Effect of Mederma on Hypertrophic Scarring in the Rabbit Ear Model

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Currently accepted conservative treatments of hypertrophic scars are limited to steroid injections, radiation therapy, and silicone occlusive therapy. However, the use of Mederma for these problematic lesions has become quite prevalent in the clinical setting. Little scientific evidence exists to support the efficacy of this product in reducing hypertrophic scars. The aim of this study was to study the effects of Mederma on hypertrophic scars in the rabbit hypertrophic scar model, allowing the histologic quantification of scar elevation, dermal collagen organization, vascularity, and inflammation and the gross examination of scar erythema. Full-thickness wounds down to cartilage, four per ear, were created in four New Zealand White rabbits, for a total of 32 scars. Twenty-eight days after the initial wounding, the hypertrophic scars were photographed, and treatment of half of the scars on each ear was begun with Mederma three times per day for a total of 4 weeks. The untreated scars served as control scars and were left exposed to air. After 4 weeks of treatment, the scars were once again photographed. The rabbits were then killed, and the scars were analyzed histologically. The pretreatment and posttreatment photographs were compared by using computer quantification of magenta, yellow, and cyan expression within the scars.

Histologic analysis demonstrated no significant reduction in scar hypertrophy or scar elevation index. However, a significant improvement in dermal collagen organization was noted on comparing Mederma-treated scars with untreated control scars (*p* < 0.05). No significant difference in dermal vascularity or inflammation was noted. Computer analysis of the scar photographs demonstrated no significant reduction in scar erythema with Mederma treatment. The active product in Mederma, allium cepa, has as its derivative quercetin, a bioflavonoid noted for its antiproliferative effects on both normal and malignant cells, and its antihistamine release effects. These properties could theoretically prove beneficial in reversing the inflammatory and proliferative responses noted in hypertrophic scars. Despite the authors’ inability to demonstrate a reduction in scar hypertrophy, the improvement in collagen organization noted in the Mederma-treated scars suggests it may have an effect on the pathophysiology of hypertrophic scar formation. (Plast. Reconstr. Surg. 110: 177, 2002.)

Hypertrophic scarring typically occurs as a result of a full-thickness injury to the dermis with an abnormal healing response. The development of this abnormal pattern of healing has been associated with an extended period between wounding and reepithelialization of the wound, resulting in a prolonged inflammatory phase. This may occur as a result of complications such as an infection, a foreign body within the wound, excessive tension on the wound, or persistent mobilization of the wound edges. Hypertrophic scarring may be observed in some cases, the pathogenesis of hypertrophic scarring is unknown.

Currently accepted and widely used conservative treatments of hypertrophic scarring are limited to steroid injections, radiation therapy, and topical silicone gel application. Although effective, all three of these treatments are cumbersome in some way to the patient. Steroid injections and radiation therapy require multiple office visits and can be uncomfortable. Topical silicone therapy requires the patient to keep the scar covered at all times. A treatment that is easy to apply, painless, and effective would certainly be preferred by both the patient and physician attempting to diminish hypertrophic scarring.

A number of skin-care products are produced annually with claims of diminishing skin flaws of aging, solar damage, or scarring from physical injury. Mederma Skin Care is a topical gel formulated to reportedly help scars appear softer and smoother. Since its arrival on the skin-care market, the number of patients we encounter who are using Mederma for scars is significant. The product can be obtained over
the counter and applied by the patient; it is painless, and once dry, it is not messy. It is our assessment that the public is willing to trial the product on the distributor’s claim that it is effective in reducing hypertrophic scarring.

Mederma’s active ingredient is allium cepa, an onion extract. Quercetin, a derivative of allium cepa, has been studied extensively and seems to have an antiinflammatory effect by stabilizing mast cell membranes and an antiproliferative effect in both normal and malignant cells of various types. The significance of these cellular effects of quercetin may be pertinent to the treatment of hypertrophic scars. Studies have not only associated increased histamine release with an increased production of collagen by fibroblasts but have also recognized higher histamine levels within keloid and hypertrophic scar tissues as compared with normal tissues. The antiproliferative effects of quercetin probably play less of a role in the treatment of hypertrophic scars. Fibroblasts, the key players in the accelerated dermal proliferative response of hypertrophic scarring, have been found to have normal proliferation rates when compared with normal skin fibroblasts. The excessive tissue formation is a result of increased fibroblast activity with a subsequent increase in extracellular matrix production as opposed to increased fibroblast proliferation rates. Assuming the product in Mederma has similar cellular activities as quercetin, one might speculate that treatment of hypertrophic scars with Mederma could result in decreased histamine levels, decreased inflammation, decreased collagen production, and subsequently, a less hypertrophic scar.

