Epidermal and Dermal Effects of Epidermal Growth Factor During Wound Repair

Lillian B. Nanney, Ph.D.

Departments of Plastic Surgery and Cell Biology, Vanderbilt University School of Medicine and Veteran's Administration Medical Center, Nashville, Tennessee, U.S.A.

Epidermal growth factor (EGF), a well-characterized peptide that stimulates in vitro cell proliferation, has now been shown to enhance in vivo resurfacing of porcine wounds. Topical formulations containing either recombinant EGF or placebo were applied daily to partial-thickness wounds along the dorsal surface of pigs. Following full-thickness removal of these wounds, tissues were sectioned and stained, and histologic sections were subjected to computerized morphometric analysis. A significant acceleration of epithelialization across the wound surface was noted following daily EGF treatments. EGF delivered in a variety of topical formulations also produced a marked increase in the cellularity and thickness in the neodermis. A dose-responsive increase in the thickness of the granulation tissue was also observed. In conclusion, topical application of EGF stimulates epithelialization of partial-thickness wounds and produces a positive impact on the underlying dermis during the early phases of wound repair. *J Invest Dermatol 94:624–629, 1990*

A potential candidate for the control of proliferation and differentiation of epidermis was first described as epidermal growth factor [1]. Over 25 years of predominantly in vitro studies indicate that EGF and several other peptide growth factors operate by interacting with the EGF receptor (EGF-R) [2–4]. With the cloning and large-scale production of growth factors such as EGF, it became feasible to conduct more extensive examinations into the in vivo responses of EGF on wound repair.

Wound healing is a complex biologic event involving inflammation, chemotaxis, cellular proliferation, differentiation, and remodeling [5]. The significance of growth factors on this cascade of events is still at its infancy [6]. To date, studies using exogenous applications of EGF have generally reported an enhancement of epithelialization [7–10]; however, several negative reports have also been published [11–13].

Resident cell types which comprise the skin, such as keratinocytes and fibroblasts, have a proved growth responsiveness to EGF in tissue culture [14–17]. The necessary receptor, EGF-R, for binding of EGF or TGF-α is present in normal human skin [18]. These data suggest that wounded skin could respond to either endogenous or exogenous application of EGF. Published studies of other proliferative conditions indicate that EGF, TGF-α, or the EGF-R are greatly increased in skin [19–26]. In psoriasis, a benign hyperproliferative disease, EGF-R are increased [24] and TGFα expression is increased [25]. In malignant melanoma, production of TGFα is associated with an increase in benign proliferative skin disorders and a concomitant increase in EGF-R and urinary TGFα [26]. Thus, several in vitro and limited in vivo reports suggest that a proliferative process such as wound healing should be responsive to exogenous manipulation using EGF.

The current study was conducted to document the in vivo effect of recombinant EGF in a porcine wound repair model. The partial-thickness injury in our studies was designed to evaluate both epidermal and dermal responses to topical application of EGF. In addition, the wounds in this animal study were selected to closely mimic the human donor site wound which has been previously reported [27].

MATERIALS AND METHODS

Surgical Wounding The porcine wound-healing model used in these studies was a modification of that described by Eaglstein and Mertz [28]. Specific pathogen-free domestic male and female pigs (24–54 kg) (Mr. Mike Snyder, Rt. 1, Box 195, Dawson, AL 35963) were anesthetized using an intramuscular injection of ketamine (0.22 mg/kg) and rompun (0.22 mg/kg). On the day of wounding the dorsal region was clipped, shaved, washed with mild soap and water, and disinfected with Betadine. Ten to 12 surgical wounds measuring 2.5 × 2.5 cm were produced with a modified Padgett Dermatome (Kansas City, MO). The actual cutting depth of the dermatome was 1254 ± 20 microns. This determination was obtained by measurement of the removed skin grafts. These paraffin sections were then cut and stained and the depth of injury assessed with quantitative morphometric analysis (see below). The wounding procedure with the dermatome created a donor site injury devoid of surface epithelium and superficial dermis, but which spared the lower dermis containing epithelial appendages such as hair follicles and sweat glands. After hemostasis was achieved, various placebo or EGF containing formulations were applied to the partial-thickness wounds. The dorsal surface of the pig, excluding the non-treated wounds, was then covered by a semi-occlusive bandage to prevent wound contamination and to hold the formulations in

Manuscript received September 27, 1989; accepted for publication January 10, 1990.

Presented in part at the Society of Investigative Dermatology, San Diego, CA, May 1987.

This work was supported by funds from Ethicon, Inc., GM 40437 and funds from the Veterans Administration.

Reprint requests to: Lillian B. Nanney, Ph.D., Plastic Surgery Research Laboratory, Medical Center North, S-2206, Vanderbilt University School of Medicine, Nashville, Tennessee 37232.

Abbreviations:
EGF: epidermal growth factor
EGF-R: epidermal growth factor receptors
FGF: basic fibroblast growth factor
PDGF: platelet-derived growth factor
TGF-α: transforming growth factor-alpha

0022-202X/90/$03.50

position (Op-site, Smith & Nephew, Massillon, Ohio). Subsequent daily dosings, dressing changes, and wound removals were accomplished while the animals remained under ketamine/rompun anesthesia. On days 3–7 after wounding the dermatome was adjusted to harvest a deeper wound (minimum 1500 microns) for histologic analysis. Two wounds were removed, a placebo- and an EGF-treated wound. Excess normal skin at the wound margins and underneath the partial-thickness wounds was included in the removal of these full-thickness biopsies.

