Topical Retinaldehyde on Human Skin: Biologic Effects and Tolerance

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The present study was designed to explore if retinaldehyde, a natural metabolite of vitamin A, has any biologic activity and is tolerated by human skin.

Biologic activity was shown by the induction of cellular retinoic acid–binding protein type 2 (CRABP 2) mRNA and protein; the rank order for CRABP-2 increase was retinoic acid > retinaldehyde > 9 cis retinoic acid > retinol > β carotene.

In volunteers treated 1–3 months with 0.5, 0.1, and 0.05% retinaldehyde, there was a dose-dependent and significant increase in epidermal thickness, keratin 14 immunoreactivity, and Ki67-positive cells. The area of distribution of involucrin, transglutaminase, and filaggrin immunoreactivity was also increased in a dose-dependent manner, and keratin 4 immunoreactivity was induced in the upper epidermis. In pilot clinical tolerance studies, 229 patients received topical retinaldehyde at different concentrations; the 1% preparation was tolerated by up to 70% of the treated subjects; tolerance of the 0.5% preparation was slightly better, whereas both 0.1 and 0.05% preparations applied on facial skin were well tolerated and allowed prolonged use (up to 3 years) in patients with inflammatory dermatoses.

These findings indicate that topical retinaldehyde has biologic activity and is well tolerated on human skin. Key words: retinoic acid/9 cis retinoic acid/cellular retinoid binding proteins/retinol/β carotene. J Invest Dermatol 103:770–774, 1994

Retinoic acid (RA) is widely used for topical therapy of several skin diseases; it also improves chronic solar damage [1]. Topical RA causes irritation of the skin, which precludes its use in some skin diseases that respond to systemic retinoids [2]. It is still not established if all the therapeutic activities of topical RA are mediated by nuclear receptors, or if irritation is necessary for obtaining these effects. Irritation might be explained, in part, by an "overload" of the RA-dependent pathways with non-physiologic amounts of exogenous RA in the skin [3].

The use of immediate RA precursors, such as retinol, retinaldehyde, and β carotene may prevent this "overload." Human keratinocytes transform retinol into retinaldehyde and then into RA by two enzymatic steps involving dehydrogenases. The first step is rate limiting and reversible; retinaldehyde can be converted enzymatically into either RA or retinol by human keratinocytes, both in vivo [4,5] and in vitro [6]. The rate of conversion depends on the state of keratinocyte differentiation; differentiating keratinocytes are able to convert retinaldehyde into RA at a higher rate than are non-differentiated keratinocytes [6]. Epidermal cells have a weak capacity to transform retinol into retinaldehyde [4,5]; β carotene is not converted into retinoids by epidermal cells (Siegenthaler G, unpublished observations).

We hypothesized that retinaldehyde may be a precursor for topical use because i) it bypasses the first rate-limiting step of retinol oxidation into RA and ii) only epidermal cells at the pertinent stage of differentiation and capable of oxidizing retinaldehyde would generate active ligand(s). This would result in more controlled delivery of ligand(s) and possibly weaker side effects compared to direct application of RA or synthetic analogs.

Because no published data are available concerning the topical use of retinaldehyde in humans, we have performed pilot studies to explore its biologic effects and tolerability.

MATERIAL AND METHODS

Patients and Volunteers The protocols have been approved by the committee for ethics of our institution (Commission d'éthique du département de médecine et dermatologie, University Hospital, Geneva).

To study the induction of cellular retinoid-binding proteins, healthy volunteers were treated for 4 d with 1, 0.1, and 0.05% retinaldehyde under occlusion as previously described [7,8]. Healthy volunteers were treated once daily for 1 to 3 months (see results) with 0.5, 0.1, or 0.05% retinaldehyde on one forearm and the vehicle on the other forearm. Biopsies were taken from both arms at the end of the period of treatment and processed for histology, immunohistochemistry, and electron microscopy.

Subjects with various skin problems gave their written consent to receive one application per day of retinaldehyde. Duration of the application period and concentration of retinaldehyde (from 0.05 to 1%) depended on the type of skin problem and are detailed in Results. Follow up was performed twice weekly in subjects treated with the highest concentrations (1% and 0.5%) and once monthly in subjects receiving the 0.1% and 0.05% preparations. Tolerance was evaluated subjectively by the subjects, and objectively by the authors concerning signs of irritation. Tolerance was graded on a 0 to 3 scale: 0, no subjective or objective signs; 1, mild burning and no objective signs; 2, stinging burning and visible erythema with subsequent fine scaling; 3, frank burning and erythema with edema. Photographs were taken at each visit.

