Topical Calcipotriol for Preventive Treatment of Hypertrophic Scars

A Randomized, Double-blind, Placebo-Controlled Trial

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Objectives: To evaluate the efficacy of topical application of calcipotriol to healing wounds in preventing or reducing hypertrophic scar formation and to investigate the biochemical properties of the epidermis associated with hypertrophic scar formation.

Design: Randomized, double-blind, placebo-controlled trial using the reduction mammoplasty wound-healing model.

Setting: University Medical Center Groningen.

Patients: Thirty women who underwent bilateral reduction mammoplasty.

Interventions: For 3 months, scar segments were treated with either topical calcipotriol or placebo.

Main Outcome Measures: Three weeks, 3 months, and 12 months postoperatively, the scars were evaluated and punch biopsy samples were collected for immunohistochemical analysis.

Results: No significant difference in the prevalence of hypertrophic scars was observed between the placebo-and calcipotriol-treated scars. Only scars with activated keratinocytes 3 weeks postoperatively became hypertrophic (P = .001).

Conclusions: Topical application of calcipotriol during the first 3 months of wound healing does not affect the incidence of hypertrophic scar formation. We observed a strong association between keratinocyte activation and hypertrophic scar formation.

Trial Registration: trialregister.nl Identifier: NTR1486

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A hypertrophic scar can be considered an abnormal end point of wound healing, despite its relatively frequent occurrence.1,2 Besides an increased incidence in predisposed individuals and susceptible regions, hypertrophic scar formation seems to occur more often after delayed reepithelialization3-6 due to deep wounding7 or wound infection.8 Although it has often been described as a disorder of the dermis, histologic evaluation of hypertrophic scar tissue has also revealed epidermal abnormalities.9-12 Increased numbers of activated keratinocytes are present in young and mature hypertrophic scar tissue but not in normal scars.9,10 Activated keratinocytes express keratin 6 and keratin 16 and are normally found mainly during the proliferative phase of wound healing. They show augmented biochemical activity, such as an increased proliferation rate and increased production of cytokines, thereby intensifying their communication with other cells.13 Also, hypertrophic scar formation is associated with an increased number of epidermal Langerhans cells, which have an antigen-presenting function.12

Keratinocyte and Langerhans cell abnormalities strongly resemble epidermal alterations observed in the skin disorder psoriasis.13,14 Psoriasis can be effectively treated with topical application of calcipotriol, a synthetic derivative of vitamin D.15,16 Calcipotriol stimulates keratinocyte terminal differentiation, inhibits keratinocyte proliferation, and suppresses the number and antigen-presenting function of epidermal Langerhans cells.17,18 Moreover, it has anti-inflammatory potency.

Considering all of this, we hypothesized that topical application of calcipotriol could prevent hypertrophic scar for-
scar thickness was measured, and a punch biopsy specimen was obtained. The extremes, represent points where the scar aspect was evaluated.

horizontal inframammary scars are divided into 4 scar segments: right breast/lateral aspect, right breast/medial aspect, left breast/medial aspect, and left breast/lateral aspect. The diamonds, located 3 cm from the extremes, represent points where the scar aspect was evaluated, scar thickness was measured, and a punch biopsy specimen was obtained.

The application frequency was twice daily, with a total treatment duration of 3 months. To verify the data, participants were questioned during the last evaluation about the way the ointment had been applied. This resulted in a minor adaptation of data in 1 patient because the ointment had been applied oppositely. After the final evaluation of the last participant, the key to the exact contents of the packages was revealed and inserted into the data file.

**METHODS**

**STUDY DESIGN AND PATIENTS**

The bilateral reduction mammoplasty wound-healing model was used. In this model, the inframammary scars are divided into 4 separate scars (right breast/lateral aspect, right breast/medial aspect, left breast/medial aspect, and left breast/lateral aspect), thereby doubling the total number of scars per patient (Figure 1). This model has proved to be valuable in comparing different treatment modalities because it offers the unique opportunity to compare symmetrically located scars in the same individual. For studying the effect of treatment, the scars are cross-paired (right lateral with left medial and left lateral with right medial). This controls for the possible differences in local predisposition for hypertrophic scar formation, the site-dependent stitching preference of the surgeon, and the presence of a less-skilled surgical assistant. Treatment with calcipotriol or placebo of the paired scars was randomized. Approval of the medical ethics committee of the University Medical Center Groningen was obtained, and all of the participants gave written consent.

