Effect of Vascular Endothelial Growth Factor on Skin Graft Survival in Sprague-Dawley Rats

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Objective: To examine the effect of exogenous vascular endothelial growth factor (VEGF) on skin graft survival in Sprague-Dawley rats.

Design: Dorsal full-thickness skin grafts were harvested from 18 Sprague-Dawley rats. To simulate human full-thickness skin grafts, the panniculus carnosus muscle was removed from the undersurface of each graft. The recipient beds were delivered subfascial injections of recombinant human VEGF or isotonic sodium chloride solution in 12 animals before replacement of the grafts (6 in each group). Grafts were replaced without injections in 6 sham control animals. Using planimetry, grafts were analyzed for necrosis along epidermal and dermal surfaces on postoperative day 7. Results were compared between groups. To determine the role of the panniculus carnosus muscle in graft survival, 12 Sprague-Dawley rats underwent the same procedure with an intact panniculus carnosus muscle and with subfascial injections of VEGF or physiologic isotonic sodium chloride solution (6 in each group). Analyses were performed on postoperative day 14. The mean microvascular density was determined in each graft after staining with anti-factor VIII antibody.

Results: The mean percentage of dermal necrosis in VEGF-treated skin grafts (10.0%) was significantly lower than that in saline-treated grafts (26.7%) or in control grafts (18.9%). Reduced, but not significant, epidermal necrosis was found in VEGF-treated rats vs saline-treated rats. No difference was found in VEGF-treated grafts vs saline-treated grafts when the panniculus carnosus muscle was left intact. Increased microvascular density was observed in VEGF-treated grafts vs saline-treated grafts, which did not reach statistical significance ($P = .17$).

Conclusion: Exogenously administered VEGF may improve the outcome of full-thickness skin grafts by decreasing dermal necrosis.

VASCULAR ENDOTHELIAL growth factor (VEGF) is an endothelial cell mitogen and a permeability factor involved in the complex cascade of wound inflammation and repair. Increased levels of VEGF protein have been observed at sites of soft tissue injury and have been shown to promote nutrient flow, angiogenesis, and vascular remodeling necessary for survival.\textsuperscript{1-4} Because of its role in wound repair and neovascularization, exogenous administration of VEGF and its precursors has been exploited in models of soft tissue injury to stimulate vascular growth and recovery of injured tissues.\textsuperscript{5,6} The role of VEGF has also been investigated in reconstructive surgery in which survival of transplanted tissue is dependent on the integrity of its vascular supply. This remains a challenge in reconstructive surgery and has spawned interest in molecular mechanisms in wound repair and neovascularization to increase the viability of transplanted tissues. Administration of recombinant VEGF protein to local flaps and their recipient beds has recently been shown to improve the percentage of surviving tissue.\textsuperscript{5-14} However, the effect of VEGF on skin graft viability is unknown. Full-thickness skin grafts (FTSGs) are widely used in head and neck reconstructive surgery and are essentially avascular in nature. Theoretically, FTSGs could benefit from a molecule that improves survival by facilitating revascularization and nutrient supply. This concept led us to examine the effect of VEGF on FTSGs in Sprague-Dawley rats (SDRs).

METHODS

ANIMALS

Thirty adult SDRs weighing 450 to 550 g were used in this study. Animals were approved for use by the Institutional Animal Care and Use Committee at the University of Arkansas for Medical Sciences, Little Rock. Standard living...
conditions and environmental control measures were monitored by University of Arkansas for Medical Sciences animal laboratory personnel with the use of day-night light cycling and standard rat chow. All animal procedures were performed under sterile conditions, with minimal variation in performance.

SURGERY

After overnight fasting, 18 SDRs underwent harvesting of dorsal FTSGs. Animals were anesthetized using an intraperitoneal injection of pentobarbital sodium (40 mg/kg of body weight) and were placed in a prone position on a standard surgical platform with the dorsum exposed. The dorsum was shaved, and a 3×3-cm rectangle was centrally outlined with a marker. This area was prepared and draped using standard sterile surgical technique. Perioperative antibiotics (30 mg/kg of cefazolin sodium) were administered, and an incision was made along the previously outlined mark. This was carried just below the panniculus carnosus muscle (PCM) to the deep fascia layer (Figure 1). Once the grafts were harvested, the PCM was dissected from the overlying dermis to simulate the FTSGs (Figure 2). Subfascial injections of recombinant human VEGF protein (1.2 mL of 1.5 µg/mL; R&D Systems, Minneapolis, Minn) or isotonic sodium chloride (1.2 mL) were randomly performed in the recipient beds of 12 animals (6 in each group). The recombinant human VEGF dose was equivalent to that used in previous work that has shown improved flap survival using subfascial injections.10 The dose was distributed evenly among 12 equally spaced injections of 0.1 mL using a 30-gauge needle. Six additional sham control animals underwent skin graft harvesting, but they received no subfascial injections in the recipient beds. The previously procured skin grafts were sutured onto the dorsal recipient beds using nonabsorbable material. Six tacking sutures were placed centrally to secure the grafts to the underlying fascia and to prevent seroma or hematoma formation. The dorsal arch of SDRs in the normal anatomical position also secured the grafts to the recipient beds. On postoperative day 7, SDRs were again anesthetized, and the skin grafts were removed. The rats were killed using intraperitoneal injections of pentobarbital sodium (200 mg/kg).

