Dermatology

Dermatology 1999;199(suppl 1):43–48

Repair of UVA-Induced Elastic Fiber and Collagen Damage by 0.05% Retinaldehyde Cream in an ex vivo Human Skin Model

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Key Words

Retinaldehyde · Normal human skin · Ex vivo model · Organ culture · Collagen · Elastic fibers · Ultraviolet light · Photoaging

Abstract

Background: Cellular effects of UV exposure are implicated in cutaneous aging. UV radiations induce structural and cellular changes in all the compartments of skin. ***Aim:*** To study the antiaging efficacy of a cream containing 0.05% retinaldehyde with an ex vivo technique using human skin in order to approximate in vivo metabolic conditions. ***Methods:*** Human skin explants were maintained alive in organ culture for 18 days and subjected to UVA exposure, thus simulating skin photoaging. Retinaldehyde cream was then applied to the surface of the epidermis for 2 weeks and the results were compared with those of nontreated skin explants. Dermal repair was analyzed histologically with quantification of collagen and elastic fibers, and biochemically by the measure of newly synthesized collagen as shown by adding tritiated proline to the culture medium. ***Results:*** UVA exposure induced significant alterations of collagen and elastic fibers as shown by morphometric analysis. In all UVA-exposed and then retinaldehyde-treated skin specimens, collagen and elastic fibers were restored to the level of nonexposed skin. UVA exposure induced a decrease in collagen synthesis, whereas in retinaldehyde-treated UVA-exposed skin the synthesis was similar to that of unexposed skin. ***Conclusion:*** It has been shown that retinaldehyde has many of the properties of tretinoin in its biological and beneficial effects on photoaging. We have verified some of these previous observations, especially on dermal connective tissue, by obtaining significant repair of elastic fibers and collagen alteration induced by UVA exposure.

Introduction

The effectiveness of topical tretinoin (all-*trans*-retinoic acid) in treating the consequences of photoaging in human skin is now well known, demonstrated by animal, clinical as well as by in vitro studies [1, 2]. Indeed, histological epidermal changes have been described in tretinoin-treated human subjects, i.e. increased epidermal thickness, increased granular layer thickness, decreased melanin content and stratum corneum compaction [3]. At the dermal level, improvement of connective tissue was observed in tretinoin-treated photodamaged skin [4] as well as increased glycosaminoglycans

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1018–8665/99/1997–0043$17.50/0

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[5], new collagen formation (types I and III) [6, 7] and improvement of elastic fibers [4].

Retinaldehyde, an intermediate between retinol and retinoic acid, is known to have biological activity close to that of retinoic acid in mouse [8] and human skin [9]. Retinaldehyde has also been shown in vivo to improve photoaged skin [10] and to achieve this improvement on a par with retinoic acid in a double-blind study but with a better patient tolerance [11].

To analyze some biochemical events that can explain these findings, we have tested a 0.05% retinaldehyde cream in an ex vivo model, thereby avoiding the need for animal testing and reducing the need for further in vivo testing. This model consisted of full-thickness normal human skin fragments obtained from plastic surgery and maintained in long-term organ culture for 21 days and exposed to UVA, thus simulating skin photoaging [12]. With this method, we have induced alterations of elastic fibers and collagen [12] similar to that observed during the acute phase of UV-induced alterations [13].

Materials and Methods

Organ Culture of Human Skin Specimens

Our original culture method is based on previous studies [14, 15]. We adapted these methods to obtain full-thickness skin surviving for 18 days in cultures with both epidermal and dermal structures resembling normal in vivo skin [16]. Eight normal human skin fragments were obtained from plastic surgery in women 35–45 years of age. Skin fragments were cut into 1-cm^2 full-thickness pieces and washed three times with antibiotics. Subcutaneous fat and lower dermis were mechanically removed under a stereomicroscope using a surgical scalpel.

Skin explants were placed with the epithelium uppermost, at an air/liquid interface, on culture inserts (filter pore size 12 μm; Costar, Poly-Labo Paul Block, France). These inserts were set on 12-well plates (Costar) for 18 days at 37 °C in a humidified incubator with 5% CO_2. Cohesion between skin and insert was obtained with a polysiloxane vinyl seal in such a way that no skin retraction or lateral passage of the cream towards the dermis was possible.

