Cachexia – an intrinsic factor in wound healing

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ABSTRACT
Systemic diseases are intrinsic factors that alter and may impair the wound healing process. Cachexia is a manifestation of systemic, often chronic, diseases and is characterised by systemic inflammation, appetite suppression and skeletal muscle wasting. Anorexia in cachectic states is commonly associated with malnutrition. Malnutrition may cause impaired healing. Therefore, it would follow that cachexia could influence wound healing because of reduced food intake. However, the lack of response to measures to reverse cachexia, such as supported nutrition, would suggest that a direct causal link between anorexia and weight loss in cachexia is too simple a model. To date, there is no published literature that examines the role of cachexia in human wound healing specifically. This article aims to demonstrate that cachexia is an intrinsic factor in wound healing. The role of the common mediators in wound healing and in cachexia are compared – specifically inflammation, including the nitric oxide synthase pathway, collagen deposition and reepithelialisation.

Key words: Cachexia • Collagen deposition • Inflammation • Reepithelialisation • Wound healing

INTRODUCTION
The key stages of wound healing are haemostasis, inflammation, proliferation, maturation and remodelling (1). Various mediators exert their influence on each stage at the appropriate time. Disruption of the balance or timing of mediators at any stage may result in the progression of an acute to a chronic wound (2). Numerous factors may affect the process of wound healing. These can be divided into two categories – intrinsic and extrinsic factors. Intrinsic factors may be further categorised into systemic and local factors (3).

Systemic diseases are intrinsic factors that alter and may impair the wound healing process. Cachexia is a manifestation of systemic, often chronic, diseases such as neoplasms, rheumatoid arthritis, chronic heart failure and chronic obstructive pulmonary disease; and is characterised by systemic inflammation, appetite suppression, and skeletal muscle wasting (4). It is acknowledged that anorexia in cachectic states is commonly associated with malnutrition. Malnutrition may cause impaired healing. Therefore, it would follow that cachexia could influence wound healing because of reduced food intake. However, the extent of protein catabolism and malnutrition, and the lack of response to measures to reverse cachexia, such as supported nutrition, would suggest that a direct causal link between anorexia and weight loss in cachexia is too simple a model (5). Those with systemic diseases may also require multiple medications, and both systemic diseases and medications may synergistically cause wound healing problems (6).

The premise of this essay is that cachexia is an extrinsic factor that affects wound healing via anorexia and undernutrition, and cachexia is an intrinsic factor because its mediators are similar to those essential in wound healing. To date, there is no published literature that examines the role of cachexia in human wound healing specifically. One obvious reason for this is ethical issues pertaining to the recruitment of participants from a vulnerable population who are often at the terminal stages of their disease. This article will
Key Points

- This article will compare the role of the common mediators in wound healing and in cachexia – specifically inflammation, including the nitric oxide synthase (NOS) pathway, collagen deposition and reepithelialisation.

INFLAMMATION

Inflammation is an early phase of wound healing and is mediated by cytokines (7) – tumour necrosis factor α (TNFα) promotes inflammation, transforming growth factor β (TGF-β) directs epidermal migration and extracellular matrix synthesis and interleukins (ILs) influence fibroblast proliferation and matrix synthesis (8). A review of catabolism in cachexia by Durham et al. (9) identified the same three cytokines as key mediators.

Interleukin 6 (IL-6) is involved in acute inflammation and immunity during wound healing, and is implicated in many disease processes (10). IL-6 knockout mice had delayed wound healing and impaired reepithelialisation compared with their wildtype counterparts (11). A follow-up study utilised polymerase chain reaction to quantify matrix metalloproteinase (MMP) expression following exposure of IL-6 knockout mice dermis to IL-6 on collagen matrices (12). The introduction of IL-6 significantly dampened the expression of MMP 2, and thus matrix degradation by MMP 2 is increased in IL-6 knockout mice leading to reduced granulation (12). Both murine studies are experimental. Collagen matrices approximate in vivo conditions, however, fibroblasts cannot differentiate. Thus the findings do not apply beyond the proliferative phase of wound healing.

