Pathogenesis of Blepharoconjunctivitis Complicating 13-cis-Retinoic Acid (Isotretinoin) Therapy in a Laboratory Model

Robert W. Lambert and Ronald E. Smith

Systemic treatment of adult male New Zealand albino rabbits with 13-cis-retinoic acid (isotretinoin) resulted in a reduction in the size of the meibomian gland. Clinical signs of toxicity included weight loss, alopecia, dry skin and mild conjunctival erythema with crusting on the eyelid margin. Histopathologic findings included thickening of duct and ductule epithelium, decrease in acinar tissue, accentuation of basaloid cells and evidence of periacinar fibrosis. The model presents the first experimental data to indicate that systemic 13-cis-retinoic acid effects meibomian gland structure in a laboratory model. Future functional studies of this model may yield important insights into the relationships between meibomian gland morphology, function, the ocular surface and the pathogenesis of blepharoconjunctivitis. Invest Ophthalmol Vis Sci 29:1559-1564, 1988

Isotretinoin (13-cis-retinoic acid) is used for the treatment of acne vulgaris and keratinizing dermatoses. Therapeutic effects of the drug as used for dermatologic conditions include decreased sebum production, changes in the surface lipid composition and inhibition of keratinization. One of the more common side effects of this therapy is blepharoconjunctivitis, which has been reported in 20-50% of such patients. Although Fraunfelder and colleagues suggested that meibomian gland dysfunction may be implicated, to date there has been no evidence to support this hypothesis. Since studies in patients undergoing isotretinoin therapy for acne have revealed a reduction in the size of skin sebaceous glands, and since there are similarities between skin sebaceous glands and the meibomian glands, we studied animals fed 13-cis-retinoic acid to determine if pathologic changes occur in the meibomian gland. Herein we describe the histopathology of the rabbit meibomian gland after systemic treatment with Accutane® (Hoffman-La Roche, Inc., Nutley, NJ).

Materials and Methods

Eighteen male New Zealand albino rabbits were treated with isotretinoin at the following doses: 1-2 mg/kg/day (Group I, n = 6), 10 mg/kg/day (Group II, n = 6), and 20 mg/kg/day (Group III, n = 6) for a period of 12 weeks. The low dose was chosen to approximate the clinical dose, while the medium and higher doses were chosen based on the data of Kamm. The drug was administered by oral intubation, using peanut oil as a vehicle. A control group of six additional animals received the vehicle only. Prior to initiation of treatment, the lids of each animal were evaluated by biomicroscopy. All meibomian glands appeared normal.

13-cis-retinoic acid (RO 4-3780) was a gift from Hoffmann-La Roche, Inc. It was supplied as a pure dry powder that was stored at −20°C. Prior to its use, the 13-cis-retinoic acid was brought to room temperature and dissolved in a known quantity of the vehicle.

At the end of the 12-week treatment period, the animals were killed and the eyelids were removed and placed in fixative for 24 hr. The tissue was then dissected into rectangular blocks containing two or three glands per block and processed for histologic examination. Only glands taken from the central portion of the lipid were sectioned for morphometric analysis. This was done to minimize the error introduced by regional differences in the size of the rabbit meibomian glands from temporal to nasal.

At least three glands per animal were serially sectioned (10 μm section thickness, 150–200 sections/
Fig. 1. (A) Normal rabbit eyelid. The central duct (*) extends the length of the gland and connects to acini by means of short ductules (arrowhead). C = conjunctiva. Original magnification X50, hematoxylin and eosin. (B) Tracing of an outline of the meibomian gland illustrated above.

Gland); every tenth section was stained with hematoxylin and eosin, assessed by light microscopy and subjected to morphometric analysis. This study conformed to the ARVO Resolution on the Use of Animals in Research.

Morphometric Analysis

The Micro-Plan II Digitizer (Donsanto Corp.) was used to determine cross-sectional area. The section was projected onto a digitizing tablet and an outline...
of the entire gland was traced with a cursor (Fig. 1). The following parameters were determined:

1. Cross-sectional area (A) in mm² for every tenth section of a serially sectioned gland.
2. Mean cross-sectional area (A) as the mean of all cross-sectional areas measured for a particular group.
3. Meibomian gland volume (V) in mm³ for each serially sectioned gland, according to the Delesse principle:
   \[ V = \frac{\Sigma A_i T}{T} \]
   \[ \Sigma A_i = \text{sum of all cross-sectional areas for gland} \]
   \[ T = \text{thickness of tissue section represented by a single slide, ie, } T = 0.1 \text{ mm as every tenth section was sectioned.} \]
4. Mean meibomian gland volume (V) as the mean of all gland volumes calculated for a particular group.

Results

At the time of sacrifice, all animals treated with 13-cis-retinoic acid showed signs of systemic toxicity. These included weight loss, alopecia of the chest, abdomen and distal extremities, dry skin and mild erythema with crusting on the eyelid margins. Animals in the control group showed no signs of toxicity. Histopathologic examination of the control tissue revealed no structural abnormalities. The “normal” rabbit meibomian gland is a simple branched acinar gland that extends the approximate length of the tarsal plate (Fig. 1). A central duct courses throughout the length of the gland and connects with individual acini by short ductules. Acinar cells can be identified at various stages of holocrine differentiation (Fig. 2).

