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EGF and TGF-α in Wound Healing and Repair

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Abstract Wound healing is a localized process which involves inflammation, wound cell migration and mitosis, neovascularization, and regeneration of the extracellular matrix. Recent data suggest the actions of wound cells may be regulated by local production of peptide growth factors which influence wound cells through autocrine and paracrine mechanisms. Two peptide growth factors which may play important roles in normal wound healing in tissues such as skin, cornea, and gastrointestinal tract are the structurally related peptides epidermal growth factor (EGF) and transforming growth factor alpha (TGF-α). EGF/TGF-α receptors are expressed by many types of cells including skin keratinocytes, fibroblasts, vascular endothelial cells, and epithelial cells of the GI tract. In addition, EGF or TGF-α are synthesized by several cells involved in wound healing including platelets, keratinocytes, and activated macrophages. Healing of a variety of wounds in animals and patients was enhanced by treatment with EGF or TGF-α. Epidermal regeneration of partial thickness burns on pigs or dermatome wounds on patients was accelerated with topical application of EGF or TGF-α, and EGF treatment accelerated healing of gastroduodenal ulcers. EGF also increased tensile strength of skin incisions in rats and corneal incisions in rabbits, cats, and primates. Additional research is needed to better define the roles of EGF, TGF-α, and their receptor in normal wound healing, to determine if alterations have occurred in the EGF/TGF-α system in chronic wounds, and to optimize vehicles for effective delivery of peptide growth factors to wounds.

Key words: growth factors, wound healing, receptors

Wound healing has been recognized for many years to occur primarily as a localized biological event which progresses through three general phases of inflammation, wound cell migration and mitosis, and extracellular matrix production and remodeling. Understanding of these biological phases of wound healing remained at this generalized level until recently when a molecular theory of wound healing emerged that is based upon the synthesis and release of several specific peptide growth factors at the site of injury which then act through autocrine and paracrine pathways to regulate healing. Evidence for this theory comes from a variety of experiments, and data is accumulating to indicate that two structurally related peptides, epidermal growth factor (EGF) and transforming growth factor alpha (TGF-α), play important roles in the natural mechanism of wound healing. A corollary to this molecular theory of wound healing is that insufficient levels of peptide growth factors and their receptors in a wound could cause retarded healing which could be reversed by treatment with exogenous growth factors. Although many details of this molecular theory of wound healing remain controversial, peptide growth factors may eventually be an additional adjuvant in wound management.

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BIOCHEMICAL PROPERTIES OF EGF, TGF-α, AND THEIR RECEPTOR

TGF-α and EGF are members of a family of peptide growth factors that also includes the proteins vaccinia growth factor and amphiregulin [1,2,3]. All the members of this gene family share substantial amino acid sequence homology including the conserved placement of three intrachain disulfide bonds (Figure 1). In addition, all the factors of this family bind to a 170,000 mw transmembrane glycoprotein receptor and activate the tyrosine kinase activity in the receptor's cytoplasmic domain [4]. Although the complete postreceptor messenger system for the EGF receptor is not known, the receptor kinase activity is essential for inducing the bio-