The application of these findings to humans is limited because IL-6 is normally low in healthy humans (13). Elevated IL-6 is pathological, but there are no studies that investigate the direct link between elevated IL-6 levels and wound healing (10). Mokart et al. (14) compared 30 patients undergoing major surgery for cancer with healthy controls and found elevated IL-6 levels were significantly associated with the development of sepsis. Mei et al. (15) studied a group of 65 patients undergoing coronary bypass grafting and found high IL-6 levels to be significantly associated with multi-organ dysfunction. It can be inferred that systemic infection and multi-organ dysfunction compromise conditions for wound healing such as a clean wound bed, adequate perfusion and oxygenation of tissues (16).

The role of IL-6 in cachexia has been explored in experimental animal models and clinically in humans. Baltgalvis et al. (17) compared cachectic mice with their wildtype counterparts and found significantly higher IL-6 levels and significantly lower physical activity in severe cachexia despite similar food intake. IL-6 was found to be significantly increased in patients with cancer cachexia for lung (18), oesophageal (19) and prostate cancer (20) compared with controls. Witte et al. (21) and Deans et al. (22) did not find an association between weight loss and IL-6 levels. Witte et al. (21) was a small study of nine cachectic individuals with chronic heart disease and Deans et al. (22) was a prospective study of over 200 patients with oesophageal cancer. One reason for the conflicting findings is the definition of cachexia. Examples of definitions were 6% weight loss in 6 months (21), 5% weight loss in 3 months (19) and 10% weight loss from the patients’ recollection of their stable weight (22). Poor quality evidence links IL-6 with wound healing outcomes in humans in the current literature. Studies on the role of IL-6 in cachexia are hampered by the lack of consensus of how cachexia is defined and measured.

The NOS pathway plays a key role in the release of nitric oxide, resulting in smooth muscle relaxation during inflammation (23,24). The presence of nitric oxide in the central nervous system reverses the orexigenic effect of hormones such as neuropeptide Y, which therefore results in anorexia seen in cachexia (23).

In vitro studies with human bronchial epithelial cells showed that nitric oxide enhanced wound closure by epithelial migration in a 24-hour period (25). However, no statistical analysis was carried out in this study; therefore, it is difficult to conclude if the improvement was significant. Mice fed a NOS inhibitor had statistically significant reduction of wound tensile strength compared with control mice when the inhibition was exaggerated (Chi-square test, \( P < 0.05 \)) (26). The other two NOS isoforms were not investigated and it is unclear if the high levels of inhibition occur physiologically. Muangman et al. (24) investigated the role of substance P, which induces the nitric oxide release via the NOS pathway, in murine wound healing. Substance P speeds healing, possibly at
the stage of wound epithelialisation (27), which is in keeping with Bove et al. (25). Muangman et al. (24) had shown that NOS knockout mice had a dampened inflammatory response and took longer to heal compared with the control group, but this was reversed with substance P. Chronic failure of epidermal keratinisation in conditions such as diabetes mellitus was reversed with the use of substance P in humans. The majority of patients healed with the treatment; however, there was no control group and the numbers treated were small (28).

The NOS pathway is also regulated by sex hormones. Oestrogen, for example, influences the rate of wound healing (29). A systematic review of megestrol acetate, a female sex hormone, in cancer cachexia demonstrated an improvement of appetite and weight gain (30). Megestrol acetate is thought to upregulate neuropeptide Y, a known orexigenic agent, via NOS pathway (23).

The role of the NOS pathway in cachexia has been investigated in animal models (31,32). Both Buck and Chojkier (31) and Wang et al. (32) had control groups and used similar NOS inhibitors. NOS inhibition prevented weight loss and maintained myosin expression in cachectic mice (31), but there was no increase of food intake by cachectic mice (32). The later experiment lasted 2 weeks, as opposed to at least 3 weeks by Buck and Chojkier (31). It is uncertain if prolonging the exposure to NOS inhibition may eventually increase food intake.

There are no studies in the current literature that explore the effect of the NOS pathway on healing and cachexia in humans. Findings from animal studies may not readily translate to humans (33). Clinically, substance P improves healing in humans and perhaps NOS inhibitors may be the next step in tackling impaired healing and reducing cachexia.