Animals treated with 13-cis-retinoic acid showed a marked reduction in the size of the meibomian glands (Fig. 3); this was confirmed by morphometric analysis. The mean cross-sectional surface area of the meibomian gland was reduced by 15% in Group I, by 21% in Group II and by 26% in Group III. This was reflected in a similar reduction of the meibomian gland volume; ie, mean volumes were reduced by 18% in Group I, by 25% in Group II and by 28% in Group III. Results are presented in Table 1.

Meibomian acinar tissue appeared to be diminished and histopathologic changes were observed in all experimental animals treated with isotretinoin. These changes included thickening of the epithelium lining the ducts and ductules, decrease in the number and size of acini and a reduction in the frequency of lipid-laden acinar cells (Fig. 3). Meibomian glands of animals treated with a high dose of isotretinoin (Group III) showed evidence of a periacinar fibroblastic activity replacing acinar tissue (Fig. 4). Casts of acinar cells were frequently observed in the acinar
Fig. 3. Treated meibomian gland (Group II, 10 mg isotretinoin daily ×12 weeks). Epithelium lining the duct and acini is thickened (arrowhead) and acini are reduced in size. Original magnification ×65, hematoxylin and eosin.

Discussion

Treatment of adult New Zealand albino rabbits with 13-cis-retinoic acid (1–20 mg/kg daily for 12 weeks) resulted in clinical signs of toxicity in all animals. These signs were present in animals receiving the clinical dose (Group I) and progressed in severity to Group III, where all animals showed clinical signs similar to a generalized hypervitaminosis A. The histopathology and statistical analysis of gland volume showed clearly that systemic 13-cis-retinoic acid affects the morphology and reduces the size of the meibomian gland at each dose studied. Isotretinoin appears to inhibit the ability of the meibomian acinar cell to differentiate, while stimulating the epithelium lining the ducts and acini to proliferate.

Histopathologic changes observed in our animal model are compatible with those of Landthaler and associates, who demonstrated an inhibitory effect of 13-cis-retinoic acid on human skin sebaceous glands. In their model, 13-cis-retinoic acid appeared to exert its action by inhibiting the differentiation of sebaceous gland acinar cells. This results in a diminution of skin sebaceous gland size and, presumably, decreases the production of sebum. In the sebaceous gland, a reduction in sebum production is believed to be the therapeutic basis for the treatment of acne vulgaris. In our animal model, the consequence of a reduction in gland tissue may be mirrored in the adverse ocular side effects associated with isotretinoin therapy, the most common being blepharoconjunctivitis, which has been reported in 20 to 50% of pa-

Table 1. Rabbit meibomian gland cross-section areas (A) in mm² and volume (V) in mm³ following oral administration of 13-cis-retinoic acid

<table>
<thead>
<tr>
<th>Group</th>
<th>A (mm²)</th>
<th>V (mm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.85 ± 0.07</td>
<td>3.93 ± 0.25</td>
</tr>
<tr>
<td>Group I</td>
<td>1.55 ± 0.04†</td>
<td>3.23 ± 0.4†</td>
</tr>
<tr>
<td>13-cis-retinoic acid 1–2 mg/kg/bw</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group II</td>
<td>1.50 ± 0.07*</td>
<td>2.97 ± 0.16*</td>
</tr>
<tr>
<td>10 mg/kg/bw</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group III</td>
<td>1.42 ± 0.16*</td>
<td>2.87 ± 0.15*</td>
</tr>
<tr>
<td>20 mg/kg/bw</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are mean ± SD, n = 6.
* P < 0.005 (paired t-test).
† P < 0.01 (paired t-test).
Several hypotheses have been suggested to explain retinoic-induced blepharoconjunctivitis. According to Fraunfelder, La Braico and Meyer, this type of blepharitis arises from abnormal meibomian gland function. They suggest that treatment with 13-cis-retinoic acid results in a decrease in the meibomian gland acini (20 mg isotretinoin daily ×12 weeks). A periacinar fibrosis appears to be replacing acinar tissue (arrowhead). Original magnification ×350, toluidine blue.

Casts of intact differentiated holocrine cells are seen in the lumen of the gland (arrow). Original magnification ×425, toluidine blue.
mian lipid component of the tear film which decreases tear break-up time and consequently destabilizes the tear film, leading to a "dry eye" syndrome.

Our present study does not address the meibomian lipid component of the tear film; however, it seems likely that the histopathologic changes observed in retinoid treated rabbit meibomian glands might influence this factor. Additional studies would be required to determine if our model provides a basis for the hypothesis that changes in meibomian gland morphology result in decreased meibum excretion and a subsequent reduction in the lipid component of the tear film. Alternatively, Rismondo and Ubels have detected the presence of 13-cis-retinoic acid in the tear fluid of patients undergoing retinoid therapy for acne. They suggested that a lipolytic action of the retinoid may further destabilize the tear film, compounding the effects described by Fraunfelder and associates.

The experimental evidence we have presented indicates that 13-cis-retinoic acid affects meibomian gland structure in a laboratory model. This is not only of potential importance in understanding the pathogenesis of retinoid-induced blepharoconjunctivitis, but also in contributing to our understanding of the relationship between the meibomian gland and the complex question of lid margin disease. Future functional studies in our animal model may yield important insights into the relationship between meibomian gland structure, function, and the ocular surface. Similar studies have not yet been performed to confirm our findings in man.

Key words: blepharoconjunctivitis, meibomian gland dysfunction, morphometry, toxicity, hypervitaminosis A

References