MATERIALS

Reagents Retinol and retinoic acid were purchased from Sigma [11,12-3H] all-trans retinoic acid (50.6 Ci/mmol) were obtained from Dupont.
Figure 1. Topical retinaldehyde induces CRABP 2 mRNA. Northern blot analysis of CRABP 2 response to 4 d occlusive treatment with 0.05% retinaldehyde (R) versus vehicle (V). A, relative increase compared to vehicle; densitometric analysis. B, results of two representative patients are shown. C, ethidium bromide staining of RNA samples after gel electrophoresis.

Figure 2. Topical retinaldehyde induces CRABP 2 protein. CRABP 2 protein following 4 d occlusive treatment; all compounds were applied at the concentration of 0.05% except β-carotene (0.5%). Densitometric analysis of polyacrylamide gel electrophoresis autoradioblotting; mean ± SD.

trophoresis, the gel was either divided into lanes and cut into 2-mm bands, which were then assayed for the determination of the radioactivity profiles, or transferred to a nitro-cellulose sheet and analyzed by direct autoradiography [13]. The relative amounts of CRABP 1 and 2 were estimated by densitometric evaluation of band intensity using a computing densitometer (Molecular Dynamics Ltd, Kent, UK).

RNA Isolation and Analysis: Total RNA was isolated from snap-frozen keratome biopsies using the RNA extraction kit purchased from Pharmacia (Pharmacia LKB Biotechnology AG, Dudendorf, Switzerland) according to the manufacturer’s instructions. Twenty micrograms of total RNA were hybridized in parallel to CRABP 1 and CRABP 2 cRNAs as previously described [10]. After quantification using a phosphorimager (Molecular Dynamics, Kent, UK), the membranes were stripped and rehybridized to human 28S ribosomal cRNAs for normalization according to standard procedure [14].

Histology: Frozen and Bouin (or Duboscq Brasil)–fixed paraffin-embedded biopsies were used for histology with the following staining: hematoxylin and eosin, Gordon and Sweets, periodic acid Schiff reaction, and Rinehart Abri’s method. Slides were analyzed using a light microscope by two blinded investigators. Epidermal thickness measured on frozen sections in

Table I. Induction of CRABP-2 Protein Following 4 d of Occlusive Treatment with Retinaldehyde in Human Volunteers

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Vehicle (mean ± SD)</th>
<th>Retinaldehyde (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5%/1 month</td>
<td>75 ± 22</td>
<td>138 ± 7*</td>
</tr>
<tr>
<td>Epidermal thickness</td>
<td>K167 cells/mm</td>
<td>57 ± 16</td>
</tr>
<tr>
<td>0.1%/1 month</td>
<td>58 ± 11</td>
<td>103 ± 22</td>
</tr>
<tr>
<td>Epidermal thickness</td>
<td>K167 cells/mm</td>
<td>78 ± 13</td>
</tr>
<tr>
<td>0.05%/1 month</td>
<td>5</td>
<td>36 ± 11</td>
</tr>
<tr>
<td>Epidermal thickness</td>
<td>K167 cells/mm</td>
<td>65 ± 17</td>
</tr>
<tr>
<td>0.05%/3 months</td>
<td>44 ± 29</td>
<td>59 ± 37*</td>
</tr>
</tbody>
</table>

* p < 0.01, paired Wilcoxon’s test.

Table II. Epidermal Thickness (µm) and Epidermal Ki-67–Positive Cells (per mm length of epidermis) Following Daily Open Use of Topical Retinaldehyde as Compared to Vehicle-Treated Side

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Vehicle (mean ± SD)</th>
<th>Retinaldehyde (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% retinaldehyde</td>
<td>30.9 ± 8.3b</td>
<td>7.1 ± 3.5</td>
</tr>
<tr>
<td>0.1% retinaldehyde</td>
<td>24.1 ± 6.3c</td>
<td>3.1 ± 5</td>
</tr>
</tbody>
</table>

* Values (pmol/mg protein) are mean of three polyacrylamide gel electrophoresis assay duplicate experiments ± SD.

