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# Epithelial Wound Healing Enhanced by Transforming Growth Factor–α and Vaccinia Growth Factor

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**Epidermal regeneration following middermal injuries to skin requires both proliferation and migration of keratinocytes. Epidermal growth factor (EGF) stimulates the proliferation of keratinocytes in culture, and topical administration of EGF accelerates epidermal regeneration of partial thickness burns or split-thickness incisions in vivo. Transforming growth factor–alpha (TGF-α) and vaccinia growth factor (VGF) have substantial sequence homology with EGF, and all appear to bind to the same receptor protein. Whether TGF-α or VGF can affect epidermal wound healing in vivo is not known. The present studies show that topical administration of TGF-α or VGF in antibiotic cream to partial thickness burns (second degree) accelerated epidermal regeneration in comparison with untreated or vehicle-treated burns. Low levels of both TGF-α and VGF (0.1 microgram per milliliter) appeared to be more effective than EGF in stimulating epidermal regeneration. Regenerated epithelium from burns treated with TGF-α or VGF appeared normal histologically. This finding suggests that topical application of selected growth factors may be useful in accelerating healing of partial thickness injuries.**

PEPTIDE GROWTH FACTORS MAY PLAY important roles in the body's response to injury by promoting wound healing. Epidermal growth factor (EGF) present in saliva is thought to accelerate healing of cutaneous injuries in mice as they lick their wounds (*1*). Topical application of EGF accelerated epidermal regeneration of middermal skin injuries (*2*), corneal abrasions (*3*), and increased tensile strength of corneal incisions (*3, 4*). Transforming growth factor–α (TGF-α) (*5*) and vaccinia virus growth factor (VGF) (*6*) have substantial sequence homology to EGF, and all three growth factors appear to bind to and activate a common tyrosine kinase receptor (*7–9*). Whether TGF-α and VGF have activities in vivo similar to those of EGF in promoting wound healing is not known. We now report that TGF-α and VGF both accelerate epidermal regeneration of middermal thermal skin injuries.

Human epidermal keratinocytes express specific high-affinity membrane receptors for EGF (*10*), and EGF increases the lifetime of cultured epidermal cells by stimulating division and minimizing differentiation (*11, 12*). Since regeneration of the epidermal layer after a middermal injury occurs by mitosis and migration of epidermal cells from residual epidermal appendages within the wound and from intact epithelium surrounding the injury (*13*), agents that accelerate mitosis of epidermal cells could accelerate epidermal regeneration of middermal wounds. The effects of TGF-α and VGF on epidermal regeneration in vivo were tested on middermal thermal wounds (second degree burns) on the dorsal thorax of adult pigs. Chemically synthesized rat TGF-α (rTGF-α) or human TGF-α (hTGF-α) (Peninsula Laboratories; Belmont, California) were applied twice a day in a water-miscible antibiotic cream (Silvadene). After 9 days of treatment, the eschar was removed, burns were photographed, and areas of regenerated epithelium were measured by two evaluators using computerized planimetry of enlarged photographs. The methods of treatment were unknown to the evaluators. The three burns treated with different concentrations of rTGF-α (0.1, 1.0, and 10 μg/ml) all showed a substantially larger area of regenerated epithelium than the parallel untreated or vehicle-treated burns (Fig. 1). Similarly, all three burns treated with different concen-

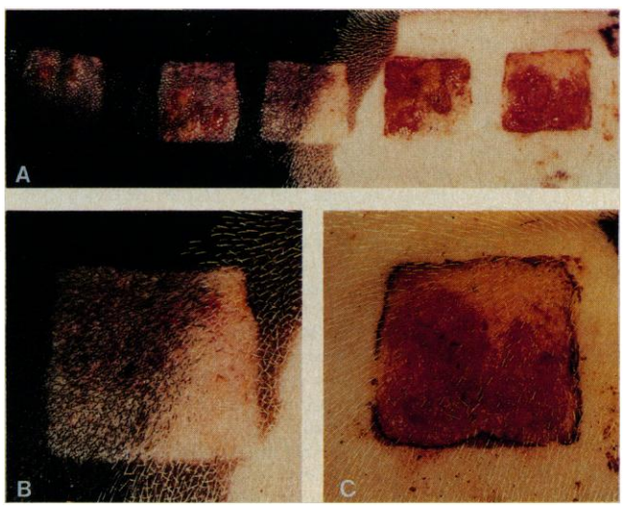


**Fig. 1.** (**A**) Photograph of middermal thermal wounds (second degree burns), with eschar removed, at 9 days (from left to right) after daily treatment with TGF-α at 10, 1, and 0.1 μg/ml in a water-miscible ointment (Silvadene), no treatment, or treatment with vehicle alone. Enlargements of burns treated with (**B**) TGF-α at 0.1 μg/ml in Silvadene or (**C**) with Silvadene alone.