Treatment Groups The recombinant EGF and basic FGF for this study were obtained from Chiron, Inc. (Emeryville, CA). It was formulated and the activity of the EGF tested by Ethicon, Inc., Somerville, NJ, a subsidiary of Johnson & Johnson, Inc. With a few exceptions, most of the studies reported herein were performed using a topical solution consisting of either EGF or placebo vehicle. These topical solutions (1 ml per wound) were saturated into an absorbent, non-adhering dressing (Release, Johnson & Johnson, Inc., Patient Care Division). On the right side of the animal, six wounds received a daily EGF treatment, and six paired wounds on the left side of the pig were treated with placebo. A total of 37 pigs were included in the present study. For each data point, n = 3 pigs, except for the 10 μg/ml daily dose, where n = 7 pigs.

The dose-response studies were conducted using 0.1, 10, 30, and 50 μg/ml of EGF in daily aqueous solutions. A single-dose study was also conducted. In this latter study, dressings were left in place for 48 h before the wounds were cleansed and redressed with op-site. In the dermal-depth evaluations, different proprietary formulations with or without growth factor were administered daily. These formulations included powder, cream, aqueous solution, or two types of gels.

Histologic Preparation Following the harvesting of wounds on days 3–7, each biopsy was divided into three pieces. These specimens were fixed in 10% neutral buffered formalin and embedded in paraffin. Nine random sections were cut from each wound and stained with either hematoxylin and eosin or Gomori's Trichrome stain. Microscopic sections were viewed and photographed on an Olympus Vanox Light Microscope.

Computerized Morphometric Analysis To assure objectivity, nine random sections were measured in a blinded fashion and an average mean determined from each wound [29]. Each microscopic wound section was placed on the stage of the microscope, where the image was then displayed on a video screen via a camera interfaced to an IBM AT computer. The wound length (distance across the wound surface) drawings were superimposed over the video image of the wound using a cursor and digitizing tablet. The planar morphometry computer software was purchased from Southern Micro Instruments, Inc. (Atlanta, GA). The percentage of wound resurfacing was determined by measuring the distance from the right wound margin to the left wound margin. Then the length of new epithelium across the surface of the wound was determined. This length was defined as the sum of the islands of new epithelium growing out from regenerating hair follicles and the new epithelium at the wound margins.

Dermal depth (granulation tissue) for each wound was the average of 30 measurements randomly collected from the nine sections taken from each wound. These dermal depth determinations were not taken near the wound margins, nor were they taken immediately adjacent to hair follicles. A dermal depth measurement was defined as the distance from the bottom of the migrating epidermis to the interface of the granulation tissue (neodermis) with the underlying non-wounded dermis. This parameter of wound healing could not be reliably determined before the third day of wound repair because insufficient new epithelium was present to determine the future level of migrating epidermal cells. All data in the current report were subjected to statistical analysis of the means using the Student paired t test (two-tailed).

RESULTS

Quantitative Evaluation of Epidermal Repair

Daily Treatment: Our initial studies with EGF indicated that 10 μg/ml EGF was an effective dose for wound epithelialization [10]. Accordingly, daily dosages of liquid 10 μg/ml EGF in an absorbent, non-adherent dressing were used in the treatment of seven pigs. Partial-thickness wounds were evaluated for the percentage of wound resurfacing (epithelialization) on days 3–7 after surgery (Fig 1). The data indicated that daily EGF treatment induced an enhancement in wound resurfacing. Epithelialization was complete by day 5 in the EGF-treated wounds, whereas wounds treated with placebo treatments reached 100% resurfacing by day 6. Interestingly, the statistically significant differences between EGF and the liquid vehicle were most marked on days 3 and 4, where $p < 0.05$ and $p < 0.001$, respectively. Earlier data points could not be collected in our split-thickness wound model due to lack of sufficient new epithelium (see *Materials and Methods*). Although EGF treatment resulted in 100% resurfacing at an earlier date than its comparable placebo, growth-curve patterns for both treatment groups were similar. In addition, several wounds received no treatment (no liquid vehicle, growth factor or semiocclusive bandage) (Fig 1). The extent of epithelialization in these non-treated wounds at days 4 and 5 was markedly less than wounds receiving either growth factor or placebo. This finding was unremarkable, for it has long been known that hydration of wounds in and of itself enhances wound epithelialization.

Single Treatment: A single initial dose of 10 μg/ml of hEGF was also evaluated in a small number of pigs (n = 3). The percentage of epithelialization following the one-time application of EGF was notably different from repeated daily applications of the identical EGF formulation (Fig 2). On day 3 after injury, the EGF-treated wounds showed more epithelial coverage than placebo-treated wounds. Subsequently on days 4, 5, and 6, the placebo-treated wounds showed greater resurfacing than the EGF treatment groups. There was no acceleration in the total number of days until complete healing was observed using a single dose of EGF.