Medium was added to the wells so that the surface of the medium was level with the filter. Organ cultures were performed with Dulbecco's minimal essential medium (Gibco BRL) containing antibotics (100 U/ml penicillin and 100 μg/ml streptomycin; Gibco BRL, USA), 200 μg/ml *L*-glutamine (Gibco BRL), bovine pituitary extract, growth factors and fetal calf serum (DAP, France) [14–18]. All supplements were freshly made at each medium change every 2 days.

UVA Radiation

To obtain premature aging of the skin with dermal alterations, we used UVA radiation, known to induce changes in the middle and deep dermis [12]. The source of UV radiation was a Vilbert Lourmat stimulator (France) fitted out with a UVA irradiation source (365 nm) composed of Vilbert Lourmat tubes T-20, L-365 (no UVB and no UVC emission) mercury vapor tubes, low pressure, hot cathodes with a Vilbert Lourmat RMX-365/312 radiometer. The radiometer was associated with a microprocessor programmable in energy (mJ/cm^2), with time basis enabling 6 irradiation measurements per second for controlling the energy received by the skin fragment. For skins receiving UVA from day 0 to day 4, 2 irradiations were administered at 12 J/cm^2 so that they received in totality 24 J/cm^2. This UV radiation is sufficient to induce reproducible alterations in the dermis, as previously described [12].

Application of 0.05% Retinaldehyde Cream

From day 4 to day 18 following UVA irradiations, a formulation containing 0.05% retinaldehyde (Ystheal® cream, Pierre Fabre) was applied to the epidermis 5 days a week at the dose of 2 mg/cm^2, followed by a slight massage.

Analysis of Dermal Repair

After 18 days, skin fragments were removed from the culture inserts and the effects of retinaldehyde cream were studied both histologically and biochemically.

Histological Study of Elastic Fibers and Collagen Bundles. Skin fragments were fixed in Bouin's solution and embedded in paraffin. Serial sections of 4 μm thickness were obtained and stained for the elastic fiber and collagen study. Five sections were compared for each skin fragment. The elastic fiber network was revealed with (+)-catechin staining [19]. Collagen was stained with a picric acid solution containing 0.1% sirius red [20].

For a quantitative analysis, a computerized image analysis was made. The stained slides were examined by a microscope (Leitz; magnification × 160) connected with a camera unit (XC-75 CE type) and with a microprocessor (Q520). Approximately, 15–25 fields were analyzed for each skin section. For elastic fiber analysis, two regions were studied: the superficial dermis, reaching to the dermoepidermal basement membrane, containing mainly oxytalan and elaunin fibers, and the middle dermis containing small horizontal reticular elastic fibers, as defined by Cotta-Pereira et al. [21].

The surfaces of elastic fibers and collagen bundles were measured per square micrometer. Then, the relative elastic fiber or collagen content of the dermis was expressed as percentage of surface: area of elastic fibers or collagen per unit area of analyzed dermis [22, 23].

Collagen Synthesis. Fibroblastic activity for collagen synthesis was analyzed after 18 days survival of ex vivo cultures. Skin biopsies were removed from inserts, put directly in the wells and 20 μCi/ml of *L*-proline-/2, 3-^{3}H) (Amersham, France, 1 mCi/ml, specific activity 43 Ci/mmol) with 100 μg/ml ascorbic acid and 50 μg/ml β-aminopropionitrile were added in the culture medium for 24 h. Extracellular ^{3}H-proline-labeled collagen was extracted by the addition of 1 mg/ml pepsin in 0.5 *M* acetic acid on the biopsies over 48 h at 4 °C. Then, the ^{3}H-proline-labeled collagen was purified by Webster's method consisting of successive salt precipitations at acid and neutral pH [24]. Radioactivity in each precipitate was measured in a liquid scintillation counter and expressed in disintegrations per minute (dpm). Total protein concentration was measured by spectrophotometric determination with the Pierce BCA protein Assay Reagent kit and finally results were expressed in dpm per milligram protein.

Statistical Analysis

Mean values and standard deviations were calculated for each parameter. The statistical significance of changes recorded in the parameters were determined with paired Student's t test ($p < 0.05$).