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trations of hTGF-α (0.1, 1.0, and 5 μg/ml) showed a larger area of regenerated epithelium than did the untreated or vehicle-treated burns. Light microscopy of biopsy specimens from the burns treated with TGF-α displayed no evidence of dysplasia or metaplasia. Also, wounds treated with rTGF-α appeared to have more granulation tissue subjacent to the epidermis than did wounds treated with vehicle.

**Table 1.** Epithelial regeneration in middermal burns treated with TGF-α or VGF. Middermal thermal injuries were made on the dorsal thorax of anesthetized female Yorkshire pigs (15 kg) whose backs had been shaved and depilitated with commercial hair cream remover (*2*). A brass template (3 by 3 cm; 147 g) was equilibrated in a 70°C water bath and placed in firm contact with the skin for exactly 10 seconds, and the resulting blister was removed. Five middermal burns were placed on each side of the spine and were separated from each other by approximately 2.5 cm. Burns were treated twice a day with approximately 3 ml of vehicle cream (Silvadene) alone or vehicle cream containing growth factor or were left untreated. Both human and rat forms of synthetic TGF-α were purified to homogeneity by reversed-phase chromatography as described (*5*). VGF was purified from 24-hour supernatants harvested from vaccinia virus–infected African Green monkey cell cultures (*14*) and, in addition, was also eluted from a $C_{18}$ μBondapak column (Waters Associates, Millford, Connecticut) with acetonitrile in 0.05% trifluoroacetic acid (*p*H 1.9). The concentration of biologically active growth factors was determined by comparison to standard EGF competitive curves measuring $^{125}$I-labeled EGF ($1 \times 10^{10}$ cpm/nmol) binding to Formalin-fixed monolayers of A431 human epidermoid carcinoma cells (*14*). After 9 days of treatment with TGF-α or 10 days of treatment with VGF, the pigs were anesthetized, eschar was removed from burns, and the burns were photographed. Biopsies were taken in each burn from reepithelialized areas. Areas of burns that had regenerated epithelium were outlined by two observers on enlarged photographs and the percentage of the original burn area that had healed was measured by computer-assisted planimetry. Values reported are the mean and range of the two evaluations.

| Treatment | Percent of original burn area with regenerated epithelium | |
|---|---|---|
| | Right side | Left side |
| | *Animal 1* | |
| Untreated | 10 ± 2 | 6 ± 4 |
| Vehicle | 68 ± 6 | 76 ± 1 |
| VGF | | |
| 0.1 μg/ml | 96 ± 2 | 92 ± 1 |
| 0.5 μg/ml | 95 ± 1 | 90 ± 2 |
| 1.0 μg/ml | 85 ± 3 | 68 ± 4 |
| | *Animal 2* | |
| Untreated | 30 ± 6 | 17 ± 3 |
| Vehicle | 45 ± 5 | 58 ± 2 |
| rTGF-α | | |
| 0.1 μg/ml | 98 ± 2 | |
| 1.0 μg/ml | 73 ± 1 | |
| 10.0 μg/ml | 90 ± 1 | |
| hTGF-α | | |
| 0.1 μg/ml | | 86 ± 4 |
| 1.0 μg/ml | | 84 ± 4 |
| 5.0 μg/ml | | 66 ± 6 |

Vaccinia virus codes for a 140-residue polypeptide with approximately 35% homology to both EGF and TGF-α, and cells infected with vaccinia virus secrete a polypeptide of approximately 24 kD that binds to the EGF receptor and stimulates its autophosphorylation (*8, 9*). The biological activity of this protein, called VGF, in promoting epidermal regeneration in vivo has not been previously evaluated. Using the model of middermal thermal wounds described above, we tested VGF purified from vaccinia-infected monkey cell supernatant (*14*) at three concentrations in a water-miscible vehicle (Silvadene). Substantial increases in the area of regenerated epithelium were measured for duplicate wounds treated with 0.1 and 0.5 μg of VGF per milliliter of vehicle compared to vehicle-treated wounds or untreated wounds, and one of the two duplicate wounds treated with VGF (1 μg/ml) had a larger area of regenerated epithelium than did controls (Table 1). Light microscopy of biopsy specimens treated with VGF revealed typical patterns of normal regenerating epidermis and dermis. No evidence of dysplasia or metaplasia was observed in burns treated with VGF.