Dose Testings: A dose-response study was conducted using a daily treatment regime. The dose-response evaluations were conducted using a daily EGF treatment with 0.1 μg/ml, 1.0 μg/ml, 30

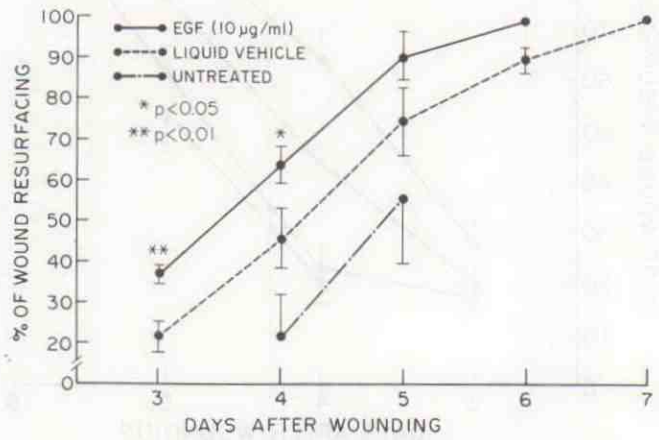

Figure 1. Effect of daily EGF application on resurfacing of porcine wounds. Ten split-thickness wounds resembling human donor sites were treated every 24 h for 7 d with either 10 μg/ml EGF in aqueous solution or placebo (see *Materials and Methods*). Two additional wounds remained untreated with either formulation or occlusive bandage. The EGF-treated wounds showed significant acceleration in epidermal resurfacing on post-wounding days 3 and 4. Wound resurfacing was completed 24 h sooner with the EGF-treated wounds than the placebo wounds. n = 7.

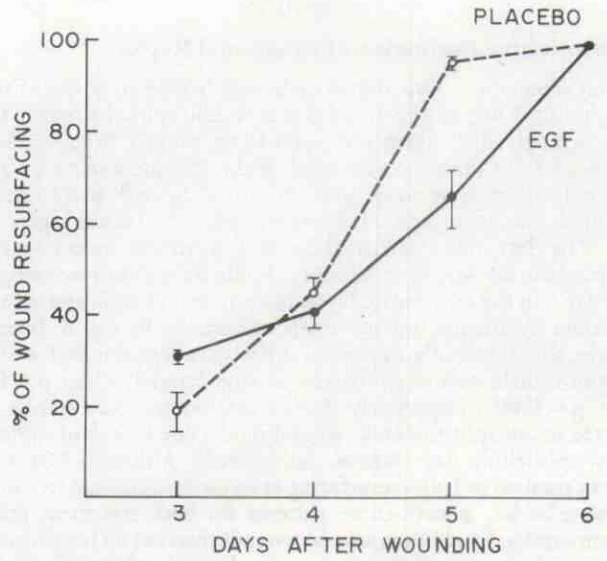

Figure 2. Effect of a single application of 10 μg/ml EGF on the resurfacing of porcine wounds. Ten split-thickness wounds resembling human donor sites were treated with a one-time dose of EGF in aqueous solution or placebo (n = 3). This dose was delivered immediately after wounding according to *Materials and Methods*. EGF treatment did not significantly hasten the resurfacing of porcine wounds as compared to the placebo treatment.

μg/ml, 50 μg/ml. The data from the 50 μg/ml (n = 3) and 0.1 μg/ml (n = 3) doses are shown in Fig 3 while the 10 μg/ml dose data are shown in Fig 1. The highest dose tested, 50 μg/ml, induced a slightly greater percentage of resurfacing than its comparable liquid placebo on day 4. However, the total time until epithelial closure was no different than its placebo. The percentage of wound

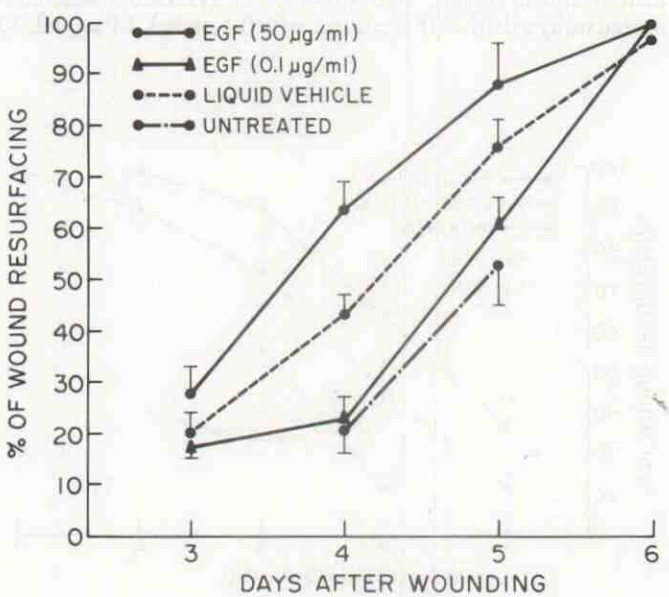

Figure 3. Dose-responsive effect of daily EGF application on the resurfacing of porcine wounds. Split-thickness wounds resembling human donor site injuries were treated with 0.1 μg/ml EGF (n = 3) or with 50 μg/ml EGF (n = 3) according to the *Materials and Methods* section. Placebo treatments and untreated wounds were included in each pig. While a trend toward an increase in epithelial resurfacing in the 50-μg dose was noted, no statistically significant improvement in the time until 100% recoverage of the wound was noted with either 50 or 0.1 μg/ml of EGF.

resurfacing appeared inhibited at the lowest-dose EGF tested, 0.1 μg/ml, at day 4 after wounding. Epithelial healing was complete by six days whether the wounds were treated with the highest dose, lowest dose, or placebo.

