IL-1RN*2** contains 2 repeats)	49.4	44.0	1.24 (0.81-1.91) NS	1.31 (0.84-2.05) NS

BAT1=HLA-B-associated transcript 1; CI=confidence interval; IL-1 RN=interleukin-1 receptor antagonist; MMP=matrix metalloproteinase; OR=odds ratio; PAI1=plasminogen activator inhibitor 1; TNFA=tumour necrosis factor-α (gene).

* Bold type indicates postulated high-risk allele

† Carriers include both heterozygotes and homozygotes

Δ Adjusted for age and gender. NS = not significant (p>0.05).

genetic differences (gene polymorphisms) may contribute to an individual's susceptibility. From the results of the studies on inflammatory cytokines and MMPs, we hypothesised that functional polymorphisms in these genes, or genes involved in their regulation, may be risk factors for the development of venous leg ulcers. Functional polymorphisms are those which alter the level of gene expression or protein function.

A case-control study was conducted to compare the frequency of several gene polymorphisms in 181 Caucasian patients with a history of confirmed venous leg ulceration and 181 age- and gender-matched healthy controls, also of Caucasian background [21, 22]. Results showed that the risk of venous leg ulceration is elevated in carriers of polymorphisms in the promoter of the TNF-α gene (TNFA-308A) and in intron 10 of the BAT1 gene (c insertion, *2) (Table 1), linked genes located in the central major histocompatibility (MHC) complex. This is a region where conserved blocks of genes are maintained, known as ancestral haplotypes (AHs). Seventy percent of Caucasian individuals carrying TNFA-308A, and >90% of individuals carrying BAT1 intron 10*2, also carry part or all of the 8.1 AH (HLA-A1, B8, DR3, DQ2), associated with numerous immunopathological disorders. Recent data from extended fine-mapping [23], shows that a region of the 8.1 AH from HLA-B8 to the TNF-α gene (including the BAT1 intron 10*2 polymorphism), increased the risk of venous ulceration. These results could be used to develop an indicator of risk of venous leg ulceration.

A retrospective study is currently being undertaken to determine clinical and genetic risk factors for the development of severe post-thrombotic syndrome (lipodermatosclerosis and leg ulceration). This will include testing whether the TNFA-308A and BAT1 intron 10*2 polymorphisms are risk factors for ulceration in individuals who have had a previous deep vein thrombosis (secondary venous insufficiency). A new study will examine if these polymorphisms are risk factors for leg ulceration in individuals with primary venous insufficiency.

Clinical trials

Approximately 20 clinical trials have been undertaken over the past 20 years, many sponsored by industry.

A randomised double-blind placebo controlled trial of topical autologous platelet lysate in venous ulcer healing (n=86) found that platelet lysate prepared and delivered by the method used in the study had no influence on the healing

of chronic venous ulceration [24]. An important study with direct clinical application was a randomised controlled study showing that graduated compression elastic stockings reduce lipodermatosclerosis and ulcer recurrence [25].

New clinical trials are about to commence to test the safety and effectiveness of VitroGro™, a synthetic growth factor-extracellular matrix complex developed at Queensland University of Technology by Zee Upton and colleagues, for the treatment of venous leg ulcers. The complex comprises insulin-like growth factors (IGF) and IGF-binding proteins bound to vitronectin, and have been shown to enhance keratinocyte protein synthesis and migration [26].

Conclusion

The effective integration of research activities into a tertiary referral centre for chronic leg wounds has attracted significant funding from both government and industry sources for the past 20 years. The capacity to undertake basic and translational research results from access to large numbers of patients at a single site with wounds that have been fully assessed for the cause of ulceration, appropriate clinical and laboratory facilities, and a broad range of staff expertise. However, future studies, in particular genetic studies and clinical trials, will require large numbers of subjects to provide adequate statistical power. Additional funding and leadership is required to increase the numbers of centres across Australia with the capacity to participate in multicentre studies, and to facilitate effective collaborations between hospital- and community-based wound care providers, research institutes and industry.

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