Topical Treatment of Pressure Ulcers with Nerve Growth Factor
A Randomized Clinical Trial

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Background: The prevalence of pressure ulcers of the foot is a major health care problem in frail elderly patients. A pressure sore dramatically increases the cost of medical and nursing care, and effective treatment has always been an essential nursing concern. Management options for pressure ulcers include local wound care; surgical repair; and, more recently, topical application of growth factors.

Objective: To examine the effects of topical treatment with nerve growth factor in patients with severe, noninfected pressure ulcers of the foot.

Design: Randomized, double-blind, placebo-controlled trial.

Setting: Teaching nursing home of Catholic University of the Sacred Heart, Italy.

Patients: 36 persons with pressure ulcers of the foot.

Intervention: 18 patients received nerve growth factor treatment, and 18 patients received only conventional topical treatment.

Pressure ulcers are one of the major causes of morbidity in older people and the most important care problem in nursing home residents (1, 2); they dramatically increase the cost of medical and nursing care (3). In particular, pressure ulcers of the foot are very common and are difficult to heal among elderly immobilized patients. Pressure ulcers at the malleolus, heel, or both develop as a result of pressure, shear, or friction concentrated on a small area over a bone prominence that lacks subcutaneous tissue. Regardless of stage, prompt treatment is essential. An untreated pressure sore may worsen and lead to cellulitis, chronic infection, or osteomyelitis. Management options for pressure ulcers include local wound care; surgical repair; and, more recently, topical application of growth factors.

Nerve growth factor is a polypeptide that is involved in a variety of biological activities in several mammalian cells, both in vitro and in vivo (4). It is the first and best-characterized member of a family of neurotrophic factors and was originally noted for its part in developing peripheral sensory and sympathetic neurons (4). Subsequent studies showed that this molecule is also critically involved in neurite outgrowth, cell survival, and functional activity of forebrain cholinergic neurons and plays a crucial role in aging and memory processes (5, 6). Nerve growth factor can promote the regeneration of injured cells that express nerve growth factor receptors, in both the peripheral and central nervous systems (5).

There are also strong indications that nerve growth factor plays an important role in wound healing. Li and colleagues (7) reported the first evidence supporting this hypothesis: the observation that removal of the submaxillary glands, tissue storing large amounts of nerve growth factor, substantially affects recovery of wound healing in mouse skin. Other studies showed that fibroblasts and epithelial cells are able to produce nerve growth factor and are receptive to its specific action (5, 8). These and other findings support the hypothesis that nerve growth factor, by stimulating growth and differentiation of epithelial-derived cells, might be clinically useful in wound healing after skin damage. This hypothesis was strengthened by recent observational studies showing that topical application of nerve growth factor speeded recovery from skin ulcer in humans (9, 10) and induced prompt healing and restoration of corneal sensitivity in patients with corneal neurotrophic ulcers (11). We evaluated the efficacy of topical application of nerve growth factor compared with conventional topical treatment in patients with severe noninfected pressure ulcers of the foot.

Methods
Study Sample
We performed a randomized, double-blind, placebo-controlled trial with 6-week follow-up. We screened all patients admitted to the teaching nursing home of Catholic University of the Sacred Heart, Fontecchio, Italy, between 1 September 2000 and 30 May 2002 for enrollment in the study. Patients were eligible if they had a pressure...
ulcer of the foot that ranged from 1 cm² to 30 cm² in total area \((n = 70)\). We excluded 6 patients who had developed the lesion more than 1 month before admission, 6 patients with terminal illnesses, 10 patients with diabetes, and 5 patients with peripheral vascular diseases. Five patients declined to participate. Therefore, 54% of the initial patients \((n = 38)\) were considered eligible for and were enrolled in the study. Subsequently, 1 patient in the treatment group died and 1 patient in the control group was lost to follow-up because of admission to the hospital for myocardial infarction. As a result, the final analysis sample consisted of 18 patients in the treatment group and 18 patients in the control group (Figure 1).

The steering committee of the Catholic University of the Sacred Heart and the institutional review board at the clinical center approved and monitored the study. All patients provided written informed consent at enrollment for prerandomization activities and at the initiation of treatment for follow-up activities and treatment. If patients were cognitively impaired, informed consent was obtained from their families.