When the three growth factors were compared at 0.1 μg/ml (Fig. 2), VGF and TGF-α appeared to produce a more rapid epithelialization of the wound area than did other forms of treatment tested. The present experiments, as well as those described earlier (*2*), indicate that the optimal concentration of EGF in this system is 1 to 10 μg/ml. Both TGF-α and VGF produce a maximal response at 0.1 μg/ml.

The similarities in sequence homology of EGF, TGF-α, and VGF—especially in relative positioning of the six conserved cysteine residues—and binding to the EGF receptor and subsequent induction of receptor autophosphorylation suggest the possibility that these peptides would have similar biological effects in vivo. TGF-α and EGF caused similar inhibition of histamine-stimulated acid secretion from guinea pig gastric mucosal strips (*15*) in vitro. However, TGF-α was reported to be a more potent angiogenic mediator than EGF in the hamster cheek pouch bioassay (*16*) and in the formation of osteoclast-like cells in human marrow cultures (*17*). Furthermore, TGF-α, but not EGF, stimulated resorption of fetal rat limb bones after 60 hours of exposure in vitro (*18*). Barrandon and Green (*19*) described a system for measuring the effects of growth factors on the proliferation of human epithelial cell clones in culture. They reported that both EGF and TGF-α serve as potent stimulators of epithelial cell proliferation and that TGF-α is significantly more active than EGF. Thus, it seems that TGF-α (and possibly VGF) may, in certain systems, act as superagonists. The basis for the difference in activity of TGF-α and VGF as compared to EGF is not yet understood; however, this difference in activity is seen in various assay systems and may reflect the ability of the growth factor receptors on certain cell types to distinguish between these closely related effector molecules. Although most wound repair models, including the wound repair system we have described, do not allow for precise quantitation, the results presented in this report do indicate that TGF-α and VGF accelerate epithelial regeneration in middermal thermal injuries and thus exhibit actions similar to those of EGF (*2–4*) in the whole animal as well as in cell culture systems.

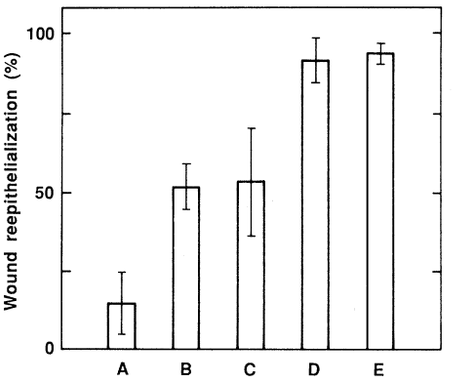


**Fig. 2.** Relative abilities of various treatment regimens to facilitate epithelial wound healing. The growth factors were all tested at 0.1 μg/ml. This concentration has previously been shown to be suboptimal for EGF (*2*). Conditions are (**A**) untreated; (**B**) Silvadene alone; (**C**) Silvadene and EGF; (**D**) Silvadene and TGF; (**E**) Silvadene and VGF. Results were scored at 9 or 10 days and are the average of two or more experiments with different test animals. Results are mean ± standard deviation.


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# Antidepressant and Circadian Phase–Shifting Effects of Light

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**Bright light can suppress nighttime melatonin production in humans, but ordinary indoor light does not have this effect. This finding suggested that bright light may have other chronobiologic effects in humans as well. Eight patients who regularly became depressed in the winter (as day length shortens) significantly improved after 1 week of exposure to bright light in the morning (but not after 1 week of bright light in the evening). The antidepressant response to morning light was accompanied by an advance (shift to an earlier time) in the onset of nighttime melatonin production. These results suggest that timing may be critical for the antidepressant effects of bright light.**

WE SHOWED EARLIER (*1*) THAT bright (2500 lux) light is necessary for suppression of nighttime melatonin production in humans, whereas other animals respond to light of ordinary intensity (*2*). This suggested that humans could have biological rhythms cued to natural daylight that would remain unperturbed by the use of ordinary indoor light (*3*) and that bright light could be used to manipulate these rhythms. We first tested this idea during the winter of 1980 when we successfully treated a patient with recurrent winter depression by exposing him for several days to light at an intensity of 2000 lux from 0600 to 0900 and from 1600 to 1900 (*4*). Since then, many such patients have been similarly treated (*5, 6*).