Women older than 18 years undergoing planned bilateral reduction mammaplasty at the University Medical Center Groningen received written information about the study before surgery. The exclusion criteria for participation were current or recent (within 6 months) use of topical corticosteroids (except for those used by dermatologists), use of immunomodulatory drugs, severe cutaneous reaction to any of the study drugs, planned pregnancy in the first year after surgery and postoperative complications (eg, hematoma and infection).

**TREATMENTS**

Ten days after surgery (after reepithelialization had been established), participants received a pair of ointment packages marked “R” and “L.” These packages were randomly filled with either calcipotriol, 50 μg/g (Daivonex in vehicle: Leo-Pharma, Ballerup, Denmark), or placebo (vehicle only). Participants and observers were unaware of the content of the ointment packages. Participants were carefully instructed to apply the contents of the package marked “R” on the right lateral and right medial scars. Accordingly, the contents of the package marked “L” had to be applied on the right lateral and left medial scars. The application frequency was twice daily, with a total treatment duration of 3 months. To verify the data, participants were questioned during the last evaluation about the way the ointment had been applied. This resulted in a minor adaptation of data in 1 patient because the ointment had been applied oppositely. After the final evaluation of the last participant, the key to the exact contents of the packages was revealed and inserted into the data file.

**EVALUATIONS**

Evaluations occurred under standardized circumstances 3 weeks, 3 months, and 12 months after surgery and were performed by the same observer (I.E.W.). During all of the evaluations, the clinical aspect of the scars was scored as normotrophic (when flat) or hypertrophic (when raised above the surrounding skin level). Scoring and measuring were performed 3 cm from the lateral and medial extremes of the scars. Because 1 patient did not participate in the 3-week evaluation, the clinical aspect of only 116 scars (29 patients) was scored during the first evaluation. All 120 scars (30 patients) were scored 3 and 12 months postoperatively.

To quantify the macroscopic properties of the scar, its thickness was measured ultrasonographically 3 and 12 months postoperatively using a 7.5-MHz probe (model SSD-680 EX/STD; Aloka Co Ltd, Tokyo, Japan). To obtain a reliable image, a gel pad (Aquaflex; Parker Laboratories Inc, Fairfield, New Jersey) was placed between the probe and the skin. The distance (in millimeters) between the echogenic stratum corneum and the subcutaneous tissue (appearing as a black circle) was measured. This method has proved to be valuable not only in the morphologic assessment of the skin but also in distinguishing normal from hypertrophic scars. The ultrasonographic data of 118 of the 120 scar segments 3 months postoperatively and of 99 scar segments 12 months postoperatively were suitable for analysis, as the most mature normotrophic scars and the surrounding skin were indistinguishable.

During all of the evaluations, 3-mm punch biopsies were collected consecutively 5, 4, and 3 cm from the lateral confines of both lateral scars after local anesthesia with 5 mL of lidocaine hydrochloride (10 mg/mL) combined with epinephrine (0.01 mg/mL). After collection, all of the samples were snap frozen in liquid nitrogen and stored at −80°C until further processing.

