Review Article

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A Review of the Prevention and Treatment of Hypertrophic Scars:Part II. Experimental Studies

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Abstract

The understanding of the pathophysiological events of wound healing is very important to carry out the prevention or treatment of hypertrophic scars. Wound healing is a complex process consisting of the overlapping events, and the researchers have focused on the pathophysiology of the scar formation. The purpose of this article is to review the recent experimental studies about hypertrophic scars, and to analyze the substances focused on by recent experimental studies.

We analyzed the researches for new hopeful treatment modalities as well as the substances that are important to wound healing in the second part of this extensive review. The researchers have tried to find a way to scarless wound healing, and it seems likely that new therapies will be available within the next few years.

Key words: *Experimental studies, hypertrophic scar, prevention, treatment*

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Introduction

Wound healing is a complex process involving inflammation, cell proliferation and tissue remodeling, and normally ends in a mature scar. Impairment of the early steps of wound healing can result in pathological responses, such as hypertrophic scarring [1]. Hypertrophic scars are characterized by proliferation of dermal tissue with excessive deposition of fibroblast-derived extracellular matrix protein, especially collagen, over long periods, and by persistent inflammation and fibrosis [2]. Excessive collagen deposition occurs relative to normal wounds. Type I collagen is the most abundant type of collagen in normal dermis (approximately 80% to 90%). Normal skin contains type I and type III collagen in a 4:1 ratio. In hypertrophic and immature scars, the percentage of type III collagen may be as high as 33% [3,4,5]. In other words, the scars remain immature with an abnormally high content of type III collagen [3,4]. Continuous deposition and/or abnormal turnover of collagen or the ratio of collagen type I/type III results

in hypertrophic scar formation [6].

Growth factors and cytokines have also been involved in scar formation, and these factors are targeted for potential therapeutic use in scar management [2]. Transforming growth factor-beta (TGF-β) has been linked clinically and experimentally to dermal proliferative disorders. Polo and colleagues found an abnormal dose response by fibroblasts of proliferative scars to TGF-β2 stimulation. Anti-transforming growth factor-beta (anti-TGF- β) was used to decrease scarring in experimental animals. Tregret describes antagonizing the proliferative effects of TGF-β2 and histamine with interferon-α2b [4]. Neutralization of TGF-β1 and β2 reduced scarring from experimental wounds in a rat model and the aim of many experimental anti-fibrotic therapies was to reduce TGF-β1 production [1, 7].

The pathophysiology of wound healing is strictly related to the regulation of inflammation that relies on a number of cell types secreting growth factors, cytokines and chemokines [1]. Recent experimental studies have focused on the pathophysiology of the scar formation, and researchers are trying to modify collagen deposition. The surgical and non-surgical therapeutic modalities were discussed in the first part of this extensive review [8], and the purpose of this current article is to review the recent experimental studies about hypertrophic scarring, and to analyze the substances focused on by these studies.

Activin A and Follistatin: Activin A is a member of the TGF- β family. It is strongly induced after tissue and organ injuries, probably by serum growth factors released upon hemorrhage and by macrophage-derived cytokines, and it plays a role on growth and differentiation of various cell types during organogenesis as well as on the repair process [1,9-13]. It has also been implicated in the pathogenesis of liver, pancreas, lung, cornea and kidney fibrosis, in Crohn's disease and in rheumatoid arthritis [1,14-20]. It shares the same intracellular Smad signaling pathway with TGF-β, but binds to its own specific transmembrane serine/threonine kinase receptors and to follistatin, a secreted protein that inhibits activin by sequestration [1,21-23]. Transgenic mice overexpressing activin A show strongly hyperthickened epidermis, accelerated wound healing and enhanced scarring [1,24]. Conversely, in follistatin-transgenic mice, wound closure is delayed and scar formation is reduced [1,25]. These findings suggest that activin A and follistatin influence scar formation and wound healing quality. Recently, a role of activin A in keloid pathogenesis has been reported [1,26]. In their more recent study, Fumagalli et al. investigated the role of activin/follistatin balance in HS genesis [1]. Multiple findings obtained in their study provided evidence for a role of activin A in pathologic scar formation and evolution. Numerous activin+ cells were consistently observed in dermis from AHS. These data were substantiated by the constitutive release by AHS fibroblasts of higher levels of activin A and lower levels of follistatin, compared to RHS and NS fibroblasts [1]. TGF- β1 is considered the most important regulator of myofibroblast differentiation in the context of dermal wound healing and is the most abundant isoform in keloids and HS [1,27]. They presented evidence that activin has TGF-β1-like functions on AHS fibroblast activation [1,28]. Furthermore, their study revealed that TGF-β3 inhibits the scarring response [1,7].

