Profilometric and Morphometric Response of Murine Skin to Cosmeceutical Agents

Tapan K. Bhattacharyya, PhD; Jeannie Linton, MD; Lily Mei, MD; J. Regan Thomas, MD

Objective: To investigate whether topical antiaging compounds can reduce wrinkle depth as noted at replica profilometry with comparable changes in histologic findings in hairless mice.

Methods: Commercial retinoic acid cream, a peptide lotion, and a soy cream were applied to the dorsal skin for 4 weeks. Silicone-negative replicas of treated and untreated skin surface were photographed and evaluated for traditional features of surface roughness. Skin samples were processed using histomorphometry and immunohistochemistry of proliferating cell nuclear antigen. Quantitative light microscopic data were acquired for estimating replication of epidermal keratinocytes, epidermal thickness, and depth of dermal collagen bundles.

Results: Data were analyzed by comparing means with 1-way analysis of variance, and significant changes in all measurements were noted. Augmented keratinocyte proliferation and thickening of viable epidermis were observed with all 3 compounds, although a greater effect was found in the retinoic acid and peptide treatment groups. A similar trend was noted with respect to widening of the collagen layer. Epidermal surface roughness manifested maximum smoothing after treatment with the peptide compound.

Conclusion: The pronounced effects noted with all 3 compounds indicate that topical agents other than retinoic acid may have comparative stimulating effects on the skin in nonirradiated mice.

Arch Facial Plast Surg. 2009;11(5):332-337

Within recent years there has been increased interest in noninvasive topical treatments to reverse the effects of photoaging on human skin. Over-the-counter cosmetics and antiaging products generate billions of dollars in commerce, and the goal of many of these products such as retinoic acid (RA), α-hydroxy acids, and vitamin creams is to reduce wrinkles and improve skin appearance. Newer cosmeceutical products are being marketed, and there is a need for scientific studies about these antiaging products and validation of their claims using clinical testing.1

Among the topical treatments, RA and its derivatives have been most extensively investigated over the last few decades, and its binding to retinoid receptors and its clinical improvements have been well documented.2-3 In the hairless mouse model, RA can repair the damage to skin caused by UV radiation.4 For stimulating structural reorganization of aged skin, other products such as α-hydroxy acid, vitamin C serum, and soybean products also have been marketed to consumers. Recently, peptide preparations have become popular as antiwrinkle agents as new options to treat aging skin.5 These products have been tested in human patients5-6; however, they have not been compared with other antiwrinkle agents in a single experiment to test their relative efficacy to resurface the skin and affect histopathologic factors. The present study was conducted to compare basic histologic effects of 3 topical commercial products, that is, RA, soy cream, and a peptide product, in the skin of the hairless mouse model. Most experiments on topical effects of these products have been conducted in the irradiated mouse model. It was deemed of interest to determine whether these products elicit effects in the intact animal on the surface profile and whether they can be correlated with their histopathologic effects. These data would serve as a baseline for our future studies of similar products on the irradiated hairless mouse. These observations may be helpful to make a clinical decision about selecting an appropriate antiaging substance for rejuvenation of aging skin.

METHODS

All animal procedures were performed per approved animal protocol from the institutional animal welfare committee. A group of 7 Skh-1 hairless mice (retired breeders aged...
9-12 months) obtained from Charles River Laboratories, Wilmington, Massachusetts, International were used for this experiment. The following antiwrinkle agents were used for daily topical application during 4 weeks: (1) RA (Renova [tretinoin emollient cream, 0.05%]; Ortho Dermatological, Skillman, New Jersey); (2) peptide lotion (LotuSculpt peptide complexes with pentapeptides and heptapeptides; LotuSculpt, distributed by Tracie Martin International LLC, New York, New York); and (3) soy cream (soy rejuvenating serum; Reviva Labs, Inc, Haddonfield, New Jersey). The dorsal skin was marked with a template drawing 9 × 9-mm square with tattoo ink for applying the products. The left side of the animal was treated with Renova, with peptide lotion in the anterior and posterior regions, respectively, with appropriate spacing, and the right side of the same animal was treated with soy cream. Untreated areas of the skin were used as control samples. The animals were humanely killed 1 day after conclusion of the treatment period.