QUANTITATIVE EVALUATION OF DERMAL REPAIR

Another aspect of wound repair, the depth of the granulation tissue or neodermis, was evaluated at 4 days after injury. This interval after injury was chosen because the growth-factor effect on epithelialization was most pronounced by this day. In addition, the dermal depth parameter itself was defined as the distance from the basal surface of the epidermis to the interface of the neodermis in these partial-thickness injuries. Therefore, a dermal depth could not be accurately determined until sufficient new epithelium was present.

Data displaying the dermal effect induced by daily EGF treatments are shown in Fig 4. A comparison of the EGF effect when delivered in various proprietary formulations including gels, liquids, powders, and creams is also illustrated in Fig 4. Each growth-factor treatment or column (n = 3) is paired to its placebo formulation. Also included are unpaired dermal depth data for untreated wounds (column A), untreated wounds under occlusion (column H), and a single-dose treatment with an aqueous solution of 10 μg/ml EGF (column B). The results as shown in columns B–G indicate that the vehicle for drug delivery by itself has a highly variable impact on the thickness or depth of granulation tissue in partial-thickness wounds. Several placebo formulations (columns D, E, F, and G) without the addition of growth factor appeared to be inhibitory in comparison with treatment with occlusive bandage alone (Fig 4). Columns C, D, E, and F indicate that a dose of 10 μg/ml EGF delivered as a daily treatment serves to increase the thickness of the repairing dermis as compared with its paired pla-

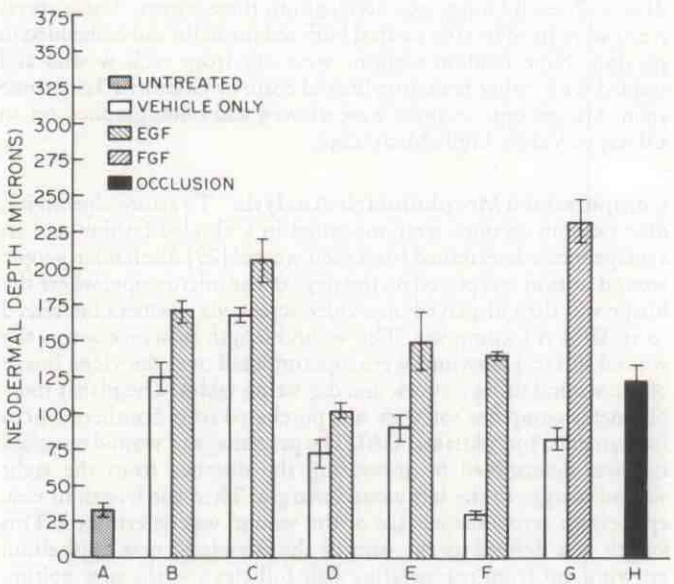

Figure 4. Effect of varied drug delivery on the thickness of granulation tissue in split-thickness wounds on day 4. Neodermal thickness was determined by computerized morphometric analysis as described in *Materials and Methods*. *Group A:* pooled data from the few untreated wounds on pigs (n = 10). *Group B:* single-dose treatment with liquid 10 μg/ml EGF (n = 3). *Group C–F* received a daily dosing with 10 μg/ml EGF in various formulations. *Group C,* daily powder (n = 3); *Group D,* gel #1 (n = 3); *Group E,* gel #2 (n = 3); *Group F,* cream (n = 3); *Group G,* liquid bFGF (n = 3); and *Group* H, pooled data from wounds receiving an occlusive dressing (n = 10). The EGF-induced effect on the thickness of the granulation tissue was dependent on the drug-delivery system. Several formulations appeared to have inhibitory effects in comparison to occlusive bandage alone. EGF treatment resulted in a thicker granulation tissue in comparison to wounds receiving placebo vehicle. FGF-treated wounds showed an increase in dermal thickness.

cebo. In columns D, E, and F, it would appear that EGF treatment can actually overcome the negative effects of the placebo. Furthermore, basic fibroblast growth factor (bFGF), which has reported dermal effects, was included in these comparative studies of dermal depth (granulation tissue). The positive effect of bFGF (n = 3) on the thickness of the granulation tissue is shown in column G (Fig 4). A 10-μg/ml dose of bFGF in a gel was administered daily to each wound in a gel formulation (1 ml/wound).

Neodermal (granulation tissue) depth was also evaluated in the dose-response trials (Fig 5). A highly variable placebo effect was attributed to pig variability because only three pigs were used for each dose with the exception of 10 μg/ml, where n = 7 pigs. Significant increases in dermal depth were apparent in wounds which received either 10 or 30 μg/ml of the daily aqueous EGF treatment. The 30-μg/ml dose produced a positive increase in the thickness that was almost twice that seen in its paired placebo (Fig 5). Essentially, no effect on dermal thickness was observed at the highest dose tested (50 μg/ml) as compared to its placebo.