The increasing use of Mederma in our clinical population prompted us to study this product in a controlled manner by using an animal model of hypertrophic scarring. Our laboratory has been successful in consistently reproducing hypertrophic scars in the rabbit ear, as described in a prior publication. Previous studies from our laboratory have demonstrated the efficacy of steroid injections and silicone sheeting in this model. The model also demonstrates the age-dependent changes of hypertrophic scarring noted in human hypertrophic scars. In this study, Mederma was applied to hypertrophic scars on rabbit ears for 28 days. Histologic analysis was used to determine whether the product had an effect on scar hypertrophy (represented as the scar elevation index), dermal collagen organization, vascularity, or inflammation. Photographic digital color analysis was used to identify any changes in scar erythema between Mederma-treated scars and untreated control scars.

**MATERIALS AND METHODS**

**Hypertrophic Scar Model and Treatments**

Hypertrophic scars were created in four young adult female New Zealand White rabbits as previously described. The animals were kept under standard conditions in the Northwestern University Animal Care Center. The animals were anesthetized with ketamine (60 mg/kg) and xylazine (5 mg/kg), and four wounds were created down to bare cartilage on the ventral surface of each ear by using a 7-mm biopsy punch. A dissecting microscope was used to ensure removal of the epidermis, dermis, and perichondrium in each wound. It is the removal of the perichondrial layer and subsequent delay in reepithelialization of the 7-mm defect that results in hypertrophic scar formation in this model. Hemostasis was then obtained by applying pressure, and each wound was individually covered by using a Tegaderm dressing (3M, St. Paul, Minn.). The wounds were kept covered until postoperative day 12 or until the entire wound appeared reepithelialized on gross examination.

On postoperative day 28, the rabbits were sedated, and the scars were photographed with a Kodak Color Control Patch (Eastman Kodak Company, Rochester, N.Y.). After the photographs were obtained, treatment of two of four scars per rabbit ear was begun with Mederma. The remaining two scars per ear remained untreated and exposed to air to serve as control scars. The treated scars were treated three times per day for a total of 4 weeks. We treated these fresh scars for 4 weeks on the basis of the claim by the distributors of Mederma that scar softening and a decrease in prominence should be evident by 4 weeks. The product was applied in generous amounts and rubbed gently into the scar area. On postoperative day 56, after 4 weeks of Mederma treatment, the animals were sedated, and the wounds were photographed by using the same aperture, shutter speed, and light source as was used on postoperative day 28 before the initiation of Mederma treatment. The Kodak Color Control Patch was again included in the photographs to control for color changes. The animals were then killed, and the scars were harvested.
Tissue Preparation

On postoperative day 56, the rabbits were killed, and the scars were harvested. The scars were bisected through the point of maximum height of the hypertrophic scar on palpation. A 0.5-cm margin of surrounding unwounded tissue was harvested with each wound. One-half of each wound was fixed in 4% neutral-buffered formaldehyde, dehydrated, embedded in paraffin, cut in 4-μm sections, and stained with Masson’s trichrome stain. Four-micrometer sections were taken from the bisected free edge, ensuring analysis of the center-most or highest portion of the scar.

Quantification Methods

Photographs. Kodak Ektachrome 35-mm slides of Mederma-treated scars and untreated control scars from postoperative days 28 and 56 were scanned into a Kodak Sprint slide scanner and imported into Adobe Photoshop 4.0 (Mountain View, Calif.). Once digitized, the area of the scar was highlighted and values were assigned, indicating the quantity of magenta, yellow, and cyan measured in both treated scars and control scars, using Adobe Photoshop 4.0. All values were then normalized to the color magenta on the Kodak Color Control Patch included in each photograph. To demonstrate the change in scar erythema over the 28-day treatment period, scars from postoperative day 56 were expressed as a percentage of change from their baseline expression of normalized magenta, yellow, and cyan values on day 28. Mederma-treated scar color values of magenta, yellow, and cyan were then compared with untreated control scar color values. Comparisons between the two groups were made using Student’s t test. Statistical significance was accepted at p < 0.05. All data were compiled and analyzed by using Microsoft Excel 97 (Seattle, Wash.) and Primer of Biostatistics Version 4.0 (Los Angeles, Calif.).