Three groups were compared: a normal group (untreated skin), a control group (UVA-exposed skin) and a treated group (UVA-exposed

Table 1. Morphometric analysis of elastic fibers

	Superficial dermis		Mid dermis	
	surface, μm^2	%	surface, μm^2	%
Nonexposed nontreated skin	4,385 ± 1,315	4	5,316 ± 1,087	4.9
Skin exposed to UVA	3,065 ± 441	2.8	4,488 ± 945	4.1
Skin exposed to UVA and treated by retinaldehyde	4,233 ± 1,291*	3.88	5,518 ± 772*	5

*$p < 0.005$: difference statistically significant in comparison with UVA-exposed skin (paired Student's t test). Surface (μm^2) and percentage of surface occupied by elastic fibers per square micrometer of dermis ± SD.

skin, then treated with 0.05% retinaldehyde). Comparison was made between normal and control groups to verify the validity of the method. For evaluation of the retinaldehyde cream efficacy, comparison was made between control and treated groups.

Results

Computerized Image Analysis of Elastic Fiber Network and Collagen Bundles

Elastic Fibers. As shown in table 1, UVA exposure induced alterations of connective tissue, particularly on elastic fibers. There was a decrease in the elastic fiber network, with fragmentation of elastic fibers (fig. 1). This observation was confirmed by morphometric analysis: after UVA radiation only 2.8% of the dermal area was occupied by elastic fibers, in contrast to 4.0% in nonexposed skin.

In all UVA-exposed and then retinaldehyde-treated skin specimens, elastic fibers stained intensely positive for catechin and tended to be longer and thicker (fig. 2) as compared with UVA-exposed, nontreated specimens. These results were confirmed by morphometric analysis: the surface occupied by elastic fibers in UVA-exposed and retinaldehyde-treated skins was significantly higher (3.88%) than in altered, nontreated skin in the superficial dermis (2.8%; $p < 0.05$). We obtained similar results in the middle dermis, where the elastic fiber network was significantly increased and better organized.

Collagen Bundles. As shown in table 2 UVA exposure induced important alterations of collagen bundles (fig. 3). Histologically, they became thinner and disorganized in the dermis. The surface occuppied by collagen decreased after UVA exposure (52.25%) in comparison with normal skin (66.75%; $p < 0.05$). The surface occupied by collagen in UVA-altered and retinaldehyde-treated skins is significantly higher (71.15%) than in UVA-altered skin ($p < 0.05$; fig. 4).

Table 2. Morphometric analysis of collagen

	Surface μm^2	Surface occupied by collagen, %
Nonexposed nontreated skin	72,750 ± 20,521	66.75
Skin exposed to UVA	56,962 ± 14,493[a]	52.25
Skin exposed to UVA and treated by retinaldehyde	77,542 ± 11,810[b]	71.15

[a]$p < 0.05$: difference statistically significant in comparison with nonexposed skin (paired Student's t test); [b]$p < 0.05$: difference statistically significant in comparison with UVA-exposed skin (paired Student's t test). Surface (μm^2) and percentage of surface occupied by collagen per square micrometer of dermis ± SD.

Table 3. Collagen synthesis (dpm/mg protein)

Nonexposed nontreated skin	37,128 ± 11,157
Skin exposed to UVA	25,105 ± 10,866[a]
Skin exposed to UVA and treated by retinaldehyde	38,014 ± 10,182[b]

[a]$p < 0.05$: difference statistically significant in comparison with normal skin (paired Student's t test); [b]$p < 0.05$: difference statistically significant in comparison with UVA-altered skin (paired Student's t test).

Collagen Synthesis

The results of collagen synthesis are given in table 3. Extracellular ^{3}H-proline-labeled collagen measured by the Webster method was significantly decreased after UVA radiation in comparison with normal skin. In UVA-exosed and retinaldehyde-treated skin, collagen synthesis reached a higher level than in UVA-exposed, nontreated skin.

1

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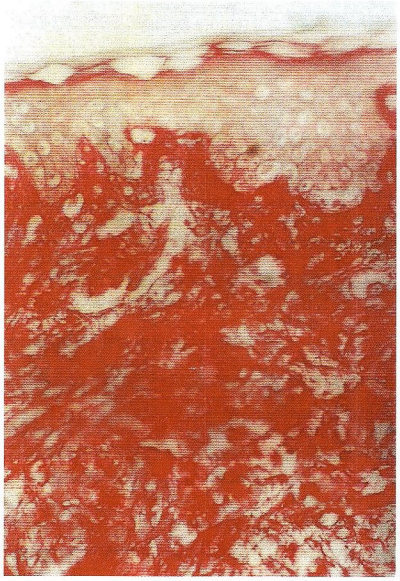

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