Follistatin, by inhibiting activin-induced fibroblast proliferation and type I collagen expression, could act as a 'brake' on AHS formation, but it is underexpressed in AHS. This view is strengthened by the finding that transgenic mice overexpressing activin A showed dermal fibrosis, whereas scar formation was decreased in transgenic mice overexpressing follistatin [1,24,25]. On this basis, it can be assumed that activin antagonists should be effective in the treatment of AHS. Recent findings support follistatin potential as an activin-inhibiting therapeutic tool for diseases characterized by an interconnection of immunologic and fibrotic disorders [1,29]. Follistatin has been found effective in treating acute lung injury and bleomycin-induced fibrosis, as well as attenuated experimental colitis in mice [1,30,31]. Moreover, exogenous follistatin administration significantly improved liver fibrosis. Based on these reports and on the recent study of Fumagalli et al., activin A could be considered a promising new target for AHS treatment strategies [1].

As mentioned before, transgenic mice overexpressing activin in the skin were characterized by strongly enhanced wound healing, but also by excessive scarring. These negative consequences of activin overex-

pression were not observed in the follistatin (Fst) mutant mice [32]. In their most recent study, Antsiferova et al. explored the consequences of targeted activation of activin in the epidermis and hair follicles by generation of mice lacking the activin antagonist follistatin in keratinocytes. They observed enhanced enlarged hyperproliferative epithelium in the tail epidermis of these animals. However, granulation tissue formation and scarring were not affected. Their results demonstrated that limited activation of activin in the epidermis enhances reepithelialization without inducing excessive scarring. In other words, the free activin that is generated in the absence of keratinocyte-derived follistatin is obviously insufficient to enhance granulation tissue formation and subsequent scarring. This may be because of the lower concentration of free activin, compared to the activin-transgenic mice [32].

Recombinant human TGF-β3 (avotermin): Transforming growth factor-β (TGF-β) has three isoforms and its ratio is important for optimizing a scar outcome. TGF-β1, TGF-β2, and TGF-β3 have differential temporal effects during the wound healing process [2]. The balance between TGF- β1 and TGF-β3 may be an important regulator of scar formation. The anti-TGF-β approach for scar therapy might be the most important discovery in the area of scar prevention and treatment, which has already been translated from scientific discovery derived from an animal study [33,34] into clinical trials [33,35]. The addition of exogenous TGF-β1 in fetal wounds could convert the scarless healing into a scar-forming healing pattern [33,36,37]. An increased production and release of TGF- β1 during wound healing may lead to the formation of scars [38]. To translate this discovery into applied research, Shah et al. [33,39] later developed a scar reduction approach using TGF-β1 and β2 antibodies to neutralize TGF-β1 and β2 in rat incision wounds, and they successfully reduced wound scarring [38,40-42].