**IMMUNOHISTOCHEMICAL ANALYSIS**

Before immunohistochemical staining, the frozen biopsy samples were further processed into 3-μm cryostat sections and were embedded on glass slides. Monoclonal antibodies were used for staining the sections: anti–Ki-67 (MIB-1; Dako Denmark A/S, Glostrup, Denmark), anti–cytokeratin 16 (K8.12; Sigma-Aldrich Corp, St Louis, Missouri), and anti-CD1a (NA 1/34; Dako). Staining of the sections involved a 3-step immunoperoxidase technique. After drying at room temperature for at least 12 hours, fixation in acetone (10-15 minutes), and washing in a phosphate-buffered saline solution (pH 7.4; 5 minutes), sections were first incubated with the proper dilution of antibody in a phosphate-buffered saline solution (1 hour). The second step involved incubation with 1% peroxidase-conjugated rabbit anti-mouse immunoglobulin antiserum. In the third step, sections were incubated with 1% peroxidase-conjugated goat anti-rabbit immunoglobulin antiserum. Both antisera were supplemented with 1% human AB serum. Sections were washed in a phosphate-buffered saline solution for 5 minutes after every step. Sections were developed with 3-amino-9-ethylcarbazole (10 mg/50 mL) and hydrogen peroxide (0.03%) as the chromogenic substrate for the peroxidase reaction. Finally, the sections were counterstained with Mayer hematoxylin. The Ki-67 sections were counterstained strongly, making differentiation between positive and negative cells possible.
HISTOLOGIC ANALYSIS

The cryosections were independently and randomly evaluated by 2 observers (W.M.V. and X.E.J.) using 100- and 200-fold magnification (Figure 2). The scar epidermis, characterized by the absence or minimal presence of rete ridges, was evaluated. The epidermal layers (acanthosis) were counted in the hematoxylin-eosin–stained sections (Figure 2A). The percentage of proliferating basal keratinocytes was evaluated in anti–Ki-67–stained sections by counting the total number of basal keratinocytes (hematoxylin stained) and the number of Ki-67–positive basal keratinocytes (Figure 2B). The presence or absence of activated keratinocytes was evaluated in anti–cytokeratin 16–stained sections by scoring suprabasal staining as absent or present (Figure 2C). The percentage of epidermal Langerhans cells was calculated by means of computer-assisted microscopic evaluation using NIS-Elements AR version 2.3 (Nikon Inc, Melville, New York). After a representative part of the epidermis was outlined, running from basal membrane to stratum corneum, the surface area of CD1a-positive tissue in the selection was expressed as a percentage of the total area in the selection (Figure 2D).

RESULTS

STUDY GROUP

During the enrollment period, 103 patients met the inclusion criteria. Of these patients, 69 refused to participate, mainly because of aversion to undergo tissue specimen collection. Consequently, 34 women enrolled in the study. Four participants discontinued treatment owing to local adverse effects (skin irritation in 3 participants) or psychological discomfort (1 participant). The remaining 30 participants completed follow-up. The mean age at enrollment was 35.1 years (age range, 18-61 years). Table 1 provides the patient characteristics. Of the 30 patients, 27 (90%) developed at least 1 hypertrophic scar during follow-up. Table 2 gives the number of hypertrophic scars in patients 3 and 12 months postoperatively.

CLINICAL ANALYSIS

Because almost all of the scars were normotrophic after 3 weeks, these scars were omitted during analysis of the clinical evaluation. After 3 months, 119 scars were suitable for...
HISTOLOGIC ANALYSIS

To analyze the biochemical effects of calcipotriol on scar formation, the scar cryosections were analyzed and compared for histologic differences. After 3 weeks, a significantly higher mean (SD) percentage of proliferating basal keratinocytes (31.1% [21.3%] vs 22.5% [15.1%]; \( P = .03 \), Wilcoxon signed rank test) and mean (SD) number of epidermal layers (13.8 [4.3] vs 11.5 [4.1]; \( P = .02 \), Wilcoxon signed rank test) was noted in the CT group compared with the PT group. After 3 months, these differences were no longer present. The percentage of proliferating basal keratinocytes correlated well with the number of epidermal layers at 3 weeks (0.273; \( P = .04 \), Spearman nonparametric correlation coefficient) and 3 months (0.278; \( P = .04 \), Spearman nonparametric correlation coefficient). Also, there seemed to be a (nonsignificant) decrease in the mean (SD) percentage of epidermal Langerhans cells in the CT group vs the PT group (12.4 [6.7] vs 16.1 [8.4]; \( P = .06 \), Wilcoxon signed rank test). No significant difference between the percentage of epidermal Langerhans cells (\( P = .53 \), Wilcoxon signed rank test) and the presence of activated keratinocytes (\( P = .08 \), Pearson \( \chi^2 \) test) was found. In 12-month-old scars, no further immunohistochemical differences between the 2 groups could be ascertained.