P values are as follows: * p < 0.01, paired Wilcoxon’s test.

p < 0.05, paired Wilcoxon’s test.

p < 0.02, paired Wilcoxon’s test.

NEN, Germany. Analysis of the retinoids was performed under yellow light. Purity of labeled and unlabeled retinoids was checked by high-performance liquid chromatography analysis and consistently exceeded 95%. All ethanol solutions of retinoids contained butylated hydroxytoluene. The other chemicals used were of best grade commercially available.

Topical Retinaldehyde Preparations: Retinaldehyde (Eastman Kodak, Rochester, NY) was prepared in lotion and cream vehicles at the concentrations of 1, 0.5, 0.1, and 0.05% and did not contain detectable amounts of retinol and retinoic acid.

Hybridization Probes: Full-length (740 bp) cellular retinoic acid–binding protein type 1 (CRABP 1) and (830 bp) mouse CRABP 2 cDNAs were kindly provided by Professor P. Chambon, CNRS LGM, Inserm U 184, Strasbourg, France, and RNA hybridizations were done as previously described [9,10]. Human 28S ribosomal RNA cDNA was purchased from The American Type Culture Collection (Rockville, MD) and 32P labeled by random-priming using the Amersham Multiprime DNA labeling kit (Amersham, Buckinghamshire, England) according to the manufacturer’s instructions.

Analysis of CRABP-1 and -2 Expression: CRABP 1 and 2 were analyzed at the mRNA and protein [11–13] levels after the in vivo short-term occlusive test previously described [7]. Keratome biopsies were obtained after 4 d exposure to the following compounds prepared in the same cream vehicle: retinol, retinoic acid, 9-cis retinoic acid, or retinaldehyde (0.05%), or β-carotene (0.5%). In each volunteer, one buttock was treated with the compound, and the other received the vehicle. In another series of experiments, CRABP 2 protein induction was studied after application of 1% or 0.1% retinaldehyde.

Polyacrylamide Gel Electrophoresis and Autoradioblotting: The tissue samples were washed with phosphate-buffered saline (PBS) to remove traces of blood before being frozen and lyophilized. Protein extractions were performed as previously described [11]. Binding proteins were analyzed after incubation of 100 µL of supernatant (500 µg of protein) with all trans [11,12-3H]retinoic acid at 600 nM for 16 h at 4°C in the presence or absence of a 200-fold excess of unlabeled ligand. The supernatants incubated with titrated ligand were subjected to vertical-slab non-denaturing polyacrylamide gel electrophoresis with 7.5% acrylamide, as previously described [11–13]. After electrophoresis, the gel was either divided into lanes and cut into 2-mm bands, which were then assayed for the determination of the radioactivity profiles, or transferred to a nitro-cellulose sheet and analyzed by direct autoradiography [13]. The relative amounts of CRABP 1 and 2 were estimated by densitometric evaluation of band intensity using a computing densitometer (Molecular Dynamics Ltd, Kent, UK).

RNA Isolation and Analysis: Total RNA was isolated from snap-frozen keratome biopsies using the RNA extraction kit purchased from Pharmacia (Pharmacia LKB Biotechnology AG, Dubendorf, Switzerland) according to the manufacturer’s instructions. Twenty micrograms of total RNA were hybridized in parallel to CRABP 1 and CRABP 2 cRNAs as previously described [10]. After quantification using a phosphorimager (Molecular Dynamics, Kent, UK), the membranes were stripped and rehybridized to human 28S ribosomal cRNAs for normalization according to standard procedure [14].
micrometers from the base of the stratum corneum to the basement membrane was assessed in ten high-power fields, and the mean thickness was used as described [15].

**Electron Microscopy**

Samples were fixed by 3% glutaraldehyde solutions served on a CM10 Philips electron microscope. Immunohistochemistry

Four micrometer frozen sections were incubated directly with mouse monoclonal antibodies to cytokeratin peptide (K) 14 (Sigma Chemical Company, St. Louis, Missouri), involucrin, filaggrin (Biomedical Technologies Inc., Stoughton, MA), or K67 proliferating cell antigen (Jackson Immunoresearch Laboratories Inc., West Grove, PA).