At autopsy, silicon replicas of skin samples were prepared using resin and rings for the evaluation of skin microrelief by Standard Replica Analysis (BIONET protocol published by CuDerm Corp; and SPSS version 14.0, SPSS, Inc, Chicago, Illinois). Using commercially available software (Excel, Microsoft Corp; and SPSS version 14.0, SPSS, Inc, Chicago, Illinois), measurements were made from the basement membrane to the end of the granular layer in interfollicular sites.11-14 The same techniques were used as control samples. The animals were humanely killed 1 day after conclusion of the treatment period.

RESULTS

After 1 month of topical treatment, surface profiles of experimental animals exhibited profound changes. The values of replica analysis revealed that the control skin samples had more rough texture compared with the 3 experimental samples, that is, higher Rz and lower FSpace. Values of replica analysis revealed that the control skin samples had more rough texture compared with the 3 experimental samples, that is, higher Rz and lower FSpace values (Table, Figure 1, and Figure 2). Analysis of variance of these quantitative parameters revealed a significant difference between means of 4 groups (1 control and 3 experimental groups). The Fisher least significant difference test was performed for post hoc analysis of pairwise comparisons to detect differences between group means, and the level of significance was assigned at \( P < .05 \). Statistical tests were performed using commercially available software (Excel, Microsoft Corp; and SPSS version 14.0, SPSS, Inc, Chicago, Illinois).

Table. Morphometric and Profilometric Data

<table>
<thead>
<tr>
<th>Group</th>
<th>Zb</th>
<th>Formalin</th>
<th>PCNA-I, Zb</th>
<th>Zb</th>
<th>Formalin</th>
<th>Collagen Width</th>
<th>Surface</th>
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<tbody>
<tr>
<td>Control</td>
<td>22.9</td>
<td>23.3</td>
<td>34.1</td>
<td>32.4</td>
<td>33.0</td>
<td>132.4</td>
<td>86.2</td>
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<tr>
<td>Peptide lotion</td>
<td>54.7</td>
<td>36.4</td>
<td>43.6</td>
<td>46.1</td>
<td>41.7</td>
<td>244.0</td>
<td>211.9</td>
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<tr>
<td>RA</td>
<td>49.2</td>
<td>53.0</td>
<td>48.5</td>
<td>462.3</td>
<td>429.1</td>
<td>234.0</td>
<td>202.5</td>
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<tr>
<td>Soy cream</td>
<td>278.4</td>
<td>32.5</td>
<td>42.5</td>
<td>380.6</td>
<td>421.9</td>
<td>175.0</td>
<td>146.5</td>
</tr>
</tbody>
</table>

Abbreviations: ANOVA, analysis of variance; PCNA-I, proliferating cell nuclear antigen index; RA, retinoic acid; Zb, Zamboni solution.
with RA. The 3 cellular layers became well differentiated and expanded after application of the topical agents, and hyperplasia was noted without any epidermal dysplasia or atypia (Figures 3, 4, and 5). This trend was observed in both sets of tissue samples treated with different fixatives. Immunohistochemistry of PCNA revealed an increased proliferation index of epidermal keratinocytes; the maximum response was noted with RA, and comparable values were found in skin samples treated with peptide lotion or soy cream (Figures 6, 7, and 8). Measurement of total dermis thickness demonstrated significantly higher values in all 3 treatment groups; peptide treatment produced an almost equal effect as that with RA. The collagen bundle width in the upper dermis area, which could be easily differentiated in trichrome preparations, showed almost similar expansion with RA and peptide treatment and a lower value after soy treatment (Figure 9). This trend was observed in tissue specimens processed with both fixatives.

**COMMENT**

Literature on experimental study of comparison of relative efficacy of over-the-counter topical antiwrinkle agents is limited despite popular concern about the cutaneous effects of the numerous commercially available topical non-invasive treatment products. In similar experiments, it is also imperative that surface replica profiles be simultaneously compared with histopathologic alterations elicited by various topical creams and lotions. The present study somewhat provides this information and gives a correlative account about the effects of the 3 tested agents. When comparing overall effects, all 3 agents produced discernible improvement in surface profiles and notable histologic effects on both compartments of the skin, the epidermis and the dermis. There is extensive literature about products containing RA that have been used to rejuvenate photoaged skin and to treat certain dermatologic conditions. Retinoic acid causes a myriad