QUALITATIVE ANALYSIS OF WOUND REPAIR

While many growth-factor-induced differences in wound repair, such as epidermal resurfacing or dermal depth, were amenable to morphometric analysis, other responses were subtle and difficult to quantify. Qualitative examination of histologic sections revealed dramatic differences between the wounds treated with EGF and wounds treated with placebo. In the epidermis, the degree of keratinocyte differentiation was striking in the EGF-treated wounds. In these comparisons, the EGF-treated epidermis was multilayered (16–20 cells deep) and a cornified layer was apparent in many regions on day 5 after injury (Fig 6*b*). Placebo-treated epidermis was thin (4–5 cells deep) and the cells were pale and poorly differentiated (Fig 6*a*). In other regions of the placebo-treated wounds, no epithelium was present on the surface after 5 days (Fig 1).

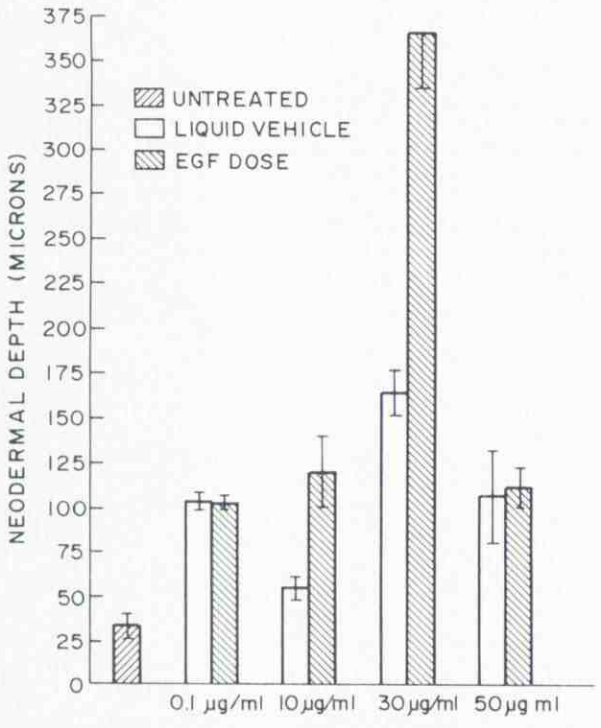

Figure 5. Dose-responsive effect of daily EGF application on the thickness of granulation tissue in split-thickness wounds on day 4. Neodermal thickness or depth of the granulation tissue was determined by computerized morphometric analysis (see *Materials and Methods*). EGF or placebo was applied in daily dosages of 0.1 (n = 3), 10 (n = 7), 30 (n = 3), or 50 μg/ml (n = 3). The maximal increase in dermal thickness was noted in the wounds treated with the 30 μg/ml dose of EGF as compared to its liquid vehicle ($p < 0.05$). A significant increase in dermal thickness was also noted with a dose of 10 μg/ml ($p < 0.05$).

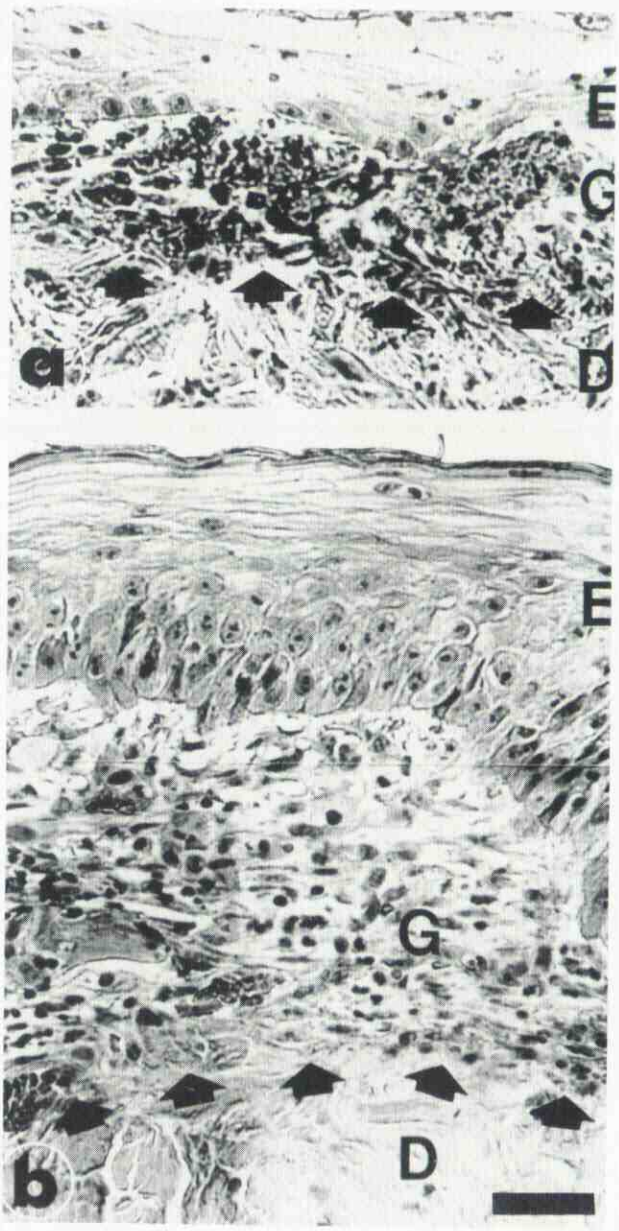

Figure 6. Micrographs of representative split-thickness wounds after 5 days of topical application of *a)* placebo or *b)* 10 μg/ml EGF. Epidermis receiving placebo treatment is poorly differentiated with only 4–5 cell layers. EGF-treated epidermis is a well-differentiated multilayered structure. Granulation tissue is thicker, more densely populated, and better organized in EGF-treated wounds as compared to placebo-treated wounds. Hematoxylin and eosin staining. *Size bar,* 50 m.