To determine the role of the PCM on FTSG survival, an additional group of 12 animals underwent the same procedure with the PCM left intact and were randomly divided into VEGF-treated or saline-treated groups (6 in each group). Full-thickness skin grafts were removed on postoperative day 14 in these animals to facilitate better penetration of VEGF or saline into the thicker graft.

PLANIMETRY

As previously described in rat models of soft tissue flaps, the areas of graft necrosis were identified using planimetry.9,10,13,16 Regions of pink and pliable soft skin with evidence of new hair growth were considered healthy, while regions of thickened, hard, contracted, and dark-colored tissue without new hair growth were considered necrotic for planimetric analyses (Figure 3A). Using transparency film, the entire dimension of each skin graft and its necrotic areas was outlined by a blinded observer (J.M.) by placing the film against the grafts. The grafts were turned over as the dermal surface was examined for necrosis. Dermal necrosis was identified by demarcated areas of dark-gray dermis surrounded by normal pearly white dermal skin (Figure 3B). In each graft, the locations of dermal necrosis reflected overlying unhealthy epidermis. Transparency film was again used to outline and measure these necrotic dermal areas. Following planimetric analysis, the grafts were placed in a 10% formalin solution for histologic examination and immunostaining.

The transparency films were digitally scanned; necrotic regions were measured and calculated as a percentage of the total graft area using computer software (Image Pro Plus, Silver Springs, Md). The calculation of necrotic areas for each graft surface was performed 3 times, and the mean of these measurements was recorded as the representative value. The mean percentage of necrosis was calculated for each group. Comparisons of graft necrosis between control, VEGF-treated, and saline-treated SDRs were performed for epidermal and dermal measurements. On postoperative day 14, VEGF-treated and saline-treated grafts with an intact PCM were removed and underwent planimetry only for their epidermal surfaces because dermal surface was not visible in these grafts. No attempt was made to remove this muscle layer to expose dermal surface because dermal surface was not visible in these grafts. No attempt was made to remove this muscle layer to expose dermal surface because of the concern of interfering with the outcome of histologic examination and immunostaining. All statistical analyses were performed using t tests, with significance set at P=0.05. Following planimetric analysis, the grafts were placed in a 10% formalin solution for histologic examination and immunostaining.

IMMUNOSTAINING

Immunostaining against anti-factor VIII, an endothelial cell marker, was performed on FTSGs lacking the PCM. Slides were pretreated and incubated with monoclonal antibody against human factor VIII–related antigen (Chemicon Inc, Temecula, Calif). Standard immunoperoxidase staining was subsequently performed. A board-certified pathologist (C.Y.F.) unaware of the
treatment groups measured the microvascular density. Slides were initially scanned under low-power microscopy (×100) to identify prominent vascular areas. These representative regions were examined at ×400 magnification as individual vessels were counted in 3 consecutive high-power fields. The mean vessel counts were compared between VEGF-treated vs saline-treated grafts lacking the PCM (postoperative day 7).

RESULTS

PLANIMETRY

The mean percentage of graft necrosis was significantly higher for epidermal surfaces than for their dermal counterparts in each group (P < .05) (Table 1). When epidermal necrosis was compared among groups, VEGF-treated SDRs had a lower percentage of graft necrosis compared with control and saline-treated SDRs. These differences approached but did not reach significance (P > .05) (Table 2).

When dermal surfaces were compared, no significant difference was identified between control and saline-treated SDRs. However, dermal necrosis was significantly lower in VEGF-treated grafts compared with control and saline-treated grafts (P = .02 and P = .04, respectively) (Table 2 and Figure 4).