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**Figure 1.** Surface profile of silicon replicas from control (A) and after topical treatment with peptide lotion (B), retinoic acid (C), and soy cream (D). Compared with the replica in the control, the experimental animals show smoother appearance and an improvement in overall skin texture.
of histopathologic changes after topical application. The pronounced epidermal thickening with RA noted in the present study can be presumed to be a result of its molecular action through nuclear receptors and activation of growth factors. Increased DNA synthesis with RA has been described in earlier articles. Use of RA also leads to production of hyaluronan, which is a glycosaminoglycan and a major component of extracellular matrix. A 2-week course of treatment with RA in the hairless mouse led to increased staining of glycosaminoglycans in the epidermis. The observed epidermal response concurs with similar stimulatory effects described in the intact or nonirradiated hairless mouse.

Despite their clinical usefulness, topical retinoid agents are known to cause skin irritation and photosensitivity. Patients reacting to tretinoin with retinoid dermatitis often must undergo a 3-month period of erythema and skin peeling for adjustment or use a lower concentration of the agent. The search for antiwrinkle agents without such putative problems has gained interest among researchers in recent years.

Peptide effects in the mouse skin were in many ways comparable to those with RA treatment under the present conditions. To reverse facial photodamage or solar scarring due to damaged collagen, topical growth factors or pep-
tide molecules have been tested in a clinical setting. Topical growth factors resulted in repaired photodamage, new collagen formation, and improved wrinkle profiles. Collagenlike peptides had notable antiwrinkle effects in women volunteers when examined using silicone replica analysis. Immunohistochemistry also showed that ex vivo human skin samples treated with collagenlike peptide demonstrated enhanced synthesis of extracellular matrix molecules. Such a mechanism may be postulated to explain the dermal responsiveness in the hairless mouse model used in the present study.

Although the commercial soy product tested in the present study had the least morphologic effect of the 3 agents, its stimulatory effects on epidermal thickness and proliferation were noticeable and are reminiscent of the beneficial effects of soy products on the skin in reported in vivo and in vitro studies. Cultured human fibroblasts showed increased collagen and hyaluronic synthesis after use of soy extracts, and isoflavone-containing emulsion topically applied on human skin increased the number of dermal papillae. Soy extract can stimulate growth of quiescent human epidermal keratinocytes under certain conditions. In our study, topically applied soy extract did not expand the width of collagen bundles as much as the RA and peptide solutions did, although there was an apparent increase in soy-mediated total dermal width. This may indicate simply that there was a hydration effect from the soy treatment without increasing collagen synthesis. However, such gross morphometric measurements do not portend collagen synthesis at the biochemical level. A 2-week course of treatment with topical isoflavones increased the density and intensity of dermal hyaluronan content in the hairless mouse model without affecting its thickness.

In the present study, wrinkle effacement was notably induced by the 3 commercial preparations, and histomorphologic alterations were simultaneously observed. This leads to speculation as to whether the morphologic changes in the epidermis or the dermis after treatment might have contributed to improved surface profiles of the mouse skin. In normal aging human skin, wrinkles...
are believed to be caused by decreased skin collagen, abnormal elastic tissue, or decreased chondroitin sulfate in the dermal matrix.24,33 Experimental studies in the hairless mouse have indicated a role of dermal collagen and epidermal keratin in wrinkle formation.34–36 These studies were conducted after repetitive UV irradiation in this animal model. Whether similar mechanisms were operating in healthy nonirradiated hairless mice treated with these 3 agents remains speculative until further immunocytochemical or histochemical analyses are conducted. The profilometric effect of soy was similar to that of the other 2 agents despite its being least effective in terms of morphologic changes.

The present study should be considered an overview of the morphologic and profilometric effects of 3 commercial antiwrinkle agents tested in the hairless mouse model. At least 2 of these agents, RA and soy, have been found to counter the damaging effects of photodamage in hairless mouse skin as reported by other investigators.2,22,30 The comparison of peptide preparations or solution in hairless mouse skin as reported by other investigators was conducted. The profilometric effect of soy was similar to that of these 3 agents remains speculative until further immunocytochemical or histochemical analyses are conducted. The morphologic and profilometric response to commercial anti-wrinkle agents in the hairless mouse. Dermatol Surg. In press.