Human recombinant transforming growth factor-beta 3 (TGF- β3) (avotermin) is a new class of prophylactic therapy for the improvement of scarring that downregulates TGF- β1 expression [38,40-42]. The role of the TGF-β family in scar-free and scar-forming healing is revealed in the current literature. Fibroblasts from fetal tissue that heal without scarring also exhibited an increased expression of TGF-β3 [38,43,44]. Briefly, in fetuses that heal without a scar, the ratio of TGF-β3 to the TGF-β1 and β2 isoforms is high, while in scar-forming healing the ratio of TGF-β3 to TGF-β1/ β2 is low [45,46]. Also, the addition of exogenous TGF-β1 to a scar-free healing model of fetal repair results in scar formation [45,47], while wounds made on early mouse fetuses genetically nulled for TGF-β3 heal with a scar [45,48]. Further insights have come from studies of the oral mucosa, a tissue characterized by rapid healing and a lack of scar formation [45,49], showing that a significantly higher ratio of TGF-β3 to TGF-β1 is present in wounds of the oral mucosa, compared to dermal wounds elsewhere in the same adult [45,50].

Exogenous addition of TGF-β3 or inhibitors of TGF-β1 and TGF-β2 to adult wounds reduces subsequent scarring [7,34,39,45]. Occleston et al. identified TGF-β3 as an important cytokine in normal skin morphogenesis and in the scar-free embryonic healing response. They then demonstrated that exogenous administration of human recombinant TGF-β3 by intradermal injection resulted in wounds that healed with an improved appearance and histological architecture in preclinical studies in animals. They then translated these findings into man, executing a series of phase I/II clinical trials. These trials demonstrated the beneficial effects of avotermin in prophylactically improving subsequent scar appearance. Subsequently, appropriately designed patient-based clinical trials demonstrated that avotermin improved the appearance of scars following scar revision surgery. To date, eight double-blind, placebo-controlled, prospective phase II clinical trials have met their primary endpoints and have demonstrated a statistically significant improvement in scar appearance with avotermin treatment [45]. Shah et al. also discovered that adding exogenous TGF- β3 could also significantly reduce wound scarring in a rat model [33,51]. In their most recent study, Honardoust et al. stated that reduced expression of TGF-β3, decorin and fibromodulin, in contrast to TGF-β1, in deep dermis may potentially influence the outcome of wound healing as well as aggravate HTS formation after injuries that involve deeper layers of skin [38]. Interestingly, Honardoust et al. showed for the first time that deep

dermal or reticular fibroblasts express significantly more TGF-β receptor type II compared to superficial or papillary fibroblasts. These data suggest that injuries to the deep dermis, which contains more TGF-β receptor type II expressing fibroblasts, are more prone to TGF- β1 fibrogenic activity and can be accounted, in part, for the development of HTS formation after wounding [38].

Role of epidermis in scar development: Multiple lines of evidence from the clinic, in-vitro experiments, and in-vivo animal and human studies, however, increasingly suggest that the epidermis plays a major role in the control of underlying dermal scarring. In the spectrum of factors contributing to dermal scarring, the epidermis and its downstream effectors offer promising new targets for the development of anti-scar therapies [51]. There is a growing body of evidence showing that the epidermis plays an important role in initiating inflammation in response to injury and continuing to mediate inflammation long after reepithelialization has occurred, until full competence of the stratum corneum as a water barrier is achieved. The outer layer of the epidermis, the stratum corneum, functions as a water barrier, and until that water barrier becomes fully competent, there is a driving proliferative signal to restore homeostasis, and those stimulatory signals have secondary effects on the dermis with a net increase in scarring [51]. Therapeutic maneuvers that mimic a competent stratum corneum (occlusive coverings) should decrease scarring by early restoration of homeostasis, as well as a reduction in proliferative or inflammatory signals. Modulation of the inflammatory state of the epidermis, especially through restoration of barrier function, is therefore a key target in the control of dermal scar formation.