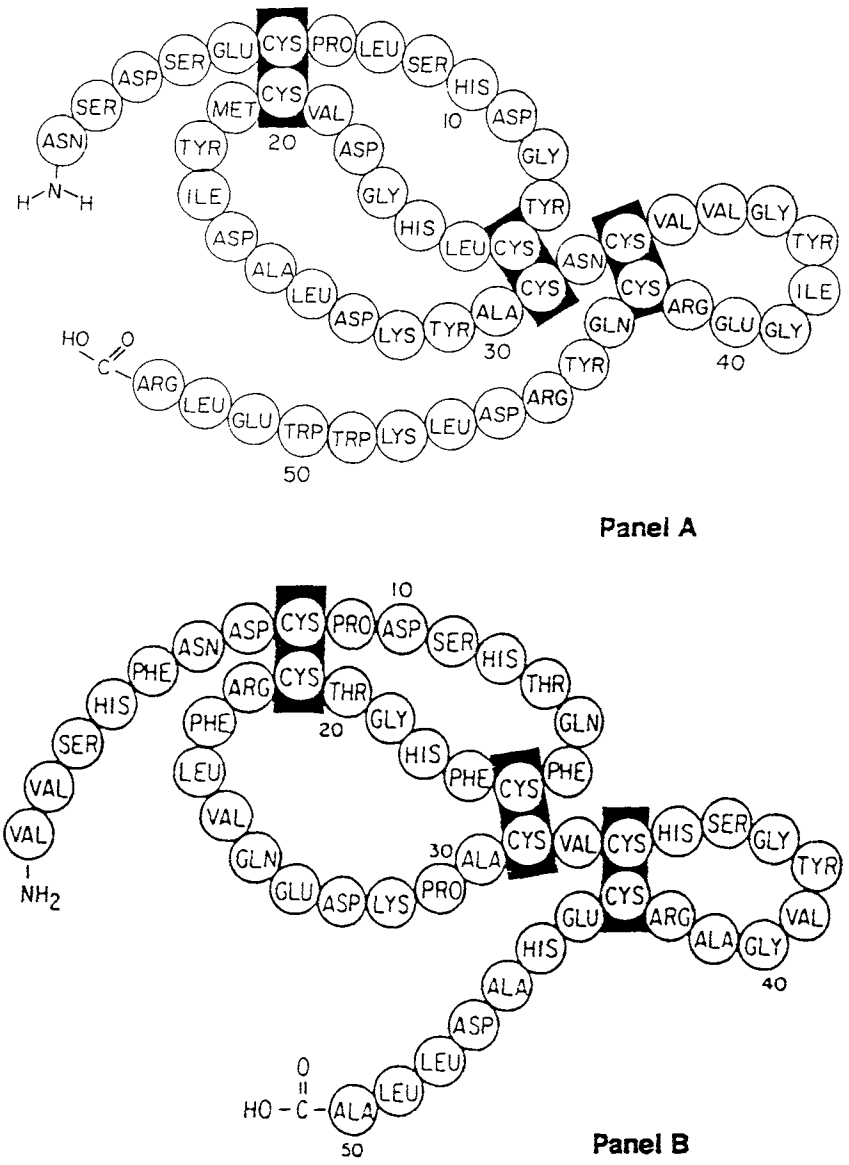

Fig. 1. Primary amino acid sequences of human epidermal growth factor (**Panel A**) and human transforming growth factor alpha (**Panel B**).

logical activities of EGF [5]. Recent data indicate that the EGF receptor system can influence several second messenger systems including the protein kinase C system and the arachidonic acid pathways by phosphorylating lipocortin I and phospholipase C [6,7].

TARGET TISSUES FOR TGF-α AND EGF

Conventional logic holds that tissues which express EGF/TGF-α receptors are potential targets for EGF or TGF-α action, and many of the cells involved in wound healing express EGF receptors. One tissue which expresses high levels of TGF-α and EGF/TGF-α receptor is skin. TGF-α mRNA and protein [8] as well as the receptor for EGF/TGF-α [9] are found primarily in the basal cell layers of normal epidermis, and TGF-α or EGF are required for growth of keratinocytes in vitro [10]. Psoriatic skin, which is characterized histologically by hyperproliferation of the epidermis, contained elevated levels of TGF-α protein [11]. Although EGF does not appear to be synthesized by normal keratinocytes, EGF was detected in secretory cells of eccrine sweat glands [12]. Thus, EGF, TGF-α, and their receptor are thought to be the major

regulators of normal keratinocyte growth and differentiation.

Other tissues which express EGF/TGF-α receptors are cornea and gastrointestinal tract. All three major cell types of the cornea, epithelial cells, stromal keratinocytes, and endothelial cells, express EGF/TGF-α receptors, and EGF stimulates proliferation of all three types of corneal cells in vitro [13]. EGF/TGF-α receptors have been detected in mucosa cells of the stomach and epithelial cells of the small intestine. Several actions of EGF/TGF-α have been demonstrated in the GI tract including inhibition of gastric acid secretion and stimulation of cell proliferation [14].

Blood cells which directly participate in wound healing also provide EGF and TGF-α to a wound site. Wound macrophages isolated from subcutaneous wound chambers implanted in mice contained TGF-α mRNA and protein [15]. Platelets contain EGF-like peptides which are released when platelets degranulate [16]. Thus, EGF-like peptides are released in wounds as soon as blood clotting occurs, and then during the inflammatory phase, TGF-α is released in the wound area by macrophages.