Other frozen sections fixed with acetone for 5 min at −20°C followed by methanol for 5 min at −20°C were incubated with mouse monoclonal antibodies to K4, K13, K19 (Bio-Science Products AG, Emmenbrücke, Switzerland), K5-6 (Boehringer Mannheim, Mannheim, D), or human transglutaminase (BC1 clone, Biomedical Technologies Inc., Stoughton, MA). The horseradish peroxidase Vectastain ABC kit (Vector Laboratories Inc., Burlingame, CA) was used according to the manufacturer’s instructions.

Quantification of immunohistochemical staining reactions was performed as follows: for K67, the number of positive cells was counted per millimeter length of epidermis on three consecutive serial sections and the mean number was used; for the other markers, the thickness of the specific epidermal staining was measured in micrometers on ten high-power fields.

**Statistical Analysis**

All treatment versus vehicle individual values were assessed with the non-parametric paired Wilcoxon’s test.

**RESULTS**

**Topical Retinaldehyde Up-Regulates CRABP 2**

CRABP 2 mRNA was, as expected, barely detectable by Northern blot analysis in vehicle-treated normal human skin but was induced following 4 d occlusive treatment with 0.05% retinaldehyde (Fig 1). CRABP 1 mRNA was not detectable in either vehicle or retinaldehyde-treated skin samples.

CRABP 2 protein was increased following 4 d occlusive treatment with retinaldehyde (Table I and Fig 2), but there was no irritation at the site of application. Figure 2 compares the CRABP 2 protein increase induced by 0.05% retinaldehyde, RA, 9 cis RA, or retinol at the same concentration in the same cream vehicle. The rank order for CRABP-2 increase was RA > retinaldehyde > 9 cis RA > retinol; interestingly, 0.5% β-carotene (i.e., a tenfold higher concentration) did not increase CRABP 2 levels.

CRABP 1 protein was reduced by 60% in 0.05% retinaldehyde-treated skin compared to vehicle alone; a reduction was also observed with RA (−42%), retinol (−43%), and β-carotene (−36%) but not 9 cis RA (not shown).

**Topical Retinaldehyde Increases Epidermal Thickness**

Skin samples obtained from volunteers treated daily on the forearm for 1 (0.5% and 0.05%) or 3 months (0.05%) with retinaldehyde were compared to contralateral skin treated with vehicle alone.

**Table II** shows that retinaldehyde induced a dose-dependent significant increase in epidermal thickness. No qualitative microscopic alterations were observed except for an increase in thickness of the granular layer in 0.5% retinaldehyde–treated samples; there was no deposition of mucin–like material.

A detailed analysis was made by electron microscopy on one specimen of skin treated for 1 month with 0.5, 0.1, or 0.05% retinaldehyde and for 3 months with 0.05% retinaldehyde. No significant ultrastructural changes were observed in the retinaldehyde-treated skin compared to vehicle; notably no deposition of mucin–like material was found.

**Topical Retinaldehyde Increases Expression of Filaggrin, Transglutaminase and Involucrin and Induces Immunoreactivity of K4**

The most important changes compared to the vehicle-treated skin were observed in specimens from subjects treated with 0.5% retinaldehyde (Tables III and IV, Fig 3): K14 immunoreactivity was confined to the basal layer in all vehicle-treated samples and was induced in the supra-basal layers in all volunteers treated with 0.5% retinaldehyde; the number of K67-positive cells in the basal epidermal layers was increased threefold (Table II); K4 immunoreactivity was not detectable in vehicle-treated samples but was present in the upper part of the epidermis in all volunteers treated with 0.5% retinaldehyde (Table III and Fig 3b), the area of distribution of transglutaminase, involucrin, and filaggrin immunoreactivities was increased in all 0.5% retinaldehyde–treated samples (Table III and Fig 3b, c, and d, respectively). In contrast, no changes were observed for K10, 5 and 6, 13, and 19 immunoreactivities.

The only consistent changes observed in specimens from subjects treated with 0.05% retinaldehyde were the slight and focal expression of K4 immunoreactivity in the upper epidermis (Table III), the slight but not significant increase in filaggrin involucrin and transglutaminase expression (Table IV), and a slight but significant increase in K67-positive cells (Table II) at 3 months.

