Understanding chronic wounds: a unifying hypothesis on their pathogenesis and implications for therapy

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Abstract

The development of new therapies for treatment of chronic wounds has not matched the availability of treatment modalities forecast by the pharmaceutical industry. This is attributable in large part to difficulties encountered in clinical trials as well as in isolating study design variables. Our hypothesis attempts to address this shortcoming. We are proposing that chronic wound pathogenesis is based on 3 fundamental factors: the cellular and systemic changes of aging, repeated ischemia-reperfusion injury, and bacterial colonization with resulting inflammatory host response. The derivation of this hypothesis is founded on the observation that the 3 primary categories of chronic wounds—pressure ulcers, diabetic ulcers, and venous ulcers, which are the overwhelming majority of chronic wounds—have these common causative factors. Our hypothesis incorporates major implications for treatment modalities based on these factors. Addressing the first issue, the cellular and systemic changes of aging, Regranex (Ortho-McNeil Pharmaceutical, Inc, Raritan, NJ), a platelet-derived growth factor drug, has shown great promise. Additional treatment modalities that address the second and third problems, repeated ischemia-reperfusion injury and bacterial colonization, include vacuum-assisted closure, warming of local tissue, and water irrigation using pulsed lavage. Additionally, treatment comprising a combination of these approaches has demonstrated success. © 2004 Excerpta Medica, Inc. All rights reserved.

Although a great deal of progress has been made in the past 15 years in both the understanding of the pathogenesis of chronic wounds and their treatment, the development of new therapies has not kept pace with the optimistic predictions of the pharmaceutical or biotechnology industries. In addition, the difficulties in performing clinical trials and isolating variables in study design have proved more challenging than most wound-healing experts predicted 15 years ago, when clinical trials in wound healing were in their relative infancy. Finally, chronic wounds do not occur in animals, and so the experimental study of chronic wounds has been difficult. Understandably, there has been a major focus on characterization of human chronic wounds and what makes them unique. There has been an implied expectation by both academic investigators and industry that for each type of chronic wound there would be a central defect that if corrected or treated, would then result in a dramatic therapeutic result.

I propose an alternative hypothesis, based on both laboratory and clinical observations. I suggest that chronic wounds can be explained largely by the coexistence of 3 major factors: (1) the cellular and systemic changes of aging, (2) repeated ischemia-reperfusion injury (often in the setting of underlying local ischemia), and (3) bacterial colonization with the resulting inflammatory host response. Animal wounds heal in a fundamentally similar way to human wounds. The reason there are no chronic wounds in animals is the relative absence of truly aged animals, as well as the absence of such conditions as neuropathy, chronic debility, or venous insufficiency that results in ischemia or ischemia-reperfusion injury. In addition, almost all animals are loose skinned, and their open wounds heal almost entirely by wound contraction (pulling normal unwounded skin over a wounded area), which occurs very rapidly. In contrast, contraction is slow and incomplete in most areas of the human body, particularly the lower leg and foot, which are the sites of many chronic wounds. Because of the multifactorial nature of virtually all chronic wounds, any single therapeutic pharmacologic intervention is unlikely to have the dramatic therapeutic result for which most companies are searching. Indeed, if other factors are not optimally treated, the therapeutic trial may well end in failure, or the benefit may be insufficient to detect in a small trial.
Chronic wound types

A very large percentage of chronic wounds fall into 3 main categories—pressure sores, diabetic ulcers, and venous ulcers—with a small fourth set of ulcers secondary to ischemia (arterial insufficiency, radiation changes). Although they have very different underlying etiologies, and the tendency has been to emphasize their differences, they do have some important causative factors in common. Excluding pressure sores secondary to paraplegia, the average age of patients in all 3 categories in clinical studies of such growth factors as platelet-derived growth factor (PDGF) for diabetic ulcers [1], PDGF for pressure sores [2], and venous ulcer trials with silver sulfadiazene [3] has been >60 years.