TGF-α and EGF also are angiogenic factors and chemotactic factors. Microvascular endothelial cells in culture expressed EGF receptors, and both EGF and TGF-α stimulated DNA synthesis of the endothelial cells in vitro. Both EGF and TGF-α stimulated new blood vessel formation in the hamster cheek pouch assay angiogenesis assay [17], and EGF increased capillary growth of embryonic chick vasculature [18]. EGF also stimulated both migration and proliferation of vascular endothelial cells in vitro [19], and stimulated migration and mitosis of wound fibroblasts in vitro [20]. Thus, TGF-α and EGF can stimulate two key processes of wound healing: neovascularization and chemotaxis of wound cells. Further understanding of the action of these factors in wound healing has been provided by treatment of experimental wounds with these factors.

TREATMENT OF WOUNDS WITH EGF AND TGF-α

Corneal Wounds

Early experiments focused on the effects of EGF on regeneration of corneal epithelial cells and demonstrated that eye drops containing EGF accelerated epithelial healing in primates [21] and patients [22]. Other experiments with corneal cells demonstrated that corneal fibroblasts expressed EGF receptors and that EGF stimulated DNA synthesis of corneal fibroblasts in culture [23]. Application of EGF in eye drops increased tensile strength of full thickness incisions in primates [21] and rabbits [23,24] even in the presence of anti-inflammatory steroids which retard wound healing [21,23].

Corneal endothelial cells perform the essential function in vivo of maintaining corneal clarity by vectorially pumping ions and water from the cornea stroma. The potential to regenerate the endothelial cell layer following trauma or disease varies in different species with human corneal endothelial cells having an extremely low capacity to spontaneously divide in vivo. However, EGF and TGF-α stimulated extensive levels of mitosis of human corneal endothelial cells in vitro, suggesting that intraocular treatment with EGF or TGF-α might stimulate human corneal endothelial cell mitosis in vivo [25].

Gastric Wounds

Conventional approaches to gastric wound healing have focused on decreasing acid production and neutralizing secreted gastric acid while regeneration of stomach cells was allowed to proceed at normal rates. EGF and TGF-α both inhibit secretion of gastric acid and stimulate proliferation of gastrointestinal cells [14,26]. Repeated administration of EGF either orally or subcutaneously accelerated healing of chronic gastroduodenal ulcers in rats with intact salivary glands and completely reversed the delay in ulcer healing in sialodectomized rats [27].

Skin Wounds

One of the first biological effects of EGF noted by Cohen was the hypertrophic development of the epidermis of skin which occurred with injections of EGF. EGF treatment of newborn mice caused early eyelid opening and incisor eruption which was characterized histologically by an increase in both the number of epidermal cell layers and the depth of keratinized layers of the skin [28,29]. Based on these observations and the demonstration that EGF stimulated growth of cultures of keratinocytes [10] and fibroblasts [30] in vitro, several attempts were made to demonstrate an effect of EGF on healing of partial-thickness skin wounds in vivo. Topical application of EGF to second degree scald burns

in rats [31,32] or to suction bullae of patients [33] failed to stimulate healing. However, in these early studies, EGF was applied to the wounds in liquid vehicles for brief periods of time, for 5 minutes in one case [33]. Since EGF was reported to require continuous exposure to fibroblasts in culture for approximately 6 to 12 hours before DNA synthesis was significantly stimulated [34], it is probable that the dosing regimens in these experiments were inadequate to significantly stimulate wound cells.

The concept that EGF required extended exposure to wound cells was further demonstrated by Buckley and colleagues [35] who reported that sustained release of EGF from pellets implanted in subcutaneous sponges in rats increased formation of granulation tissue. In contrast, EGF injected daily into the sponges was not retained and did not increase formation of granulation tissue. In a similar way, EGF placed in surgical incisions in a saline solution was rapidly lost from the incision and did not increase tensile strength. However, EGF formulated in multilamellar liposomes was retained for prolonged periods in the rat incisions and significantly increased their tensile strength [36].

Formulating EGF or TGF-α in cream vehicles also provided a method to release the growth factors to partial thickness wounds for prolonged periods. When EGF was formulated in lanolin or Silvadene and applied to partial thickness burns or dermatome injuries on pigs, it significantly accelerated the rate of epidermal regeneration [37,38,39]. These results in animal models of partial thickness wound healing led to a similar experiment in patients. In the first prospective, randomized, double-blind clinical evaluation of a biosynthetic peptide growth factor in wound healing, EGF in Silvadene vehicle, or Silvadene vehicle alone were applied topically to paired, partial-thickness dermatome injuries in patients. EGF treatment significantly accelerated healing compared to vehicle treated injuries [40] (Figure 2). Thus, these series of studies utilizing partial thickness injuries in animals and humans demonstrated that the spontaneous rate of healing in noncompromised skin wounds was not the maximum rate at which these wounds could heal since addition of exogenous growth factors accelerated healing. More importantly, however, was the possibility that peptide growth factors might be useful in the treatment of chronic wounds.