Study Design and Treatment Protocols

Baseline assessments were done before randomization during the first 2 weeks after nursing home admission. Patients were evaluated by using the Minimum Data Set instrument for nursing homes \((12, 13)\) in its validated translation \((14)\). The Minimum Data Set is a comprehensive assessment instrument whose application is now federally mandated in U.S. nursing homes. The Minimum Data Set form includes information such as demographic characteristics, functional and cognitive status, and nursing needs relevant to care planning. Two summary scales based on Minimum Data Set items have also been designed to describe patients’ performance in terms of personal activities of daily living \((15)\) and level of cognitive function (Cognitive Performance Scale) \((16)\). The assessment also included a complete list of diagnoses and medications. The following indicators of nutritional status were also measured at admission: body mass index, serum albumin level,
serum hemoglobin level, serum lymphocyte count, and serum glucose level. To exclude peripheral vascular diseases (arterial and venous disorders) and other causes of foot ulceration, arterial and venous color Doppler ultrasonography of the legs was performed in all patients before randomization. Peripheral arterial disorders were defined as the presence of atherosclerotic plaque leading to stenosis of any grade in 1 or more arterial segments along the iliac–femoral–popliteal axis; venous disorders included venous thrombosis and varicose veins.

Ulcer size and stage were recorded at baseline and every subsequent week for 6 weeks. Outcome measures were collected by a research assistant who was not involved in the study protocol and was completely blinded to patient assignments. To provide highly accurate and precise measures of ulcers, the wound perimeter was traced onto sterile, transparent block paper and the blocks were counted (17). Ulcer stage was determined by using the Yarkony–Kirk scale (18). This classification, which uses a 6-grade scaling system, takes ulcer depth (involvement of dermis, subcutaneous fat, exposed muscle, exposed bone) into particular consideration. In a previous study, this scale appeared to have good reliability and to guarantee a more thorough assessment of pressure ulcers than other scales (18). In addition, digital photographs were taken at baseline and every week during the follow-up period.

In all patients, ulcers had been present for at least 1 week before the start of the study and were characterized by a very slow response to local treatment and limited tendency to heal. After the baseline assessment, patients were randomly stratified by age group, sex, and ulcer surface area according to a computer-generated list. Eighteen patients received nerve growth factor treatment, and 18 received only conventional topical treatment. A research assistant at the Institute of Neurobiology of the National Research Council, Rome, prepared and labeled the nerve growth factor and placebo solutions. The nursing home staff who administered pressure ulcer medication were blinded to treatment assignment.

For both groups, treatment began in the absence of

Table 1. Baseline Characteristics of 36 Patients with Foot Ulcer

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Main Disease</th>
<th>Site of Ulcer</th>
<th>Area of Ulcer</th>
<th>Stage of Ulcer*</th>
<th>Duration of Ulcer</th>
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<th>Area of Ulcer</th>
<th>Stage of Ulcer*</th>
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<td>M</td>
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<td>Heel</td>
<td>655</td>
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<td>10</td>
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</table>

* Yarkony–Kirk Scale (18). Higher numbers indicate more severe stage (range, 1 to 6).
clinical signs and symptoms of active topical infection and after 2 wound cultures performed at different parts of the ulcer showed no bacterial growth. The same preventive skin regimen (a turning and repositioning program and a pressure-relieving mattress) was initiated in all patients before randomization. Thereafter, all patients received the same daily local care: irrigation with normal saline, use of debriding enzymes, and application of opaque hydrocolloid occlusive barriers. Treatment was continued until the wound healed completely or for a maximum of 6 weeks. It is important to emphasize that this treatment duration was comparable to that in previous randomized, controlled trials assessing the effect of specific treatments for pressure ulcers (19–21).

### Treatment Group

We used 2.5S murine nerve growth factor, which was purified from submaxillary glands according to the method of Bocchini and Angeletti (22) as modified by Mobley and colleagues (23). This murine nerve growth factor is closely homologous to human nerve growth factor (24). One milligram of nerve growth factor was dissolved in 20 mL of balanced salt solution, with a final concentration of 50 μg/mL. The nerve growth factor solution was dropped daily on the lesion and allowed to dry for 2 to 3 minutes.

### Control Group

A balanced salt solution was used as a placebo and was dropped on the lesion in a manner identical to the nerve growth factor solution. The control preparation was indistinguishable from the growth factor preparation in presentation, color, density, and odor. Except for the topical application of nerve growth factor solution, the same local care procedures were used to treat the ulcers in the control group.

### Statistical Analysis

Quantitative variables are presented as mean values (±SD). Differences in baseline characteristics between patients in the control and treatment groups were analyzed in several ways. Quantitative outcomes were tested by using the Student t-test after a pretest for homogeneity of variance. The Mann–Whitney test was used for cases in which the normality assumption was not reasonable. Categorical variables were analyzed by using the Fisher exact test.

Analysis of covariance was used to compare reduction in pressure ulcer area from baseline to 6-week follow-up after adjustment for baseline ulcer area, location, and duration. Because the distribution of reduction in pressure ulcer area was not normal, this analysis was performed after natural log transformation of this variable. Statistical analyses were performed by using SPSS, version 10.0 (SPSS Inc., Chicago, Illinois).