In the granulation tissues, both major and subtle differences were noted in response to growth-factor treatment. Measurable differences in dermal depth are displayed in graphic form in Figs 4 and 5, but this difference in thickness is also quite apparent in Fig 6*a,b* which was photographed at the same magnification. After 5 days of repair, the EGF-treated granulation tissue exhibits increased cellularity and more organization (Fig 6*b*) than its comparable control (Fig 6*a*).

DISCUSSION

The experiments described here reveal that pharmacologic dosages of exogenous EGF have positive effects on both epidermal and dermal components during wound repair. These data collected

from a porcine partial-thickness wound model add further substantiation to the clinical report that EGF has a stimulatory impact on cutaneous wound repair [27].

Earlier wound-healing studies with smaller mammals such as mice and rabbits suggested that EGF serves to enhance wound healing [7–10]. However, a number of attempts to show enhanced epidermal regeneration using EGF have also been unsuccessful [11–13]. With the cloning and large-scale manufacture of peptide growth factors such as EGF, PDGF, FGF, TGF-α, and TGF-β the large-scale testing of growth factors on wound-healing mechanisms became possible. A brief report from Brown et al [9] suggested that recombinant EGF enhanced epidermal regeneration in pigs. In the present study, the scope of the porcine-wound model was expanded ($n = 37$) to evaluate a variety of formulations, dosages, and treatment schemes. The porcine wounds were designed to closely resemble human donor site injuries where a definitive effect of recombinant EGF was recently reported [27].

Our report of accelerated epidermal resurfacing in partial-thickness injuries in response to exogenous application of EGF was not surprising. The responsiveness of keratinocytes to EGF has been well documented in numerous in vitro experiments [reviewed in 2,4,14–16]. Also, EGF binding and the presence of EGF receptors have been detected in the normal epidermis and epidermal appendages [18,30]. These earlier studies provided evidence that the necessary growth-factor machinery, a requirement for EGF binding, was present in the epidermis.

Although exogenous EGF produced an enhancement of epidermal resurfacing of the wounds in our experiments, its mode of action following binding to EGF-R is unknown. Historically, EGF is best known for its mitogenic action [2–4], but recent evidence also suggests that rapid resurfacing of wounds could be due to an increased rate of cell migration after EGF exposure [31]. Either or both mechanisms could explain our observed acceleration in the resurfacing of the wound surface.

The EGF-induced effect on the thickness of the granulation tissue in a split-thickness wound model was an original observation. These quantitative studies were undertaken after observing the marked stimulation of granulation tissue on histologic sections. A mesenchymal response to EGF has previously been reported from both in vitro and in vivo experiments. In vitro data with fibroblasts indicates that EGF binds to EGF-R, and increases mitosis and synthesis of proteins [17]. In granulation tissue models of wound repair, sustained release of EGF showed stimulatory effects, i. e., an increased number of fibroblasts, increased neovascularization, and accumulations of collagen [32]. Incisional wounds showed an increase in tensile strength after EGF treatment [33]. In a hamster check model, EGF served as an angiogenic mediator [34]. Thus our present finding of EGF-induced stimulation of dermal repair in a donor site wound model complements the existing literature.

In our studies, we noted a tendency for increased dermal thickness following EGF treatment using a wide variety of formulations. However, when assessing the depth of granulation tissue, care must be taken because the effect of vehicle itself can be dramatic [13]. Although several placebo formulations appeared to inhibit dermal thickness, the addition of EGF to these formulations showed a tendency to overcome this negative effect. Statistically significant increases in dermal thickness were noted at both 10 μg/ml and 30 μg/ml EGF. Because our wound-healing model so closely resembles the human partial thickness injury, our data further suggest that EGF could stimulate dermal repair in other clinical situations such as donor sites, partial thickness burns, or chronic skin ulcers.

Although exogenous EGF induced a dose-responsive effect in the neodermis, these initial studies were not designed to determine whether EGF produced a direct or indirect effect. Endogenous growth factors such as EGF, PDGF, TGFα, and TGFβ, which are most certainly delivered to the wound via platelets [35,36], may also have substantial impact on both epidermal and dermal wound repair. Various wound-repair studies with exogenous delivery of TGFβ [33,37–39], TGFα [40], PDGF [39,41,42], and platelet extracts [43,44] have shown the potential of these growth factors. Although the complexities of the untreated wound milieu are poorly understood at this time, autocrine/paracrine theories of growth-factor interaction suggest that cell types within the wound should respond to endogenously produced growth factor [45]. The production of growth factors by macrophages in wound fluid has already been reported [46]. Also in situ hybridization studies have indicated that exogenous TGFβ increases the expression of endogenous TGFβ, collagen, and fibronectin [47].