Histologic characteristics were analyzed further based on scar aspect after 3 months (normotrophic vs hypertrophic). The normotrophic group consisted of 37 scars (19 right and 18 left), and the hypertrophic group consisted of 19 scars (9 right and 10 left).

A statistically significant association was noted between the presence of activated keratinocytes in 3-week-old scars and scar outcome. Nineteen of the 3-week-old scars with activated keratinocytes (n=40) became hypertrophic 3 months postoperatively, whereas none of the 3-week-old scars without activated keratinocytes (n=16) became hypertrophic (\( P = .001 \), Pearson \( \chi^2 \) test) (Table 4). Twelve months postoperatively, 10 of the 19 scars with activated keratinocytes were still hypertrophic (\( P = .30 \), Pearson \( \chi^2 \) test). Scars with activated keratinocytes contained a higher mean (SD) percentage of proliferating basal keratinocytes compared with scars without activated keratinocytes after 3 weeks (29.3% [19.3%] vs 20.5% [16.5%]; \( P = .08 \), Mann-Whitney test) and 3 months (25.1% [19.7%] vs 14.7% [10.2%]; \( P = .03 \), Mann-Whitney test). In scars with activated keratinocytes 3 weeks postoperatively, the mean (SD) percentage of proliferating basal keratinocytes decreased considerably after 3 months (from 29.6% [19.4%] to 18.3% [17.2%]; \( P = .001 \), Wilcoxon signed rank test), which was not seen in the scars without activated keratinocytes after 3 weeks (from 21.1% [16.9%] to 19.9% [9.5%]; \( P = .73 \), Wilcoxon). In addition, 3-week-old scars with activated keratinocytes exhibited a higher mean (SD) number of epidermal layers after 3 months compared with 3-week-old scars without keratinocyte activation (10.6 [2.2] vs 13.5 [4.3]; \( P = .008 \), Mann-Whitney test). This increase in mean (SD) epidermal layers was also present in normotrophic scars with activated keratinocytes after 3 weeks (3 weeks: 11.3 [4.0] vs 14.1 [4.3]; \( P = .08 \), Mann-Whitney test).
and proliferate at higher rates. The activated state typically shows augmented production of cytokines, are migratory, and biochemical activity because these keratinocytes activated phenotype is characterized by increased biological factor family, CXCL8 (formerly interleukin 8), and interleukin 1, tumor necrosis factor, and other proinflammatory cytokines derived from macrophages most likely keep the keratinocytes in the etiology of hypertrophic scar formation because it seems that a prolonged inflammatory phase is an important prerequisite. During this prolonged inflammatory phase, relatively high concentrations of interleukin-1, from immune cells in the wound bed, are released by keratinocytes, which activates fibroblasts and adjacent keratinocytes and attracts endothelial cells and lymphocytes to the injured area. The activation of cytokines derived from macrophages most likely keep the keratinocytes to remain activated is not completely clear, but because the activated phenotype is generally present during the inflammatory phase of wound healing, it shows hypertrophy (Table 4). This prolonged activated epidermis is associated with reduced interleukin 1α and increased profibrotic platelet-derived growth factor expression compared with normal scar tissue, which may directly affect fibroblast collagen production. What causes the keratinocytes to remain activated is not completely clear, but because the activated phenotype is generally present only during the inflammatory phase of wound healing and because increased persistence of epidermal Langerhans cells has been observed in hypertrophic scars, it seems that a prolonged inflammatory phase is an important prerequisite. During this prolonged inflammatory phase, relatively high concentrations of interleukin 1α and tumor necrosis factor, and other proinflammatory cytokines derived from macrophages most likely keep the keratinocytes activated, whereas fibroblasts remain stimulated to produce extracellular matrix.