### Role of the Funding Sources

The funding sources had no role in the collection, analysis, and interpretation of the data or in the decision to submit the manuscript for publication.

### RESULTS

We studied 36 patients who had noninfected pressure ulcers of the foot (Table 1). Skin ulceration had been present for a mean of 13 days, without substantial differences between the treatment and control groups (mean [±SD], 13 ± 4 days vs. 12 ± 5 days, respectively; P > 0.2). The location of the ulcer was similar between groups: Fourteen patients in the treatment group and 15 in the control group had ulcers at the heel, and 4 patients in the treatment group and 3 in the control group had ulcers at the lateral malleolus. At baseline, patients in the treatment and control groups did not differ across demographic vari-

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Table 2. Functional and Clinical Characteristics of Patients in the Treatment and Control Groups*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Treatment Group (n = 18)</th>
<th>Control Group (n = 18)</th>
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<tbody>
<tr>
<td>Age, y</td>
<td>80.2 ± 3.0</td>
<td>80.2 ± 4.7</td>
</tr>
<tr>
<td>Women, n (%)</td>
<td>13 (72)</td>
<td>13 (72)</td>
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<tr>
<td>Activities of daily living score†</td>
<td>3.3 ± 1.0</td>
<td>3.3 ± 0.8</td>
</tr>
<tr>
<td>Cognitive Performance Scale score†</td>
<td>1.6 ± 1.2</td>
<td>1.6 ± 1.3</td>
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<tr>
<td>Medical conditions, n</td>
<td>5.0 ± 1.1</td>
<td>4.8 ± 1.7</td>
</tr>
<tr>
<td>Medications, n</td>
<td>3.8 ± 0.9</td>
<td>4.1 ± 2.0</td>
</tr>
<tr>
<td>Nutritional status</td>
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<tr>
<td>Body mass index, kg/m²</td>
<td>24.0 ± 1.4</td>
<td>23.8 ± 1.4</td>
</tr>
<tr>
<td>Albumin level, g/L</td>
<td>33 ± 3</td>
<td>33 ± 3</td>
</tr>
<tr>
<td>Hemoglobin level, g/L</td>
<td>129 ± 10</td>
<td>130 ± 10</td>
</tr>
<tr>
<td>Lymphocyte count, ×10⁹ cells/L</td>
<td>3.073 ± 0.42</td>
<td>3.117 ± 0.36</td>
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<td>Serum glucose level, mmol/L (mg/dL)</td>
<td>4.9 ± 0.6 (87.4 ± 10.3)</td>
<td>4.8 ± 0.5 (85.8 ± 9.0)</td>
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<tr>
<td>Pressure ulcer</td>
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<tr>
<td>Area, mm²</td>
<td>1012 ± 633</td>
<td>1012 ± 655</td>
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<tr>
<td>Stage‡</td>
<td>3.2 ± 0.8</td>
<td>3.0 ± 0.7</td>
</tr>
<tr>
<td>Duration, d</td>
<td>13 ± 4</td>
<td>12 ± 5</td>
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</table>

* Values expressed with a plus/minus sign are means ± SD. P > 0.2 for all comparisons.
† Higher numbers indicate greater impairment.
‡ Yarkony–Kirk Scale (18). Higher numbers indicate more severe stage (range, 1 to 6).
ables, clinical characteristics, and functional measures (Table 2).

At baseline, 3 patients (17%) in the treatment group and 3 (17%) in the control group had stage 2 ulcers, 9 patients in the treatment group (50%) and 13 in the control group (72%) had stage 3 ulcers, 5 patients in the treatment group (28%) and 1 in the control group (5%) had stage 4 ulcers, and 1 patient in the treatment group (5%) and 1 in the control group (5%) had stage 5 ulcers (P > 0.2). Similarly, the baseline mean area (±SD) of the ulcer did not differ between the 2 groups: 1012 ± 633 mm² (range, 303 mm² to 2462 mm²) in the treatment group and 1012 ± 655 mm² (range, 105 mm² to 2934 mm²) in the control group (P > 0.2).

After 6 weeks of treatment, the mean area (±SD) of the ulcers in the treatment group was 274 ± 329 mm² compared with 526 ± 334 mm² in the control group (P = 0.022) (Figure 2). The reduction in pressure ulcer area was 738 ± 393 mm² in the treatment group and 485 ± 384 mm² in the control group (P = 0.034). Ulcer location was not statistically significantly associated with reduction in ulcer area (heel, 623 ± 431 mm², and lateral malleolus, 566 ± 285 mm²; P > 0.2). After adjustment for potential confounders, which included baseline ulcer area, location, and ulcer duration, reduction in ulcer area was statistically significantly greater in the treatment group compared with the control group (natural log of area reduction, 6.5 ± 0.3 mm² vs. 5.9 ± 0.3 mm²; P < 0.001). No statistically significant interaction was observed between treatment group and ulcer location (P > 0.2).