Chronic wounds in the aged: the altered stress response

Although most wounds in the aged heal eventfully (complications of surgical procedures in the elderly are generally only minimally elevated), there have been longstanding clinical observations that the elderly do quite poorly when subjected to stress. Perhaps the greatest stress that humans can be subjected to is major burns. In considering cases of 50% total body burns, the survival rate is relatively stable beyond infancy with >50% of patients surviving (with a slight fall-off up to 60 years of age). However, beyond age 60 there is a dramatic decrease in the rate of survival: after 80 years of age, survival becomes unusual. Experimental animal studies in wound healing support these observations and suggest they can be applied to human wound healing. In rabbits, rats, and mice, wound healing is modestly delayed in terms of wound closure or wound-breaking strength in aged animals. However, when the wounds are made ischemic, wound healing is drastically impaired. This impairment begins to show up during middle age in rabbits (36 months old; expected average life span in the laboratory about 60 months) [4]. Wound-healing impairment is additive, if not synergistic, when age and ischemia are combined. When 60-month-old rabbits have ear wounds that are made chronically ischemic, a true chronic wound is produced: there is essentially no observable healing after 25 days. On the other hand, within the same time frame, wounds with chronic ischemia in young animals go on to complete healing [5].

Changes in gene expression in the elderly

The cellular changes of aging have been the object of intense investigation in many fields of biology. In culture, aged cells show decreased proliferative capacity with prolonged doubling times and eventual replicative senescence [6,7]. These changes most likely affect the altered stress response in the aged and play a role in chronic wounds [8]. Transfection of cells nearing replicative senescence in vitro with the telomerase gene (which elongates the telomere) can indefinitely delay senescence, with maintenance of the phenotype of nontransformed cells [9]. Intriguingly, transfection of aged rabbits with telomerase results in a marked improvement in wound healing (J. C. Mogford, T. A. Mustoe, unpublished data, 2003). In vitro, senescence can be induced by such stresses as oxidants or ultraviolet irradiation, but transfection with telomerase does not rescue cells from stress senescence [10]. The molecular basis of the altered aged response to stress is complex, and many pathways are undoubtedly involved, but the response to telomerase transfection in aged rabbits suggests a new therapeutic approach that may address aspects of aging that previous approaches have not encompassed.

Microarray analysis of gene expression in the elderly

When gene expression of aged cells is examined by microarray analysis, most genes are unchanged compared with young cells, but interestingly, a substantial number of stress response genes are upregulated. Observations in our laboratory on selected stress genes confirm an upregulation under normal conditions, but when placed under an oxidative stress or an ischemic stress, these genes do not change expression in contrast to young cells that show a substantial change [11]. An attractive, although speculative, interpretation of these results is that aged cells are unable to respond optimally to stress with protective pathways but are more likely instead to shut down and proceed to apoptosis or necrosis. Certainly, it can be said that at the gene expression level, altered expression of stress-related genes is a major difference between aged and young cells.

Chronic wounds: the role of ischemia-reperfusion

Many chronic wounds occur in the setting of some degree of local tissue ischemia either from chronic fibrosis, as in many pressure sores, venous ulcers, and diabetic ulcers, or atherosclerosis for many diabetic ulcers. Although the presence of underlying ischemia is well accepted, and the ischemic insult secondary to pressure is accepted for pressure sores and diabetic ulcers, the reperfusion side has not been emphasized. The ischemia-reperfusion injury has been a major area of investigation, and is accepted to underlie the pathophysiology of myocardial infarction, hemorrhagic shock, and organ transplantation [12]. However, ischemia-reperfusion has not been commonly discussed in relation to chronic wounds. Actually, for all 3 major chronic wound types, the underlying precipitating event is a period of ischemia followed by a period of reperfusion. These ischemia-reperfusion events are usually repetitive, which means the deleterious effects of ischemia-reperfusion are potentiated and eventually sufficient to cause ulceration. Appropriate offloading or adequate leg compression is necessary...
to interrupt the repetitive ischemia-reperfusion events. In venous ulcers, the ischemic event occurs when the lower extremity is dependent and the normal arterial-venous pressure gradient is no longer present. There is blood stasis and effective loss of circulation. When the leg is elevated, circulation is restored, and the inflammatory changes that occur with reperfusion worsen the injury. For both pressure sores and diabetic foot ulcers, the ischemic event is the result of prolonged pressure sufficient to prevent tissue perfusion, and when the pressure is finally relieved, the reperfusion injury occurs.