The present study was designed to evaluate the efficacy of topical application of calcipotriol to healing wounds in preventing or reducing hypertrophic scar formation and to investigate the biochemical properties of the epidermis associated with hypertrophic scar formation. Thirty healthy women who had undergone bilateral reduction mammoplasty topically administered either placebo or calcipotriol on paired segments of inframammary scars. Several biochemical observations point to involvement of the epidermis in the etiology of hypertrophic scar formation and, more specifically, to keratinocyte activation. However, topical application of calcipotriol during the first 3 months of wound healing did not reduce scar thickness or the incidence of hypertrophic scar formation.

Deep wounding and delayed reepithelialization are profound risk factors for scar hypertrophy. In response to skin injury, keratinocytes become activated, which is essential for reepithelialization. This process is initiated by prestored interleukin 1α released by keratinocytes, which activates fibroblasts and adjacent keratinocytes and attracts endothelial cells and lymphocytes to the injured area. Fibroblasts, in turn, secrete fibroblast growth factor 7 and granulocyte-macrophage colony-stimulating factor to further activate basal and suprabasal keratinocytes. The activation is sustained by other growth factors, such as tumor necrosis factor, members of the epidermal growth factor family, CXCL8 (formerly interleukin 8), and interferon γ from immune cells in the wound bed. The activated phenotype is characterized by increased biological and biochemical activity because these keratinocytes show augmented production of cytokines, are migratory, and proliferate at higher rates. The activated state typically disappears when the wound is reepithelialized, but in young and mature hypertrophic scar tissue, keratinocytes remain activated.

The present results show that even before scar hypertrophy is clinically present, activated keratinocytes in 3-week-old scars are significantly associated with hypertrophic scar formation. None of the 3-week-old scars without activated keratinocytes became hypertrophic, whereas 48% of the 3-week-old scars with activated keratinocytes showed hypertrophy (Table 4). This prolonged activated epidermis is associated with reduced interleukin 1α and increased profibrotic platelet-derived growth factor expression compared with normal scar tissue, which may directly affect fibroblast collagen production. What causes the keratinocytes to remain activated is not completely clear, but because the activated phenotype is generally present only during the inflammatory phase of wound healing and because increased persistence of epidermal Langerhans cells has been observed in hypertrophic scars, it seems that a prolonged inflammatory phase is an important prerequisite. During this prolonged inflammatory phase, relatively high concentrations of interleukin 1α, tumor necrosis factor, and other proinflammatory cytokines derived from macrophages most likely keep the keratinocytes activated, whereas fibroblasts remain stimulated to produce extracellular matrix.

Transforming growth factor β is an important etiologic factor of hypertrophic scar formation because it

Table 3. Prevalence of Hypertrophic Scar Formation per Segment 3 and 12 Months Postoperatively

<table>
<thead>
<tr>
<th>Scar Segment</th>
<th>Calcipotriol-Treated Group (n=60)</th>
<th>Placebo Group (n=59)</th>
<th>Calcipotriol-Treated Group (n=59)</th>
<th>Placebo Group (n=59)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RL</td>
<td>5 (8)</td>
<td>5 (8)</td>
<td>3 (5)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>RM</td>
<td>12 (20)</td>
<td>7 (12)</td>
<td>3 (5)</td>
<td>6 (10)</td>
</tr>
<tr>
<td>LM</td>
<td>7 (12)</td>
<td>9 (15)</td>
<td>4 (7)</td>
<td>4 (7)</td>
</tr>
<tr>
<td>LL</td>
<td>8 (13)</td>
<td>4 (7)</td>
<td>3 (5)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Total</td>
<td>32 (53)</td>
<td>25 (42)</td>
<td>14 (22)</td>
<td>13 (22)</td>
</tr>
</tbody>
</table>

Abbreviations: RL, right breast/lateral aspect; RM, right breast/medial aspect; LL, left breast/lateral aspect; LM, left breast/medial aspect.