All of the ulcers treated with topical application of nerve growth factor showed a statistically significant acceleration of the healing process. Advancement of epithelial tissue from the margin toward the center of the ulcer was already visible 2 weeks after the beginning of treatment, and 6 weeks after the beginning of treatment the ulcer had completely healed.

The ulcers in the control group, which were treated only with conventional therapy, healed more slowly. In fact, although complete healing was observed in the treatment group in 2 patients within 3 weeks, 2 patients within 4 weeks, 1 patient within 5 weeks, and 3 patients within 6 weeks, only 1 patient in the control group healed completely within 3 weeks. Overall, complete healing was documented in 8 patients in the treatment group and 1 patient in the control group (44% vs. 6%; P = 0.009). In addition, pressure ulcers improved by 3 or more stages in 5 patients in the treatment group (28%) and no patients in the control group, by 2 stages in 9 patients in the treatment group (50%) and 2 patients in the control group (11%), and by 1 stage in 4 patients in the treatment group (22%) and 8 patients in the control group (44%) (P < 0.001). Eight patients in the control group (44%) showed no improvement. It is important to note that none of the patients had systemic or local side effects during treatment with nerve growth factor or conventional therapy.

**DISCUSSION**

The healing process is characterized by an intricate organization of cellular and molecular interactions. Inflammation and coagulation are essential preliminary processes and are followed by angiogenesis, cell replication, and epithelialization (25). The complex series of events resulting
in the repair of cutaneous wounds is modulated at least in part by several polypeptide growth promoters, such as interleukins (interleukin-1, interleukin-6, and interleukin-8), insulin-like growth factor, fibroblast growth factor, epidermal growth factor, platelet-derived growth factor, and nerve growth factor (9, 26–28). Topical treatment of wounds with these exogenous growth factors has been studied in animal models and in some human models (29–31). However, their effectiveness and clinical usefulness are still doubtful.

The results of our randomized, controlled trial indicate that topical administration of nerve growth factor is effective therapy for patients with severe pressure ulcers. The first sign of wound healing in the treatment group was evident during the second week of treatment and was characterized by the advancement of epithelial tissue from the margin toward the center of the ulcer. This finding suggests that nerve growth factor acts directly on the epithelium, in agreement with the results of previous studies showing that levels of nerve growth factor increase in wounded skin tissues and that topical application of such factors accelerates wound healing (7–10). The known effect of nerve growth factor on cells of the immune system (32), on connective tissue (fibroblast and osteoblast) (10, 33, 34), and on endothelial cells (35), as well as the widespread distribution of nerve growth factor receptors in peripheral tissues, supports this hypothesis.

The mechanisms responsible for the efficacy of nerve growth factor treatment might be related to its stimulating activity on proliferation of keratinocytes and vascular neoangiogenesis. Biologically active nerve growth factor is synthesized and released by human basal keratinocytes and plays an important role as an autocrine neurotrophic molecule at the skin level (36). Increasing evidence shows that nerve growth factor stimulates the release of several neuropeptides and growth factors that can stimulate the skin healing process (37, 38).

Nerve growth factor may act indirectly by modulating neurogenic inflammation. The neuritic sprouting of sensory nerve fibers induced by nerve growth factor is typical of wound healing (39, 40), and it has been observed in neuronal cells in vitro and in vivo in denervated skin (4, 6, 41, 42). In this respect, it could be hypothesized that proliferating keratinocytes, by secreting increasing amounts of nerve growth factor, regulate skin innervation during wound healing. Nerve growth factor may also act directly on endothelial cells and probably plays an important role in angiogenic activity. Some recent studies suggest that angiogenesis may be regulated through activation of angiogenic agents directly produced by neurons on request and that nerve growth factor indirectly stimulates this process (43, 44). It also been demonstrated that nerve growth factor stimulates the production of vascular endothelial growth factor, the most powerful mitogen for endothelial cells (45).

In conclusion, the combined observations that kerato-cytes and fibroblasts produce nerve growth factor, that epithelial cells bear nerve growth factor receptors and respond to the action of nerve growth factor, and that nerve growth factor accelerates wound healing in mouse skin (5, 7, 8, 11) are consistent with the hypothesis that nerve growth factor may have a direct action on the epithelium and is implicated in functional activity in wound healing. The results of this randomized, controlled trial indicate that topical application of nerve growth factor may be effective therapy for patients with severe, acute pressure ulcers. However, further studies are warranted to better understand the benefit of topical nerve growth factor treatment in patients with chronic skin ulcers.

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