**Ischemia-reperfusion: cellular events**

The events of ischemia-reperfusion injury have been the subject of intense investigation and are nicely summarized in a recent review [10]. Briefly, during the reperfusion process after an ischemic event secondary to pressure or lower extremity dependency in the setting of venous insufficiency, leukocytes are recruited by chemokines, leukotrienes, complement factors, and other chemoattractants. The endothelial surface expresses adhesion molecules, and the leukocytes roll, stick, and migrate into the tissue. There they express proinflammatory cytokines and release destructive oxygen free radicals, and in the process nitrous oxide is downregulated, which accentuates the inflammatory cycle. The downregulation of nitrous oxide causes vasoconstriction, which may contribute to the no-reflow phenomenon (lack of tissue perfusion), which occurs in reperfusion injury. At the cellular level, the release of oxidants is a stress event. As the above discussion notes, aged cells are less equipped to deal with these events than are nonaged cells. Repeated ischemia perfusion events potentiate the cycle of inflammatory cytokines, leukocyte migration, and protease and oxidant injury with loss of tissue perfusion and resultant tissue necrosis.

**Role of bacteria in chronic wounds**

The third critical factor in the pathogenesis of most chronic wounds is a combination of the presence of bacteria, the leukocytes they attract, and the high-protease, high-oxidant environment that results. The oxidant-producing enzymes, such as myeloperoxidase, within polymorphonuclear leukocytes, which are essential to kill bacteria, have an activity proportional to oxygen within the wound environment. A partial pressure of oxygen (PO$_2$) level of 25 mm Hg will effectively generate the superoxides that kill bacteria [13]. Experimental and human studies have documented that with even moderate reductions in tissue oxygen levels as a result of temporary ischemia (such as in hypothermia during surgery), the risk of infection increases substantially [14,15].

During periods of ischemia in chronic wounds, the ability to kill bacteria counts goes down and the bacterial counts can go up. As an important cofactor, the protein exudate on the surface and any nonviable tissue serve as a culture medium and are in effect protected from host antibacterial defenses. The high leukocyte counts in the granulation tissue at the wound surface result in production of proteases and oxidants that degrade the extracellular matrix and cytokines, inhibiting cell migration and preventing wound closure. In a classic report, it has been well documented that in the setting of a bacterial count $>$100,000/mm$^3$, skin grafts will not survive [16]. In addition, there is a great deal of anecdotal clinical evidence that chronic wounds will not heal without lowering the bacterial count.

**Role of bacteria in chronic wounds: clinical examples**

Although most chronic wounds occur in the setting of multiple risk factors, the following example of a chronic wound arising from a pilonidal sinus illustrates the profound inhibition in healing that can occur with the single factor of chronic bacterial excess: A young man, aged 27 years, had a pilonidal sinus that failed to heal after 7 years and approximately 7 excisions, as well as weekly visits for wound care. He was otherwise active and in excellent health. On examination, the wound did not appear infected, but was in the setting of a moist contaminated environment complicated by the presence of hair (foreign bodies drastically reduce the patient’s host defense ability to eliminate bacteria). By instituting a regimen of hair removal by shaving, frequent washing and water irrigation, and use of an antibiotic ointment, complete wound closure was achieved in 6 weeks. I have encountered several examples of such wounds failing to heal for $\geq$1 year with conventional therapy, which went on to heal with this regimen focused on lowering bacterial count. Other related examples of chronic wounds successfully closed with attention to the single factor of lowering bacterial count include pin tracts, perirectal wounds arising from fistulae or abscesses, and multiple wounds with significant depth compared with surface area. In all of these situations, in patients with chronic wounds (ie, failure to heal significantly within 90 days) referred to our wound clinic, simple measures to remove exudate at frequent intervals with resultant decrease in bacterial count have steadily progressed to complete healing.