When VEGF-treated and saline-treated grafts with an intact PCM (harvested on postoperative day 14) were compared, the difference in the percentage of epidermal necrosis was not significant (P = .49) (data not shown). Similarly, there was no significant difference in epidermal necrosis between VEGF-treated grafts with (postoperative day 14) and without (postoperative day 7) the PCM (P = .13) (data not shown). Comparison of epidermal necrosis between saline-treated grafts with (postoperative day 14) and without (postoperative day 7) the PCM also revealed no significant difference (P = .28) (data not shown).

IMMUNOSTAINING

The mean ± SD microvascular densities for VEGF-treated and saline-treated skin grafts were 13.3% ± 4.5% and 9.5% ± 1.4%, respectively. The difference was not significant (P = .17).

COMMENT

Head and neck surgery involves the removal and subsequent reconstruction of soft tissue for various reasons. This typically involves the use of myocutaneous flaps, free tissue transfers, or skin grafts. This provides better cosmesis, homology, and health of the new tissue. Despite improvements in technology and technique, failures of soft tissue transfers still occur. This has stimulated the investigation of various factors to improve flap survival. Failures are ultimately linked to tissue ischemia during the healing process. Therefore, techniques such as delayed repair, pedicled flaps, and microvascular free flaps have been used to improve vascularity and nutrient flow to transferred tissue.

Figure 3. Epidermal (A) and dermal (B) surfaces of full-thickness skin graft before planimetric analysis. Note the areas of necrosis characterized by dark skin with distinctive demarcation (arrows).

Table 1. Comparison of Epidermal and Dermal Necrosis in Each Group*

<table>
<thead>
<tr>
<th>Group</th>
<th>% of Necrosis</th>
<th>P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidermal</td>
<td>Dermal</td>
<td></td>
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<tr>
<td>Control</td>
<td>33.7 ± 15.7</td>
<td>.01</td>
</tr>
<tr>
<td>Saline treated</td>
<td>38.2 ± 21.2</td>
<td>.006</td>
</tr>
<tr>
<td>VEGF treated</td>
<td>19.7 ± 11.0</td>
<td>.04</td>
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Abbreviation: VEGF, vascular endothelial growth factor.
*Data are given as mean ± SD unless otherwise indicated.
†Tests.

Table 2. Comparison of Epidermal and Dermal Necrosis Among Groups*

<table>
<thead>
<tr>
<th>Groups</th>
<th>Epidermal Necrosis</th>
<th>Dermal Necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control vs saline treated</td>
<td>.35</td>
<td>.18</td>
</tr>
<tr>
<td>VEGF treated vs control</td>
<td>.07</td>
<td>.02</td>
</tr>
<tr>
<td>VEGF treated vs saline treated</td>
<td>.06</td>
<td>.04</td>
</tr>
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</table>

Abbreviation: VEGF, vascular endothelial growth factor.
*Data are given as P values by t tests. Statistical significance was set at P < .05. Boldfaced values indicate statistical significance.
Others have examined the role of molecular factors to stimulate new blood vessel growth. Vascular endothelial growth factor is a homodimeric 43-kilodalton protein implicated in soft tissue growth and repair. Increased levels are found in fresh wounds, granulation tissue, tumors, and chronic ulcers. By stimulating endothelial cell proliferation and migration, VEGF has been shown to affect the development and construction of new blood vessels during embryogenesis, tumor development, and soft tissue injury. The subsequent improved vascularity is thought to result in a flux of nutrients and chemicals that are important for new growth and repair.

Four splice variants of VEGF messenger RNA have been described (VEGF 121, 165, 184, and 209). The protein products of VEGF 121, 165, and 184 promote vascular permeability and angiogenesis. A high degree of protein homology exists between species (rat VEGF 164 vs human VEGF 165) that allows for the use of animal models to investigate the role of VEGF in human physiology.

Recently, exogenous administration of VEGF has been used to improve survival of local flaps in animal models of soft tissue repair. Various routes of administration have been attempted, including dermal injections, subfascial injections, application to recipient beds, vascular pedicle infusions, and adenovirus-mediated gene transfers of VEGF complementary DNA. All techniques have led to improved survival of soft tissue flaps, with most studies using ischemic models for demonstration. In particular, Kryger et al examined the effect of VEGF protein on survival of dorsal pedicled flaps in SDRs. Different routes of administration were compared, including systemic, subdermal, subfascial, and topical applications. The mean flap survival after 5 days of recovery was significantly improved in all VEGF-treated groups compared with controls. Equally distributed subfascial injections of recombinant human VEGF 165 into the recipient beds resulted in an 88% mean survival, which was significantly better than that of controls (66%).