**COLLAGEN DEPOSITION**

TNFα is a proinflammatory cytokine integral to wound healing (8) that was previously known as cachectin, alluding to its role in cachexia (34). In vivo experiments on cultured human fibroblasts show that TNFα modulates collagen synthesis by inhibiting the action of TGF-β stimulating fibroblast collagen production (35) at messenger RNA (mRNA) transcriptional level (36). Both studies utilised Northern blot analysis to quantify type I collagen. Kähäri et al. (35) demonstrated a fivefold increase of collagen from the control group when TGF-β was present but not in the presence of TGF-β and TNFα. There were no statistical analyses undertaken; therefore, it cannot be said with certainty if the findings were statistically significant. Greenwel et al. (36) investigated the direct inhibitory effect of TNFα on collagen production. TNFα repressed mRNA expression. The findings were statistically significant compared with controls (Mann–Whitney U test, P < 0.05). Chojkier et al. (37,38) carried out two experiments on murine models of cachexia. By measuring radioactive labelled collagen and hydroxyproline levels as an indicator of collagen degradation, the two studies found that scurvy and acute fasting induced weight loss similar to cachexia and resulted in a decrease in collagen synthesis but no change in collagen degradation (37,38). A later controlled study by Buck et al. (39) also found a significant decrease in collagen synthesis and reduced extracellular matrix deposition, measured by direct histological observation and colorimetric assay. The mice studied by Buck et al. (39) were transfected with TNFα and had free access to food and water, whereas the mice studied by Chojkier et al. (38) were starved. Despite the different methodologies, all three studies reached a similar conclusion: collagen production is decreased in a cachetic model.

Buck et al. (39) also demonstrated two other important points. First, TNFα was responsible for changes in collagen production prior to the onset of weight loss. This finding may imply that the mediators of cachexia influences wound healing rather than undernutrition. Second, TNFα mice had unhealed wounds after 5 days, whereas control mice had healed upon visual inspection. The unhealed wounds precluded any attempt to test tensile strength. There is yet to be a study relating TNFα, collagen levels and cachexia in humans. Thus, findings from murine models described may not necessarily apply to humans. The development of chronic wounds may be modulated by TNFα levels, where significantly higher TNFα levels were found in chronic venous ulcer fluid compared with acute wound fluid (40). Cowin et al. (40) also found that etanercept, a commonly used anti-TNFα therapy, reduced the TNFα levels in chronic wound fluid in a dose-dependent
manner in vitro. It remains to be seen if topical application or systemic administration of anti-TNFα therapy in cachectic individuals with chronic wounds aids healing.

In clinical practice, anti-TNFα therapy modulates the immune system. Rheumatoid arthritis, an autoimmune disease which is associated with cachexia, responds well to anti-TNFα therapy (4). Anti-TNFα therapy is thought to adversely affect wound healing by increasing the risk of infections and delaying wound healing (41). However, because of the complexity of the wound healing process, evidence is emerging that it may be safe to continue anti-TNFα therapy in the perioperative period. Corrao et al. (42), in a case series of five patients, found no evidence of infection within a year of surgery despite continuing therapy during the operative period. The conclusion of the safety of this practice is based on evidence from a case series which is less robust than a cohort study, for example, (43). Ruysen-Witrand et al. (44) performed a retrospective study on a cohort of patients on anti-TNFα therapy. Their cohort underwent 127 surgical procedures. Discontinuing anti-TNFα therapy did not significantly reduce the risk of complications (44). The two main drawbacks of this study are that it was retrospective and it did not have a control group. Cachexia was not specifically addressed in either study. However, these two studies point to the possibility that anti-TNFα therapy may not suppress the immune system enough to increase the risk of infection and that lower TNFα levels is not detrimental to the wound healing process clinically.