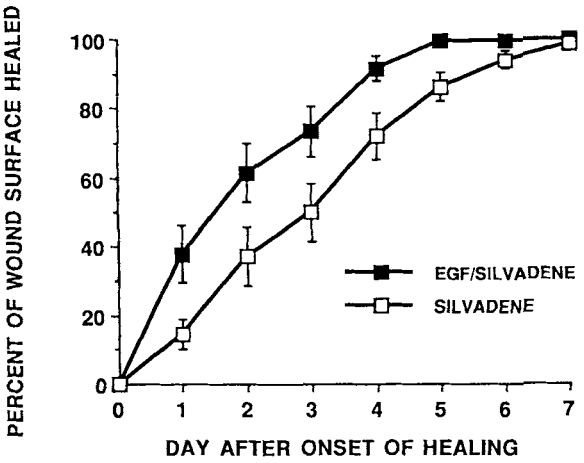

Fig. 2. Epidermal regeneration of paired, partial thickness wounds created with a Padget dermatome and treated with 10 μg EGF per gm Silvadene or treated with Silvadene alone. Significant differences ($P < 0.05$) in areas of epidermal regeneration were found at days 1 through 5 after initiation of healing.

PEPTIDE GROWTH FACTORS AND CHRONIC WOUND HEALING

The term *chronic wound* is used clinically to describe a very broad range of conditions which have a variety of contributing causes including ischemia and diabetes. Just as cancer is a heterogenous group of diseases characterized by uncontrolled cell growth, chronic wounds share a common denominator of insufficient growth of wound cells. It is reasonable to theorize that insufficient levels of peptide growth factors in chronic wounds may contribute to the inability of these wounds to heal spontaneously. If this concept is correct, treatment of chronic wounds with large doses of peptide growth factors might promote healing of chronic wounds. At present, no results have been reported from a prospective, randomized, double-blind study using biosynthetic peptide growth factors for treatment of chronic wounds. However, experience has been gained from an open label, crossover study of chronic wounds treated with EGF in Silvadene vehicle [41]. In this preliminary study, nine patients with persistent wounds which were refractive to conventual therapies were treated with Silvadene for three weeks to six months with no clinical evidence of initiation of healing. The patients were then crossed over to twice daily treatment with 10 μg of EGF per gram of Silvadene vehicle. Treatment with EGF/Silvadene induced complete healing in 8 of 9 patients with persistent wounds arising from a variety of

conditions including diabetes, rheumatoid arthritis, and old burn scars. The one patient which did not heal completely did decrease the initial wound size by approximately 80% but did not completely heal. The wounds of the eight patients that healed with EGF/Silvadene treatment did not break down for at least one year after discontinuing EGF/Silvedene treatment.

Using another approach, Knighton and colleagues [42] treated 49 patients with 80 chronic cutaneous ulcers with an extract of autologous platelets. The mean duration of conventional therapy was 4 years with a range of 8 weeks to 35 years with a wide range of etiologies including diabetes, venous stasis, pressure sores, and rheumatoid arthritis. The platelet extract was prepared from washed platelets by stimulating discharge of the platelet granules with thrombin. The platelet extract was then combined with microcrystalline collagen to produce a topical salve which was applied for 12 hours, then washed off with tap water, and Silvadene was applied for 12 hours. Successful re-epithelialization was obtained in 97% of the wounds with an average time to re-epithelialization of 8 weeks with a range of 1 to 22 weeks.

FUTURE DIRECTIONS OF RESEARCH ON EGF AND TGF-α IN WOUND HEALING

These clinical results imply that one aspect which contributes to the nonhealing condition of chronic wounds is the lack of adequate stimulation by peptide growth factors. However, there is essentially no basic information on the levels or activity of growth factors in spontaneously healing wounds and in chronic wounds which support this assumption. Thus, there is a clear need for more information regarding growth factors in the environment of healing and nonhealing wounds. In a preliminary evaluation of fluids collected from healing wounds and chronic wounds, we found that fluids collected from chest drains of healing mastectomy wounds stimulated exceptionally high levels of DNA synthesis by cultures of normal human foreskin fibroblasts. The mastectomy fluids also contained high levels of several peptide growth factors including TGF-α, IGF-I, and TGF-β. Thus, the environment of healing wounds is rich in peptide growth factors which can stimulate wound cell migration, proliferation, and regeneration of extracellular matrix.