In this preliminary study, we used subfascial administration of recombinant human VEGF 165 according to the method by Kryger et al to examine its effect on survival of FTSGs in SDRs. The mean percentage of graft necrosis was significantly greater along the epidermis than the dermal counterpart in each group, regardless of treatment to the recipient beds. In addition, less epidermal necrosis in VEGF-treated FTSGs vs control and saline-treated FTSGs was observed, but the difference was nonsignificant. Nevertheless, a dramatic reduction in dermal necrosis was found in VEGF-treated FTSGs compared with the control and saline-treated FTSGs. The findings of this study suggest that differences in skin graft ischemia may be evident along the dermal surface of FTSGs when significant external differences are not apparent. A reduction in nutrient distribution from dermal vessels may be reflected broadly in the more sensitive epidermis and may lead to larger areas of identified necrosis. Improvements in nutrient flow via VEGF may thereby reduce early dermal necrosis that is not yet apparent in the epidermis. Therefore, examining the epidermis may provide a more sensitive method for predicting future skin graft survival in animal models. Furthermore, the epidermis may be the first layer to show ischemic changes because of the increased distance from the recipient bed, as health and recovery of the graft are dependent on the underlying dermis.

These findings support observations of FTSGs in the clinical setting. Most FTSGs show some, although minute, evidence of epidermal necrosis in the early postoperative period, despite the higher final rates of graft survival. Therefore, evaluation of the epidermal surface of transplanted skin may not indicate the final outcome, as limited epidermal necrosis is repaired without sequelae in the presence of a healthy underlying dermis. This information may be useful for future studies evaluating survival of transplanted skin regardless of its vascular nature (ie, graft or flap).

In this study, we also compared the mean percentage of epidermal necrosis between VEGF-treated and saline-treated grafts with an intact PCM at 2 weeks, with the expectation that VEGF disbursement and vascular ingrowth would require more time because of the additional avascular layer of the PCM. The mean percentage of epidermal necrosis was not significantly different between VEGF-treated and saline-treated grafts with an intact PCM. Unlike grafts with the PCM removed, the difference between these grafts did not approach significance. Because of technical reasons, the dermal surface was not evaluated. However, nutrient flow may have been prevented because of the additional layer of soft tissue. This is similar to the clinical setting in which subcutaneous fat (panniculus adiposus) is left in place along the undersurface of FTSGs. Such grafts have poorer survival because of this additional layer, which is routinely removed before graft placement along the recipient beds.

Despite a reduction in dermal necrosis in VEGF-treated grafts, the difference in the microvascular density between VEGF-treated and saline-treated grafts did not achieve statistical significance. This finding is surprising considering the angiogenic effect of VEGF. However, similar published results illustrate that VEGF may not have an effect on the mean number of vessels in transplanted tissue models, despite improved survival with exposure to exogenously administered VEGF protein or VEGF complementary DNA. These authors attribute this finding to an increase in newly formed vessels within the recipient bed but not in the transplanted dermis, to increased blood flow in VEGF-exposed cap-

![Figure 4. Bar graph demonstrating significant differences by t tests in the mean percentages of dermal necrosis between vascular endothelial growth factor (VEGF)-treated and control groups (P=.02) and between VEGF-treated and saline-treated groups (P=.04).](http://www.archoto.com/06640.png)
illaries in the transplanted tissue, or to more rapid vascularization of the transplanted tissue without a change in the actual number of vessels by VEGF administration. Further research is necessary to delineate the exact mechanism of dermal improvement in skin graft and flap models.

Herein, we created a sensitive model for investigating early changes of FTSGs through the examination of their dermal surface. Although our study demonstrates a reduction of dermal necrosis in VEGF-treated grafts, it would be presumptuous to suggest that VEGF improves FTSG survival based on these results. However, a significant reduction in dermal necrosis can still be considered an improvement in graft health that can lead to faster or more complete repair of the epidermal surface. Long-term survival of FTSGs exposed to VEGF needs to be examined to answer this question.

Experiments on skin graft survival are limited because of the high percentage of graft acceptance. However, a reduction in survival of FTSGs exposed to ischemic or damaged recipient beds has been illustrated in grafts placed on irradiated tissue. Therefore, we speculate that gross improvement in graft survival after exposure to VEGF would be more dramatic in FTSGs used to repair ischemic beds that are found in patients with head and neck cancer.

CONCLUSIONS

Exogenously administered VEGF may improve FTSG survival by reducing the percentage of dermal necrosis. Ischemic changes in the epidermis may not reflect the condition of the underlying dermis for the evaluation of survival of transplanted skin in experimental models.

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REFERENCES