**Table III. Summary of Alteration in Expression of Differentiation Markers After Topical Application of Retinaldehyde in Human Volunteers Compared to Vehicle**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Vehicle</th>
<th>Retinaldehyde 0.50% 1 Month (n = 5)</th>
<th>Retinaldehyde 0.05% 1 Month (n = 5)</th>
<th>Retinaldehyde 0.05% 3 Months (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K14</td>
<td>Normal</td>
<td>Increased&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>K10</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>K5-6</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>K4</td>
<td>Absent</td>
<td>Increased&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Not induced</td>
<td>Not induced</td>
</tr>
<tr>
<td>K13</td>
<td>Absent</td>
<td>Not induced</td>
<td>Not induced</td>
<td>Not induced</td>
</tr>
<tr>
<td>K19</td>
<td>Absent</td>
<td>Not induced</td>
<td>Not induced</td>
<td>Normal</td>
</tr>
<tr>
<td>Involucrin&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Normal</td>
<td>Increased&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Increased&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Increased&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Filaggrin&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Normal</td>
<td>Increased&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Increased&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Increased&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Transglutaminase&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Normal</td>
<td>Increased&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Increased&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Normal</td>
</tr>
</tbody>
</table>

<sup>a</sup> Denotes immunoreactivity in stratum corneum and granulolam.

<sup>b</sup> Continuous staining.

<sup>c</sup> Discontinuous staining.

<sup>d</sup> Focal staining.

<sup>e</sup> See text.

<sup>f</sup> See Table IV.

**Table IV. Thickness of Immunoreactive epidermis for Markers of Differentiation (μm)**

<table>
<thead>
<tr>
<th>n</th>
<th>Involucrin</th>
<th>Filaggrin</th>
<th>Transglutaminase</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5% retinaldehyde 1 month</td>
<td>5</td>
<td>93 ± 13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>76 ± 5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Vehicle 1 month</td>
<td>5</td>
<td>18 ± 1</td>
<td>24 ± 2</td>
</tr>
<tr>
<td>0.05% retinaldehyde 1 month</td>
<td>5</td>
<td>27 ± 11</td>
<td>32 ± 13</td>
</tr>
<tr>
<td>Vehicle 1 month</td>
<td>5</td>
<td>15 ± 1</td>
<td>23 ± 1</td>
</tr>
<tr>
<td>0.05% retinaldehyde 3 months</td>
<td>5</td>
<td>13 ± 2</td>
<td>38 ± 4</td>
</tr>
<tr>
<td>Vehicle 3 months</td>
<td>5</td>
<td>12 ± 3</td>
<td>38 ± 4</td>
</tr>
</tbody>
</table>

<sup>a</sup> The thickness of the specific epidermal staining was measured in ten high-power fields.

<sup>b</sup> p < 0.01, paired Wilcoxon’s test.
Figure 3. Topical retinaldehyde induces expression of filaggrin, transglutaminase, involucrin, and keratin 4. Immunoreactivity of keratin 4 (a), transglutaminase (b), involucrin (c), and filaggrin (d) in human forearm skin treated for 1 month with 0.5% retinaldehyde (R) compared to contralateral side treated with vehicle (V). Bar, 75 μm.

Topical Retinaldehyde Is Tolerated by Human Skin Two hundred twenty-nine patients received topical retinaldehyde at different concentrations (Table V). The 1% preparation was tolerated by up to 69% of the treated subjects, mostly psoriatics treated on the trunk and limbs; grade 2 to 3 irritation leading to cessation of treatment was observed in 31%. Tolerance of the 0.5% preparation was slightly better, whereas both 0.1 and 0.05% preparations, used mostly on facial skin [16], were well tolerated (Table V).

DISCUSSION
To date we have found no report in the literature concerning the topical use of retinaldehyde on human skin. Our study was undertaken to explore whether this natural metabolite of vitamin A has a biologic activity and is tolerated when applied on human skin. Our results show that topical retinaldehyde has biologic activity. This was demonstrated through two approaches: the induction of CRABP 2 and morphologic observations.