In conclusion, the porcine wound-repair model system described herein shows the pharmacologic stimulation of both epithelialization and granulation tissue by EGF. This report provides additional histologic and quantitative data from a porcine donor site wound to complement the limited data from the human donor site trials with EGF [27]. Together these data suggest that clinical application for EGF treatment could extend to deeper cutaneous injuries such as chronic wounds or burns.

Expert technical assistance was provided by Jesse Britton, Nancy Love, Mary McKissack, and Lise McLean. We are indebted to Ethicon, Inc. for the supply of formulations and study coordination. We especially thank Barbara F. Matlaga and Amy Finkenaur from Ethicon, who made major contributions.

REFERENCES

1. Cohen S, Elliot GA: The stimulation of epidermal keratinization by a protein isolated from the submaxillary gland of the mouse. J Invest Dermatol 40:1–5, 1963
2. Carpenter G, Cohen S: Epidermal growth factor. Ann Rev Biochem 48:193–216, 1979
3. Stoscheck CM, King LE: Functional and structural characteristics of EGF and its receptor and their relationship to transforming proteins. J Cell Biochem 31:135–152, 1986
4. King LE, Stoscheck CM, Gates R, Nanney LB: Epidermal growth factor and related growth factors in Biochemistry and Physiology of the Skin. Goldsmith LA (ed.). Oxford University Press, New York, 1989 (in press)
5. Clark RAF, Henson PM (eds.): The molecular and cellular biology of wound repair. Plenum Press, New York, 1988
6. Barbul A, Pines E, Caldwell M, Hunt TK (Eds.): Growth factors and other aspects of wound healing: Progress in clinical biological research. Vol 266. Alan R. Liss, New York, 1987
7. Franklin JD, Lynch JB: Effects of topical application of epidermal growth factor on wound healing: experimental study on rabbit ears. Plast Resconstr Surg 64:766–770, 1979
8. Laato N, Niinikiski J, Gerdin B, Lebel L: Stimulation of wound healing by epidermal growth factor. Ann Surg 203:379–381, 1986
9. Brown GL, Curtsinger L, Brightwell JR, Ackerman DM, Tobin GR, Polk HC, George-Nascimanto C, Valenzuela P, Schultz G: Enhancement of epidermal regeneration by biosynthetic epidermal growth factor. J Exp Med 163:1319–1324, 1986
10. Nanney LB: Epidermal growth factor-induced effects on wound healing (abstr). Clin Res 35:706A, 1987
11. Greaves MW: Lack of effect of topically applied epidermal growth factor on epidermal growth in man in vivo. Clin Exp Dermatol 5:101–105, 1980
12. Arturson G: Epidermal growth factor in the healing of corneal wounds, epidermal wounds and partial-thickness scalds. Scand J Plast Reconstr Surg 18:33–37, 1984
13. Chvapil M, Gaines JA, Gilman T: Lanolin and epidermal growth factor in healing of partial-thickness pig wounds. J Burn Care 9:279–284, 1988
14. Rheinwald JG, Green H: Epidermal growth factor and the multiplication of cultured human epidermal keratinocytes. Nature (London) 265:421–426, 1977
15. O'Keefe E, Battin T, Payne R: Epidermal growth factor receptor in human epidermal cells: direct demonstration in cultural cells. J Invest Dermatol 78:482–487, 1982
16. O'Keefe EJ, Payne RE: Modulation of the epidermal growth factor receptors of human keratinocytes by calcium ion. J Invest Dermatol 81:231–235, 1984