Persistent epidermal activation by IL-1 may exert effects on the underlying dermis through activation of well-known downstream effectors of scars, such as TGFβ and the connective tissue growth factor (CTGF) [52]. Occlusive dressings, such as silicone gel in its various forms, or other alternatives, reduce reactive epidermal hyperplasia, and IL-1 signaling, presumably due to their ability to restore barrier function, whereby reducing transepidermal water loss (TEWL) and therefore increasing skin hydration [51]. Mechanisms for transduction of the decreased hydration state to the epidermal inflammatory cascade need to be elucidated. It is reasonable to hypothesize that changes in osmolarity play an important role in this signal transduction [51,53,54]. Delineation of the exact epidermal to dermal communication pathways and their convergence on effectors of scar formation will also be essential. Although these mechanisms have yet to be elucidated, Mustoe and Gurjala suggested that epidermal regulation of dermal scarring is a promising new target for continued therapeutic efforts at scar reduction [51].

Researches for New Hopeful Treatment Modalities

Gene Therapy: Gene therapy might also serve as a potential important anti-scarring therapy approach [33,55,56]. As examples, the adenovirus- [33,56] or retrovirus- [33,57] mediated overexpression of truncated TGF-β receptor II was found to be able to inhibit wound scarring in animal models. Because fibromodulin is a modulator of TGF-β and can inhibit TGF-β activation, adenovirus-mediated overexpression of fibromodulin was also used as a strategy for inhibiting scar formation [33,58]. In addition, TGF-β antisense oligodeoxynucleotides were also used for scar inhibition [33,59]. Gordon et al. [33,60] and Peranteau et al. [33,61] demonstrated that adenovirus-mediated or lentivirus-mediated overexpression of IL-10 in wounds could reduce scarring in healed postnatal or adult wounds. Akasaka et al. [33,62] applied the exogenous basic fibroblast growth factor (bFGF) to a rat incision wound and demonstrated that it could promote wound cell apoptosis as well as suppress granulation tissue formation. Based on this study, Ono et al. [33,63] translated the experimental discovery into clinical trials and demonstrated that topical application of bFGF could help to reduce wound scarring in human acute incision wounds. In another animal study, Ono et al. demonstrated that wound gene therapy with HGF could significantly reduce scar formation and enhance skin regeneration [33,64].

Multifactor-based anti-scarring approach: A multifactor-based anti-scarring approach has been proposed in order to achieve a combinational effect on multiple targets of wound healing and scar formation process. Theoretically, this multifactor-based approach

might be carried out with a combined application of two or more factors together in wounds or by applying different factors at different wound-healing stages to act on their own specific targets. For example, the combined use of recombinant TGF- β3 and recombinant IL-10 to both inhibit the inflammatory reaction and antagonize the scar-causing effects of TGF- β1 and β2, and this combined effect would be reasonably more effective than the effect of using either TGF- β3 or IL-10 alone [33].

Tissue-engineered wound repair: It has been known that delayed wound healing can lead to severe scarring [65]. The prolonged wound-healing process results in a series of inflammatory reactions with enhanced production of scar-causing growth factors and cytokines [33,66,67]. Hence, promoting wound closure by enhanced wound epithelialization would be one of the options to prevent HS formation. For scar reduction at the donor site of a skin graft, a chitosan–gelatin membrane was developed for use as a carrier for transferring a cultured epithelial graft to treat the wound [33,68]. The selection of the chitosan membrane was based on the fact that it is mechanically feasible for keratinocyte sheet transfer, has good compatibility with keratinocytes, and can promote wound healing [33,69,70]. After proof of efficacy in an animal experimental study, a keratinocyte-seeded chitosan–gelatin membrane was used as a tissue-engineered epidermal membrane for a clinical trial. At the donor sites of a split skin graft, half of the wound was treated with the epidermal membrane, whereas the other half was treated with routinely used Vaseline gauze as a control. Interestingly, the clinical results showed that the healing time was significantly shortened for the epidermal membrane-treated wound than for the gauze-treated wound. More importantly, the rapidly epithelialized wound eventually led to much reduced scarring than the control wound, which was also verified by the significant difference in the Vancouver scar scale analysis between the two groups [69]. The results of this study indicate that tissue-engineered wound repair is particularly important in scar reduction and prevention in large wounds, where endogenous regeneration ability may not be sufficient to repair and regenerate the epidermis in a timely manner to prevent the consequential scar-causing events.