**Therapeutic approaches to chronic wounds**

If chronic wounds occur most often in the setting of the combined risk factors of age, ischemia-reperfusion injury, and the inflammatory environment of protease and oxidant excess induced by bacterial colonization, then it seems optimistic to hope that a therapy directed at 1 of these factors will have a dramatic impact. In experimental studies in animals, although a doubling in breaking strength or new granulation tissue can be achieved at selected time points,
when wound healing is observed to complete closure, acceleration in wound healing is limited to 30% to 40% with such wound-healing agents as growth factors [17]. However, in none of the animal models to date has it been possible to introduce all 3 risk factors that occur in most human chronic wounds. Therefore, it seems unlikely that any single healing agent will have the kind of dramatic clinical effect that most companies have been hoping to achieve with their wound-healing products.

Therapeutic approaches: growth factors

The largest, most definitive, prospective randomized trials to date have been performed with Regranex (platelet-derived growth factor; Ortho-McNeil Pharmaceutical, Inc, Raritan, NJ). In >1,000 patients with diabetic ulcers, an overall 10% increase in total wound closure was achieved when all patients were included, even the dropouts. Although many have thought this was a failure of the product [18], it could also be argued that in the large pivotal trials, (1) aggressive debridement to reduce bacterial count and inflammation and to optimize local wound blood supply and (2) elimination of ischemia reperfusion injury by absolute offloading were both not done optimally. In the initial smaller phase 2 trial [1], a doubling of wound closure was achieved. This study was done with many fewer clinical trial sites, and the best outcomes with Regranex were achieved at those sites that did the most frequent debridements (D.L. Steed, personal communication, 1998). Our interpretation of these results is that optimal attention to reduction of bacterial count and inflammation, perhaps combined with more optimal offloading (often carefully selected study sites in phase 2 trials may involve more experienced physicians and more motivated patients), led to better results.

Variability in healing rates of chronic wounds

Examining the Regranex trials from another perspective, the healing rates of the control groups in the 3 large pivotal trials of >300 patients varied by 10% from study to study, as did the healing rates of the Regranex treatment groups. With the very large sample sizes, statistical significance was demonstrated overall, but this kind of variability is inherent in chronic wound studies. In smaller studies, the potential variability is even larger, and yet this is often overlooked by companies interpreting their smaller studies, and perhaps even by expert panels convened by the FDA.

Therapeutic approaches that address ischemia and bacterial burden

Looking at current and future therapeutic interventions in the field of chronic wounds, it is perhaps useful to analyze their utility in terms of their ability to address the 3 critical factors of aging, ischemia, and ischemia-reperfusion injury, and the inflammatory impact of a bacterial burden. One device that addresses both the issues of ischemia and bacterial burden has been vacuum-assisted closure (VAC; Kinetic Concepts, Inc, San Antonio, TX). It has enjoyed widespread use in recent years for many difficult acute wounds and in the treatment of pressure sores as an alternative to surgery. Although there has been a paucity of prospective randomized trials defining the efficacy, the rationale is solid. The use of a vacuum to continuously remove exudate and fluid minimizes the substrate for bacterial growth, and the reduction in edema and negative pressure enhances local blood flow. In animal studies, both a reduction in bacterial counts and an increase in blood flow have been documented [19].

Another method to enhance local blood flow and tissue perfusion has been warming the local tissue. Total body warming with secondary increased blood flow to the skin and gut has become standard therapy for surgical procedures under general anesthesia to prevent hypothermia. Landmark studies done by Kurz et al [15] showed a substantial reduction in the infection rate during routine abdominal procedures when hypothermia was prevented. For chronic wounds, increasing evidence is accumulating on the benefits of local tissue warming to increase blood flow, and a noncontact radiant bandage has been developed and is now in clinical use (Warm-Up; Augustine Medical Corporation, Eden Prairie, MN) [20,21].