Histologic analysis. Using light microscopy to examine the trichrome-stained tissue sections, the degree of hypertrophy within each scar was quantitatively measured and expressed as the scar elevation index, as described previously by this laboratory. Briefly, as the wounds were initially created, the cartilage was gently nicked with a punch biopsy to allow later identification of the most lateral junction of scar edge and normal nonscarred tissue. On harvesting of the scars, they were bisected in the center at the height of the hypertrophic scar, with a 0.5-cm rim of normal nonscarred tissue around the scar. The total area of new scar contained within the nicks in the cartilage was quantified by using a calibrated lens square reticule composed of 100 small squares in a light microscope (Nikon Inc., Melville, N.Y.). In addition, the thickness of adjacent unscarred dermis, or that lying outside the nicks in the cartilage, was measured. The index represents the ratio of total scar area to the estimated area of normal tissue had the new tissue been the same height as the sur-
rounding nonscarred dermis (Fig. 1). An index of 1 indicates that the wound healed essentially flat, with no scar hypertrophy. An index of 1.5 indicates the scar thickness or hypertrophy was 50 percent of the normal nonscarred dermal thickness, and an index of 2 indicates a wound that healed with a 100-percent increase in normal tissue dermal thickness. In this study, the scar elevation index of each scar was measured twice by a blinded examiner using a calibrated eyepiece reticule. The index values of the two readings were then av-

Fig. 2. (Above, left) No difference in scar elevation index was noted in Mederma-treated scars as compared with nontreated control scars. A score of 1.0 indicates no scar hypertrophy. (Above, right) No significant difference in number of vessels per high-power field was noted between Mederma-treated and nontreated control scars. (Center, left) No significant difference in the number of inflammatory cells per high-power field was noted between Mederma-treated and nontreated control scars. (Center, right) No significant difference was noted in scar dermal vascularity when comparing Mederma-treated with nontreated control scars. The higher value indicates a higher degree of dermal vascularity. (Below, left) Mederma-treated and nontreated control scars demonstrated a similar degree of dermal inflammation. A higher value indicates more disorganization of the collagen fibrils within the scar dermis. For all plates, T bars indicate SD (n = 16 for each group).
eraged, and the Mederma values \( n = 16 \) were compared with the nontreated control values \( n = 16 \) by means of Student’s t test.

Dermal changes of vascularity and inflammation within the scars were measured quantitatively. The number of vessels, both transverse and longitudinal orientation, were counted within a fixed grid area using a calibrated lens square reticule under 20× magnification. Each scar was counted in three different areas, and the three values were then averaged. The Mederma-treated scar values were then compared with the nontreated control values by using Student’s t test. Similarly, the number of inflammatory cells identified within a fixed grid area using a calibrated lens square reticule under 20× magnification was recorded at three different sites within the hypertrophic scar area, and these values were averaged. The number of inflammatory cells in the Mederma-treated scars were then compared with the number of cells in the nontreated control scars by using Student’s t test.

The dermis was further examined for vascularity, inflammation, and collagen organization in a semiquantitative manner. This semiquantitative assessment consisted of a visual rating, using a light microscope, on a scale of 1 to 4 of the hypertrophic scar dermal area of each trichrome slide, with the higher value indicating a more disorganized collagen, greater vascularity, or more inflammation within the scar dermal layer. These parameters were assessed twice at two different time points by a blinded observer. The values were averaged, and comparisons were made between the two treatment groups by using the Mann Whitney U test. Statistical significance was accepted at \( p < 0.05 \). All data were consolidated and analyzed using Microsoft Excel 97 and Primer of Biostatistics Version 4.0.

**RESULTS**

Color analysis of scar photographs revealed no significant difference in scar erythema between scars treated with Mederma for 28 days and untreated control scars. Analysis of scar hypertrophy revealed no significant difference in measured scar elevation index or scar hypertrophy between scars treated with Mederma and untreated control scars (Fig. 2, *above, left*).