Table 4. Associations Among Histologic Characteristics After 3 Weeks and Scar Aspect After 3 Months

<table>
<thead>
<tr>
<th>Histologic Characteristic</th>
<th>Scar Aspect 3 mo Postoperatively (n=56)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normotrophic (n=37)</td>
<td>Hypertrophic (n=19)</td>
</tr>
<tr>
<td>Epidermal layers, mean (SD)</td>
<td>12.8 (4.3)</td>
<td>12.2 (4.4)</td>
</tr>
<tr>
<td>Proliferating basal keratinocytes, mean (SD), %</td>
<td>24.6 (17.0)</td>
<td>31.0 (21.7)</td>
</tr>
<tr>
<td>Epidermal Langerhans cells, mean (SD), %</td>
<td>16.0 (8.6)</td>
<td>13.6 (7.9)</td>
</tr>
<tr>
<td>Presence of activated keratinocytes, No. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>16 (43)</td>
<td>0</td>
</tr>
<tr>
<td>Present</td>
<td>21 (57)</td>
<td>19 (100)</td>
</tr>
</tbody>
</table>

aMann-Whitney test.
bPearson χ² test.
stimulates infiltration of inflammatory cells and fibroblasts and induces fibroblast proliferation, angiogenesis, and synthesis of extracellular matrix, especially early during wound healing. However, it is also known to terminate keratinocyte activation. The significance of its effects on keratinocytes is not completely clear. It may be unrelated to its profibrotic effects or depend on concentration and the phase of wound healing in which transforming growth factor β appears.

When hypertrophic scars emerge, they show psoriasiform features, such as increased acanthosis, higher keratinocyte proliferation, and abnormal keratinocyte differentiation compared with normal scars. Calcipotriol is widely used for topical treatment of psoriasis because it has immunomodulatory effects, decreases keratinocyte proliferation, and induces keratinocyte differentiation. Calcipotriol binds to an intranuclear vitamin D receptor, which is expressed in keratinocytes. There it exerts its effects by modification of the expression of target genes. However, in contrast with its effects in psoriatic lesions, in the present study, calcipotriol significantly increased proliferation of basal keratinocytes and the number of epidermal layers compared with placebo 3 weeks postoperatively. This unexpected opposite effect may have been caused by the way keratinocytes are activated. As stated previously herein, in abnormal wound healing, the stimuli that remain activated mainly derive from persisting immune cells in the wound bed. In psoriasis, on the other hand, it has recently been shown that besides the stimuli from T lymphocytes, the keratinocytes themselves are genetically different from normal keratinocytes. Calcipotriol has its effect on the cell itself, which can explain why it has no therapeutic effect on hypertrophic scar keratinocytes in this study. Another explanation may be the biochemical inactivity of calcipotriol on healing skin, the ineffectiveness of the chosen drug provision method (topical), or skin irritation, which was also found in 3 patients in this study who were forced to stop treatment. Although an opposite drug effect was observed, it did not result in a significantly higher incidence of hypertrophic scar formation.

In conclusion, topical application of calcipotriol did not prevent hypertrophic scar formation. In contrast with its effects in psoriatic lesions, calcipotriol increased the proliferation of keratinocytes and the number of epidermal layers. In this study, the results suggest an association between the duration of keratinocyte activation after injury and hypertrophic scar formation. These findings contribute to the concept of dermal and epidermal involvement in the etiology of hypertrophic scar formation. Further research is needed to identify the processes responsible for the development of these epidermal abnormalities more precisely.

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Correspondence: Frank B. Niessen, MD, PhD, Department of Plastic and Reconstructive Surgery, VU University Medical Center, PO Box 7057, 1007 MB Amsterdam, the Netherlands (fr.niessen@vumc.nl). Author Contributions: Mr van der Veer and Drs Jacobs and Niessen had full access to all the data in the study and take responsibility for the accuracy of the data and the accuracy of the data analysis. Study concept and design: van der Veer, Jacobs, Ulrich, and Niessen. Acquisition of data: van der Veer, Jacobs, and Waardenburg. Analysis and interpretation of data: van der Veer, Jacobs, Waardenburg, and Niessen. Drafting of the manuscript: van der Veer, Jacobs, Waardenburg, and Niessen. Critical revision of the manuscript for important intellectual content: Waardenburg, Ulrich, and Niessen. Statistical analysis: van der Veer, Jacobs, and Niessen. Obtained funding: Niessen. Administrative, technical, and material support: Jacobs. Supervision: Niessen.

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REFERENCES


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