The role of antibiotics in the therapy of chronic wounds

Antibiotic therapy has always had a place in the treatment of chronic wounds when clinically infected, but the role of systemic antibiotics in treating chronic wounds is limited by the lack of blood supply to the wound surface. Systemic antibiotics have a clear role in infected wounds with surrounding cellulitis, but their role in the routine treatment of chronic wounds is limited. Topical antibiotics have been accepted as useful in the setting of high bacterial counts characterized by a highly exudative wound, but their role in colonized wounds is less clear. However, the lack of ability to define the bacterial burden by clinical examination suggests a potential role, ie, treat all wounds with the presumption that a reduction in bacterial burden in at least some wounds is beneficial. One clinical study supporting the efficacy of topical antibiotics, even in wounds with bacteria counts >100,000/mm³, is a study on venous ulcers treated with silver sulfadiazene. In this study, a modest but statistically significant increase in wound-closure rate was seen, with no other confounding variables between treatment groups [3].
Other methods to lower bacterial counts in chronic wounds

Another effective way to lower bacterial counts in tissue has been the use of water irrigation using pulsed lavage, which can lower bacteria counts by several logs [22]. Even the moderate pressure achievable by a syringe and 20-gauge catheter or hand-held shower spray irrigation at close range can be quite effective, and this single modality has been the decisive factor in achieving closure of many chronic wounds (T. A. Mustoe, unpublished clinical observations). Surgical debridement, of course, has been the classic method to remove necrotic tissue and eliminate the culture media for bacteria, which thus lowers the bacterial burden.

Combined therapies to address multiple deficiencies

Oxygen therapy in the form of hyperbaric oxygen to counteract the impact of ischemia has also been put to considerable use. However, the paucity of prospective randomized studies, and the enthusiastic use by some practitioners in a variety of conditions for which a clear-cut randomized studies, and the enthusiastic use by some practitioners in a variety of conditions for which a clear-cut reasonable rationale has been lacking, have made this therapy somewhat controversial. In recent years there has been a great deal of research defining the signal transduction pathway in response to hypoxia mediated through hypoxia-inducible factor. Correspondingly, moving from a hypoxic state to a well-oxygenated state during intermittent hyperbaric oxygen treatment also serves as a cell signal mediated through generation of physiologic levels of oxidants. The work of many investigators has begun to define these pathways and provide a theoretical rationale for the use of hyperoxygen, with a resultant increase in blood flow [23,24]. In looking to optimize therapies in the future, it is intriguing to suggest that combining therapies that act by means of different signal transduction pathways may offer better chances for therapeutic success. One example from animal studies has been the combination of hyperbaric oxygen and growth factors in ischemic animal wounds [25]. Another has been the benefit of adding an insulin growth factor–binding protein, which contains an RGD sequence to interact by means of the integrin signal transduction pathway with insulinlike growth factor–1, which acts by means of a growth factor signal transduction pathway. Neither is effective by itself in improving wound healing, but when both signal pathways are active, the combination is highly effective in enhancing wound healing [26].

For the future, it would seem fruitful to elucidate the alterations of aging in the response to ischemia-reperfusion injury and ischemia, the impact of bacterial colonization, the induced inflammatory response on ischemia-reperfusion, and the combination of all 3. There is still a great deal that can be done with currently existing animal wound models and new models to be defined that better mimic the human chronic wound. Perhaps future therapies will include a treatment to reverse some of the effects of aging (eg, gene therapy with telomerase, supply of a growth factor by protein or gene therapy), augmentation in blood supply (mediated by angiogenic agents, hyperbaric oxygen, warming therapy, or negative pressure), and novel ways of reducing bacterial counts (augmenting host defense), as well as optimal prevention of ischemia-reperfusion injury. There is room for optimism that combination therapy, acting through multiple pathways, may result in the dramatic therapeutic outcomes that to date have remained elusive.

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