Quantitative histologic analysis of dermal vascularity revealed no significant difference in the number of vessels or inflammatory cells counted per high-power field in Mederma-treated scars as compared with nontreated control scars (Fig. 2, *above, right*, and *center, left*). In accordance with this data, semiquantitative analysis of scar dermal vascularity and inflammation again did not demonstrate any differences between the Mederma-treated scars and untreated control scars (Fig. 2, *center, right*, and *below, left*). However, semiquantitative analysis of collagen organization within the scar dermal layer did demonstrate significantly more organized dermal collagen in Mederma-treated scars as compared with untreated control scars \( (p < 0.05) \) (Fig. 2, *below, right*).

**DISCUSSION**

The quest to identify conservative methods that successfully treat hypertrophic scars is ongoing. The invasive treatment methods such as intrallesional corticosteroid injections, surgical excision, and even resurfacing the scar site with skin grafts are all associated with recurrence rates of 50 percent or greater over an extended period of time.\(^ {14–16} \) These recurrence rates have been reduced some when these techniques are paired with conservative treatments such as pressure or silicone gel, but long-term success rates are still moderate at best.

The problematic nature of hypertrophic scarring, combined with the lack of a consistently successful treatment regimen, has led to much research into the biological nature of scar formation. Our laboratory’s research in this field has resulted in the development of an animal hypertrophic scar model. The rabbit ear hypertrophic scar model has enabled us to evaluate various agents and their effects on hypertrophic scars in a controlled manner by allowing us to quantify changes in scar height and scar histology induced by these agents. With eight scars per rabbit, four on each ear, treated scars can be directly compared with nontreated or placebo-treated scars within the same animal. Previously published data from our laboratory using this model has been successful in demonstrating a reduction in hypertrophic scarring in response to steroid injections, silicone occlusion, and aging.\(^ {11–13} \) We think this is an ideal model in which to test products with questionable efficacy on hypertrophic scars, possibly revealing benefits of certain products.\(^ {17} \)

In this study, we used our rabbit ear hypertrophic scar model to evaluate the effects of Mederma on hypertrophic scarring. To date, one double-blinded study exists in the litera-
ture analyzing the effects of Mederma clinically. Clarke et al. identified a significant improvement in scar softness in the Mederma-treated group (n = 52) as compared with the placebo-treated group (n = 45), as perceived by the patients after 2 months of treatment. However, the blinded plastic surgeon’s photographic evaluation of scar size and scar appearance revealed no significant difference between Mederma-treated and placebo-treated scars. As with clinical study by Clarke et al., we did not detect a significant difference in scar height or scar erythema with the use of Mederma. We did, however, identify increased collagen organization in the scar dermis of Mederma-treated scars as compared with nontreated control scars (p < 0.05). Whether an increase in mature, organized collagen is associated with a clinically softer scar, as identified by Clarke et al., has yet to be determined.

The active product in Mederma, allium cepa, is derived from a specific type of onion: Allium cepa Linn. The principal constituent of Allium cepa is quercetin, a bioflavonoid noted for its antiproliferative effects on both normal and malignant cells and for its antihistamine release effects. The antihistamine effects of the quercetin product, presumed to be present in Mederma, may play a role in down-regulating the overproduction of collagen by fibroblasts. Studies have identified increased amounts of histamine in keloid and hypertrophic scar tissues and increased production of collagen by fibroblasts in response to histamine. It seems logical that a product that blocks histamine release could perhaps normalize or at least decrease collagen production by hypertrophic scar fibroblasts, subsequently resulting in reduced dermal scar volume and relative normalization of the scar maturation process. In addition, a decrease in scar inflammation and erythema would also be expected from a product with true antihistamine effects.

Our finding of more mature, organized collagen in Mederma-treated scars indicates a transformation from hypertrophic scars, noted for their high immature collagen content, to more mature scars. With no simultaneous decrease in scar height, this finding is suggestive of two possibilities. Either the conversion of immature to mature collagen is accelerated in some fashion with no net decrease in overall collagen production by fibroblasts, or the treatment period of 4 weeks was too brief to detect a significant decrease in scar hypertrophy in this study.

In summary, our finding of modest but significantly greater collagen organization in Mederma-treated scars hints at this product’s ability to manipulate the scarring process in some fashion. Clinically, however, its antiscarring efficacy is limited by its inability to produce a clinically significant reduction in hypertrophic scar volume as demonstrated in the rabbit ear hypertrophic scar model after 4 weeks of treatment. In previous studies on this model with aged animals, steroid treatments, and topical silicone gel, a decrease in hypertrophic scar was seen as the primary marker of scar improvement. We were also unable to demonstrate any improvement in scar erythema with this topical scar treatment product.

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