Topical RA had been shown to increase CRABP 2 protein [7,12] and to induce CRABP 2 mRNA expression in human skin [8,17]; this latter effect has been proposed to be a good correlate of biologic activity [17]. We found that retinaldehyde likewise induces CRABP 2 mRNA expression and increases CRABP 2 protein. Factors that regulate the expression of CRABP 2 in vivo are not fully understood. A “ligand-dependent” [18] pathway is most likely, because an increase in RA, both from endogenous enzymatic formation [4,10] and from exogenous delivery [7,8,12,17], is associated with an increase in keratinocyte CRABP 2 mRNA and protein. This pathway implies nuclear receptors such as retinoic acid receptors (RARs) or retinoid X receptors (RXRs), because binding motifs have been identified in the mouse CRABP 2 gene that mediate RA differential transcription transactivation by RARs and RXRs [19]. Mouse CRABP 2 mRNA is also controlled post-transcriptionally by RA [20]. An apparently “ligand-independent” pathway [18] is also likely to exist, because CRABP 2 is induced in human epidermis by stimuli such as ultraviolet B irradiation, superficial epidermal wounding, and anthralin [7,12].

It is therefore not unlikely that CRABP 2 induction by topical RA, an irritant compound, may involve, at least in part,
Table V. Tolerance of Topical Retinaldehgy on Human Skin

<table>
<thead>
<tr>
<th>Concentration (%)</th>
<th>Number of Patients</th>
<th>Treatment Duration (months)</th>
<th>Irritation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>26</td>
<td>1</td>
<td>0.3 - 1.4</td>
</tr>
<tr>
<td>0.5</td>
<td>32</td>
<td>1.2</td>
<td>0.6 - 2</td>
</tr>
<tr>
<td>0.1</td>
<td>32</td>
<td>4.5</td>
<td>2 - 13</td>
</tr>
<tr>
<td>0.05</td>
<td>162</td>
<td>8.2</td>
<td>1 - 4</td>
</tr>
</tbody>
</table>

* 1% and 0.5% concentrations were used mainly on psoriatic lesions of trunk and limb, whereas 0.1% and 0.05% were used mainly on facial skin (seborrheic dermatitis, acne vulgaris, atopic dermatitis, melanoma, photaging, and keratin 6 and 13 but not keratin 1, 2, 10). The total number of subjects treated was 239; seven patients received more than one dose.

1 Shortest and longest duration of treatment.
2 Irritation was quantified using a three-point scale (see text).

paths that are not directly linked to the binding to nuclear receptors.

The molecular mechanism through which retinaldehyde induced CRABP 2 is therefore not established. Our observations favor, however, a ligand-dependent pathway, because i) retinaldehyde did not induce irritation and ii) retinol also induced CRABP 2 but not carotene. Indeed, although retinaldehyde can be formed either from retinol or carotene (Siegenthaler, unpublished observations); this is in accordance with our finding that retinal induced CRABP 2 slightly less than retinaldehyde. Because retinaldehyde binds poorly (500 times less than RA) to members of the retinoic acid receptors family [22,23], CRABP-2 induction by topical retinaldehyde must probably results from its transformation by epidermal enzymes into active ligand(s). This study has been designed to test the biologic activity of retinaldehyde, not to rank precisely this activity in comparison to the other vitamin A derivatives tested. Therefore, because the bioavailability of retinol, 9cis RA, and RA in the vehicle used has not been evaluated, one cannot make any firm conclusion on the actual potency of retinaldehyde compared to any of these compounds.

The biologic activity of topical retinaldehyde was also demonstrated by morphologic observations. At the high concentration of 0.5%, many changes, except the absence of mucin deposition, were similar to that induced by 0.1 and 0.05% topical RA or 13 cis RA [15,24,25]. The alterations induced by 0.05% retinaldehyde were milder but significant. Because this concentration induced CRABP 2 about as well as 0.05% RA, there is a dissociation between morphologic changes and CRABP 2 induction, the latter being a more sensitive indicator of biologic activity, as observed previously by others [15]. The correlation between the induction of morphologic changes and clinical irritation requires additional study [26].

Topical retinaldehyde was well tolerated and could be used at rather high concentrations, but tolerance was dependent upon concentration. The highest concentrations (1-0.1%) were tolerated by an unexpectedly high percentage of patients, and we think an improvement in delivery may even increase the tolerance of high concentrations. The 0.05% concentration was well tolerated and allowed prolonged use on facial skin in patients with inflammatory dermatoses. This indicates that this natural metabolite of vitamin A may be used as a topical agent on human skin. Its usefulness in several conditions is currently under evaluation [16].

REFERENCES


K.Tamm, D.Grand, P.Carraux, M-J.Carrier-Deldon, and M. Pisteur are acknowledged for excellent technical help and L.French for reading the manuscript.