For the prevention of extensive wound scarring, the application of tissue-engineered skin should also serve as a carrier for providing regenerative materials and signals to the wound in order to fully regenerate the skin structure, including skin appendages. Thus, Liu et al. proposed the following suggestions to be integrated into a regenerative bioengineered skin, including: (a) anti-TGF-b reagents [56], such as TGF- β3, or TGF-β antagonists, like decorin and soluble TGF-β receptors, or a TGF-β activation inhibitor or modulator, such as mannose-6-phosphate or fibromodulin; (b) other growth factors that promote regeneration and that inhibit matrix production, for example, HGF; (c) anti-inflammatory cytokines, like IL-10 or other molecules that are able to inhibit inflammation; (d) fetal ECMs that may favor skin regeneration, like hyaluronic acid; (e) regenerative signals, for example, Wnt ligands [71] or Epimorphin; [72] and (f) regenerative stem cells that have the potential to differentiate and develop skin-related structures.

Antiangiogenesis approach: Angiogenesis might be a target for preventing hypertrophic scarring. If uncontrolled growth of hypertrophic scarring can be considered as a type of neoplastic tissue, the antiangiogenesis approach would then be relevant because it has already been proven to be an efficient way to control tumor growth [73] and has been applied in clinical cancer therapy [74]. The clinical phenomenon of enhanced angiogenesis (erythema) always precedes HS formation, and also provides a rationale for using antiangiogenesis as a potential anti-scarring approach.

Based on the observation of microvascular abnormality in pathological scarring [75-77], the use of an antiangiogenesis approach has been proposed to inhibit HS formation [78]. Song et al. [78] developed a gene therapy approach for scar reduction, using adenovirus-mediated overexpression of METH1 (metalloprotease and thrombospondin 1), and the result showed significantly reduced microvessel density and microcirculatory perfusion along with reduced scarring in a rabbit ear model.

It has been reported that an important part of the therapeutic mechanism of the laser is angiogenesis inhibition [79-80]. Liu et al. proposed the use of PDT to

treat post-burn healed wounds in order to better control the angiogenic reaction secondary to wound injury, and hope to find a way to prevent HS formation [33]. Interestingly, Cai et al. have reported the PDT treatment of a hyperplastic scar in a rabbit ear model, and demonstrated that it could reduce scar formation, decrease microvessel density, and prevent excess collagen deposition at the wound site [81].

Inhibition of Collagen synthesis: Nonspecific inhibitors of collagen synthesis (such as penicillamine) are not used anymore because of its unacceptable toxicity. Instead of them, specific nontoxic inhibitors of collagen synthesis that could be applied locally have been tested in recent years [82,83]. Bilidase (a hyaluronidase preparation), pentoxifylline (the antifibrinolytic agent), and fibrostat (putrescine in a eutectic vehicle) have also been studied for the treatment of hypertrophic scars, and promising signs have been observed [2,84-86].

Conclusion

It is much more efficient to prevent hypertrophic scars than to treat them. Prevention implies using a therapy with the aim of reducing the risk of a problematic scar evolving, so treatment and prevention regimens can be similar. The other noteworthy fact, aside from prevention, is that the most successful treatment is achieved when the scar is immature, but the overlying epithelium is intact, although this is not as yet confirmed in current literature [82].

Most of the recent experimental studies have interested in the molecular events in wound healing due to the difficulty of hypertrophic scar treatment. The researchers are trying to find a way to scarless wound healing as well as fetal wound healing. It seems likely that new therapies will be available within the next few years.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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