A small, randomised trial consisting of 26 patients was undertaken by Marcora et al. (45). The study compared the effects of a 3-month course of anti-TNFα therapy and methotrexate on cachexia in patients with early rheumatoid arthritis. Anti-TNFα therapy was as efficacious as methotrexate in disease control and caused a significant increase in fat free mass (45). Fat free mass is an important indicator of cachexia. Cachexia is characterised by weight loss of over 10% body weight and gross muscle wasting (46). It is unknown if long term anti-TNFα therapy reduces the risk of cachexia development and how this affects wound healing. Further work is required, such as a longitudinal study of cachectic patients on anti-TNFα therapy undergoing surgery or with chronic wounds.

**REETEPITHELIALISATION**

TGF-β is a superfamily of peptides instrumental in fibroblasts differentiation, except in the lungs (47). TGF-β, therefore, encourages connective tissue matrix growth (5). TGF-β increases fibroblasts collagen production and encourages migration of epithelial cells by sustaining the expression of integrins essential for keratinocyte motility (48). Harrison et al. (49) reviewed the role of activin, a member of the TGF-β superfamily, and postulated that activin levels were associated with cancer development, cachexia and altered epithelialisation.

A series of murine experiments by (50–52) demonstrated the role of activin during reepithelialisation. Polymerase chain reaction determined that dermal activin mRNA levels were significantly higher during wound healing compared with unwounded mice, but activin receptors were not upregulated (50). Mice that overexpressed human activin in the epidermis had thicker epidermal and dermal layers compared with controls and their injured skin had significantly more epitelial proliferation and granulation tissue than controls (51) but the strength of repair was unknown. Overexpression of follistatin, an activin antagonist, in mice reduced granulation tissue and delayed wound healing compared with controls (52). Fumagalli et al. (53) found that there was a significant increase in activin expression in the dermis of hypertrophic scars of post-burn patients without receptor upregulation, reflecting the findings in murine models described. Fumagalli et al. (53) carried out a controlled study, but the sample size was small (n = 19 study, eight controls) so statistical significance should be interpreted with caution. TGF-β is ubiquitous in tissues but its roles vary between tissue types and between species (54); therefore, findings in these experiments may not apply to all tissue types and species.

The role of TGF-β peptides in cachexia was examined by Li et al. (54) using inhibin deficient mice. These mice had higher activin levels and cachexia. When treated with an activin antagonist, the mice maintained their weight and had better survival rates than controls. However, the survival rates may be influenced by the fact that animals with signs of wasting were sacrificed for other analyses, thus leading to bias. Costelli et al. (55) studied the effect of myostatin, another TGF-β peptide. The rats
were inoculated with tumour cells and sacrificed within a week. Tumours significantly increased the levels of myostatin and reduced body and muscle weight compared with controls. The sample size of six and short duration of this study calls into question the reliability of the statistical analysis.

Banno et al. (41) performed a microarray study on TNFα in human keratinocytes. The study revealed that TNFα upregulated genes specific to keratinocyte migration. This was confirmed by time course analysis, where keratinocyte with added TNFα had markedly increased movement compared with controls (41). There are no more recent studies that specifically address the direct role of TNFα in keratinocyte migration. The role of TNFα in cachexia has been discussed earlier in this essay.

CONCLUSION

It would seem that high levels of circulating cytokines, such as IL-6 and TNFα, and activation of the NOS pathway cause impaired wound healing and cachexia. Experimental evidence suggests TNFα suppresses collagen production and keratinocyte mobility. The effect on wound healing and cachexia in humans receiving anti-TNFα therapy is subject to debate because of the relationship between inflammation, infection and TNFα levels. In rodents, and perhaps in humans, activin and myostatin contribute to weight loss and muscle wasting in cachexia. Activin, in particular, may cause overgranulation and altered epithelial migration. A consensus of the definition of cachexia should be sought.

There is currently no successful method of countering cachexia and its effects on wound healing because of the complexity of the process and lack of research evidence. By understanding how cachexia affects wound healing in humans, it may lead to effective therapy that will improve the quality of life of those affected by cachexia.

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REFERENCES


Key Points

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- Experimental evidence suggests TNFα suppresses collagen production and keratinocyte mobility.
- The effect on wound healing and cachexia in humans receiving anti-TNFα therapy is subject to debate because of the relationship between inflammation, infection, and TNFα levels.
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- A consensus of the definition of cachexia should be sought.
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