Subsequent studies showed that dim light is not effective in treating this disorder (*5, 6*). However, there is disagreement about whether the time of exposure to light is important. Some investigators have concluded that only duration and brightness are important (*7–9*), whereas we have held that time of exposure to light is also critical (*10–12*).

We hypothesized (*10–12*) that the antidepressant effect of bright light depends on shifting the phase (timing) of circadian (24-hour) rhythms. The effect of bright light would vary according to a phase response curve (PRC) similar to those described for other animals (*13, 14*); light in the morning would advance circadian rhythms (shift them to an earlier time) and light in the evening would delay them (shift them to a later time) (*15*). We further hypothesized that the circadian rhythms of most patients with winter depression are abnormally phase-delayed and that most of these patients should preferentially respond to morning light which would provide a corrective phase advance (*11, 12*). We now present data from a study designed to test these hypotheses.

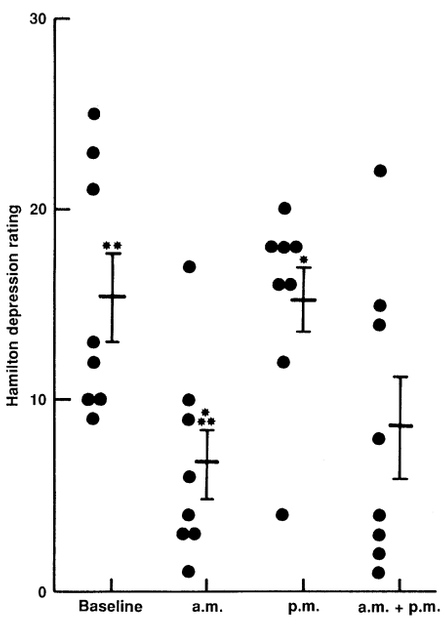


**Fig. 1.** Individual and average 21-item Hamilton depression ratings (±SEM) for eight patients with winter depression for each of the 4 weeks of the study. An analysis of variance for repeated measures indicated a significant ($P = 0.026$) difference between treatments. Only the paired $t$ tests comparing the week of morning (a.m.) light and the baseline week (**$P = 0.004$) and comparing the week of a.m. light and the week of evening (p.m.) light (*$P = 0.045$) were significant. Average depression ratings (±SEM) for the seven normal control subjects were 3.0 ± 0.9 at baseline, 2.4 ± 0.3 (a.m. light), 6.1 ± 1.6 (p.m. light), and 4.3 ± 0.9 (a.m. + p.m. light).

During a 4-week protocol, seven normal control subjects and eight patients with winter depression (*16*) stayed indoors between 1700 and 0800 shielded from bright light and slept only between 2200 and 0600. The first week was a baseline week. During the second week, subjects were randomly assigned to morning bright light exposure (0600 to 0800) or to evening bright light exposure (2000 to 2200), and during the third week these assignments were reversed (*17*). During the fourth week, all subjects were exposed to bright light both in the morning and in the evening. Under continuous dim light (*15*), blood was sampled for melatonin every 30 minutes between 1800 and 2300 on the first (prebaseline) day of the study and on the last day of each week and was subsequently assayed for melatonin by the gas chromatographic–negative chemical ionization mass spectrometry (GC-MS) technique of Lewy and Markey (*18*). Subjects were rated with the 21-item Hamilton depression scale (HAM-D) (*19*) on the evening of each blood drawing by a psychiatrist (R.L.S.) who was not aware of the treatment conditions assigned for weeks 2 to 4. Patients were told that an antidepressant response could potentially occur on any of the 4 weeks of the study, depending on the individual.

At baseline, HAM-D ratings for the patients (Fig. 1) averaged 15.4 ± 2.3, which is considered to be a moderate degree of depression (*19*). After a week of morning light, ratings were significantly lower [6.6 ± 1.8 ($P = 0.004$)] and not significantly different from those of the control subjects. Ratings after a week of evening light (15.2 ± 1.8) were not significantly different from those of the baseline week but were significantly greater than those after a week of morning light ($P = 0.045$). Depression ratings after the week of both morning and evening light (8.6 ± 2.7) were not significantly different from those of any of the preceding 3 weeks.

Average onset times of melatonin secretion for both subject groups are shown in Fig. 2. Prebaseline and baseline melatonin onset times of the patients were significantly delayed compared to those of the normal controls ($P = 0.02$ and 0.05, respectively). Morning light exposure advanced the time of onset of melatonin secretion and evening light delayed it (*20*). The combination of morning and evening light caused the melatonin onset times to shift to intermediate

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