17. Carpenter G, Cohen S: ^{125}I-labelled human epidermal growth factor (hEGF): binding, internalization, and degradation in human fibroblasts. J Cell Biol 71:159–171, 1976
18. Nanney LB, Stoscheck CM, King LE: Comparison of epidermal growth factor binding and receptor distribution in normal human epidermis and epidermal appendages. J Invest Dermatol 83(5):385–393, 1984
19. Ozanne B, Richards CS, Hendlen R, Burns D, Gusterson B: Overexpression of the EGF receptor is a hallmark of squamous cell carcinomas. J Pathol 149:9–14, 1986
20. Coffey RJ, Derynck R, Wilcox JN, Bringman TS, Goustin AS, Moses HL, Pittelkow MR: Production and auto-induction of transforming growth factor-alpha in human keratinocytes. Nature 328:817–820, 1987
21. Gottlieb AB, Chang CK, Posnett DN, Fanelli B, Tam JP: Detection of transforming growth factor-alpha in normal, malignant, and hyperproliferative human keratinocytes. J Exp Med 167:670–675, 1988
22. Nanney LB, Stoscheck CM, King LE, Underwood RA, Holbrook KA: Immunolocalization of epidermal growth factor receptors in normal developing human skin. J Invest Dermatol (in press)
23. Nanney LB, Ellis D, Dale B, Stoscheck C, Holbrook K, King LE: Epidermal growth factor receptors (EGF-R) in idiopathic and virally induced hyperproliferative skin diseases (abstr). Clin Res 36(3):678A, 1988
24. Nanney LB, Stoscheck CM, Magid M, King LE: Altered ^{125}I-epidermal growth factor binding and receptor distribution in psoriasis. J Invest Dermatol 86(3):260–265, 1986
25. Elder JT, Fisher GJ, Lindquist PB, Bennett GL, Pittelkow MR, Coffey RJ, Ellingsworth L, Derynck R, Voorhees JJ: Overexpression of transforming growth factor alpha in psoriatic epidermis. Science 243:811–814, 1989
26. Ellis DL, Kafka S, Chow J, Nanney LB, Inman W, McCadden M, King LE: Melanoma, growth factors, acanthosis nigricans, the sign of Leser-Trelat and multiple acrochordons: A role for transforming growth factor-alpha in cutaneous paraneoplastic syndromes. N Engl J Med 317(25):1582–1587, 1987
27. Brown GL, Nanney LB, Griffin J, Cramer AB, Yancy JM, Curtsinger LJ, Holtin L, Schultz GS, Jurkiewicz MJ, Lynch JB: Enhancement of wound healing by topical treatment with epidermal growth factor. N Engl J Med 321:76–79, 1989
28. Eaglstein WH, Mertz PM: New method for assessing epidermal wound healing: the effects of triamcinolone acetonide and polyethelene film occlusion. J Invest Dermatol 71:382–384, 1978
29. Chvapil M, Gaines JA, Benson D, Tellez C: An optimal morphometric method for quantitating wound epithelialization. J Surg Res 44:266–276, 1988
30. Green MR, Couchman JR: Distribution of epidermal growth factor receptors in rat tissues during embryonic skin development, hair formation, and the adult hair growth cycle. J Invest Dermatol 83(2):118–123, 1984
31. Barrandon Y, Green H: Cell migration is essential for sustained growth of keratinocyte colonies: the roles of transforming growth factor-α and epidermal growth factor. Cell 50:1131–1137, 1987
32. Buckley A, Davidson JM, Kamerath CD, Wolt TB, Woodward SC: Sustained release of epidermal growth factor accelerates wound repair. Proc Natl Acad Sci USA 82:7340, 1985
33. Brown GL, Curtsinger LJ, White M, Mitchell RO, Pietsch J, Norquist R, Schultz GS: Acceleration of tensile strength of incisions treated with EGF and TGF-β. Ann Surg 208:788–794, 1988
34. Schreiber AB, Winkler ME, Derynck R: Transforming growth factor-α: A more potent angiogenic mediator than epidermal growth factor. Science 232:1250, 1986
35. Assoian RK, Komoriya A, Meyers CA, Miller DM, Sporn MB: Transforming growth factor-β in human platelets. J Biol Chem 258:7155–7160, 1983
36. Oka Y, Orth DN: Human plasma epidermal growth factor-urogastrone is associated with blood platelets. J Clin Invest 72:249–259, 1983
37. Mustoe TA, Pierce GF, Thomason A, Gramates P, Sporn MB, Deuel TF: Accelerated healing of incisional wounds in rats induced by transforming growth factor β. Science 237:1333–1335, 1987
38. Pierce GF, Mustoe TA, Lingelbach J, Masakowski VR, Griffin GL, Senior RM, Deuel TF: Platelet-derived growth factor and transforming growth factor-β enhance tissue repair activities by unique mechanisms. J Cell Biol 109:429–440, 1989
39. Pierce GF, Mustoe TA, Lingelbach J, Masakowski V, Gramates P, Deuel TF: Transforming growth factor β reverses the glucorticoid-induced wound healing deficit in rats and is regulated by platelet derived growth factor in macrophages. Proc Natl Acad Sci USA 86:2229–2233, 1989
40. Schultz GS, White M, Mitchell R, Brown G, Lynch J, Twardzik DR, Todaro GJ: Epithelial wound healing enhanced by transforming growth factor-α and vaccinia growth factor. Science 235:350, 1987
41. Grotendorst GR, Martin GR, Poncer D, Sodek J, Harvey AK: Stimulation of granulation tissue formation by platelet-derived growth factor in normal and diabetic rats. J Clin Invest 76:2323–2329, 1985
42. Lynch SE, Nixon JC, Colvin RB, Antioniades HN: The role of platelet-derived growth factor in wound healing: synergistic effects with other growth factors. Proc Natl Acad Sci (USA) 84:7696–7700, 1987
43. Knighton DR, Ciresi KF, Fiegel VD, Austin LL, Butler EL: Classification and treatment of chronic nonhealing wounds: Successful treatment with autologous platelet-derived wound healing factors (PDWHF). Ann Surg 204:322–330, 1986
44. Carter DM, Balin AK, Gottlieb AB, Eisinger M, Lin A, Pratt L, Sherbany A, Caldwell D: Clinical experience with crude preparations of growth factors in healing of chronic wounds in human subjects. Prog Clin Biol Res 266:303–317, 1988
45. Sporn MB, Todaro GJ: Autocrine secretion and malignant transformation of cells. N Engl J Med 303:878–880, 1980
46. Rappolee DA, Mark D, Banda MJ, Werb Z: Wound macrophages express TGF-α and other growth factors in vivo: analysis by mRNA phenotyping. Science 241:708–712, 1988
47. Quaglino D, Nanney LB, Ditesheim J, Kennedy R, Broadley KN, Davidson JM: Localization of matrix gene expression in growth factor-stimulated wound repair (abstr). J Cell Biol 107(6):49A, 1988