In contrast, we have found that fluids collected from chronic wounds had low levels of peptide growth factors and failed to stimulate DNA synthesis by fibroblast cultures. Furthermore, fluids collected from chronic wounds reversibly inhibited the mitogenic activity present in fluids from healing mastectomy wounds when the two wound fluids were mixed together. Clearly, additional research is needed before general conclusions can be made concerning the status of peptide growth factors and their receptors in chronic wounds. However, if it can be established that deficiencies of peptide growth factors play a key role in preventing healing of chronic wounds, then new strategies for treatment of chronic wounds may be developed which are based on treatment with biosynthetic peptide growth factors alone or in combination with other agents such as protease inhibitors and extracellular matrix components.

Further development is also needed in designing effective vehicles for peptide growth factors. This is particularly important for EGF and TGF-α since in vitro and in vivo studies indicate that EGF requires continuous exposure to cells for prolonged periods to commit cells to divide or to stimulate healing of wounds [34]. Open skin wounds such as burns or chronic ulcers may have the simplest requirements for vehicles. Creams such as Silvadene, which slowly release EGF for at least 12 hours when applied to partial thickness keratome injuries on pigs, were effective in accelerating epidermal regeneration of patients. Other vehicles such as pluronics which are liquid at room temperature but form jells at body temperature need to be evaluated as vehicles.

Different kinds of wounds such as corneal epithelial defects or surgical incisions will require different vehicles. An interesting new potential vehicle for ocular surface delivery of peptide growth factors is the collagen shield. These contact-lens–like structures are composed of lyophilized cross-linked collagens which absorb substantial amounts of EGF when hydrated in solutions containing high levels of EGF. As the collagen shield dissolves over a period of several hours, it releases EGF in the tear film.

Surgical incisions may have the most demanding criteria for vehicles since treatment is limited to a single application. Thus, the vehicle needs to deliver adequate levels of the peptide growth factor over a period of time which can induce a significant effect on the wound cells. Simple vehicles such as saline or viscous solutions of hyaluronic acid do not retain EGF in the

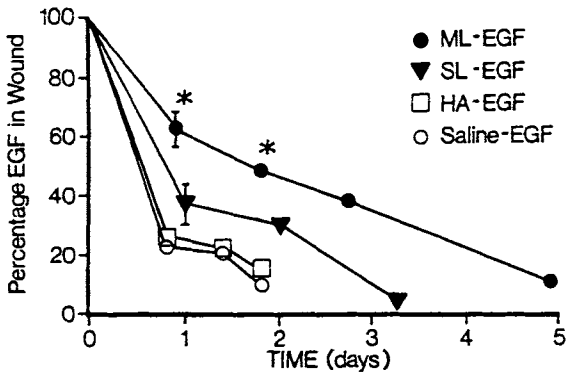

Fig. 3. Retention of EGF in incisions. Forty-eight adult male Sprague-Dawley rats were divided into four equal groups. One of four formulations containing ^{125}I-EGF and unlabeled insulin as carrier was then placed in the base of the incisions: multilamellar liposomes (ML-EGF) (●), single lamellar liposomes (SL-EGF) (▼), hyaluronic acid (HA-EGF) (□), saline (saline-EGF) (○). Values are the mean and standard deviation of radioactivity measured in three incisions. *$P < 0.001$. vs. HA-EGF and vs. saline-EGF.

tract of incisions for periods that are adequate to stimulate increases in tensile strength (Figure 3). In contrast, encapsulation of EGF in multilamelar liposomes retained EGF in incisions for extended periods and increased tensile strength of incisions in rats [36]. Although encapsulation of EGF in liposomes was successful under laboratory conditions, it is uncertain whether liposomes containing EGF could be made on an industrial scale.

Another approach which may be more practical takes advantage of polymer matrix technology developed primarily by Langer and colleagues. They reported that EGF could be combined with the carrier protein serum albumin and incorporated into a copolymer of ethylene vinyl acetate which released active EGF over a three-week period in vitro [43]. Further research needs to be done to determine if polymers can be developed that do not retard healing when placed in incisions and which release adequate levels of EGF over a time period sufficient to increase healing.

Although many fundamental questions remain to be answered concerning the roles of peptide growth factors and their receptors in normal and impaired healing, it seems highly probable that peptide growth factors formulated in well-designed vehicles will be a valuable adjuvant for the clinical management of wound healing.

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