Pathologic Changes in the Exorbital Lacrimal Gland of the Vitamin A-Deficient Rat

Ken Hayashi,*†† Chiitranjan Reddy,*†† Laila Hanninen,* George Wolf,§ and Kenneth R. Kenyon*††

Histologic changes in lacrimal glands of vitamin A-deficient (A-) and pair-fed control rats were compared. In A- lacrimal glands, secretory granules were strikingly diminished, and rough endoplasmic reticulum appeared somewhat atrophic. Nuclei of acinar cells were hyperchromatic and pleomorphic. Using alcian blue-PAS, no positive staining was present in acini of A- lacrimal glands, whereas in controls apical portions of acini were intensely stained. Thus, lacrimal tissues of A- rats were thought to be poorly differentiated as a glandular epithelium. When A- rats were supplemented with retinyl acetate, secretory granules reappeared, rough endoplasmic reticulum cisternae greatly dilated, and mitochondria proliferated, indicating accelerated secretory activity. Resupply of vitamin A can induce glandular differentiation in A- lacrimal tissues. Tear volume was not decreased in A- rats compared with pair-fed controls. Regression of secretory organelles in A- lacrimal tissues may lead to a decrease in protein and mucoprotein secretion and subsequent changes in tear composition. Invest Ophthalmol Vis Sci 31:187-196, 1990

Whether lacrimation in vitamin A deficiency is disturbed, leading to alterations of tear volume or composition, is uncertain.1-3 Mori first noted morphologic changes at the light microscopic level in lacrimal tissue of vitamin A-deficient (A-) rats and emphasized the role of these changes in causing xerophthalmia.4,5 However, other studies reported minimal changes of lacrimal tissue.6,7

Vitamin A is a potent inducer of epithelial differentiation.6,8,9 Its deficiency causes alteration of epithelial tissues with a decrease in the number of mucus-secreting cells10-12 in addition to squamous metaplasia,6,7,13 and keratinization.2,13,14 In contrast, vitamin A excess can induce mucous metaplasia15 and inhibit epithelial keratinization.16 Thus, vitamin A plays an important role in modulating epithelial differentiation.

Lacrimal tissue consists primarily of glandular epithelium arranged in acini. The acinar cells secrete various proteins into the aqueous layer of tear fluid and secrete mucosubstances20,21 necessary for stabilizing the tear film.22 Importantly, Ubels and associates provided evidence that the lacrimal gland is the major source of retinol in rabbit tear fluid,23,24 although the contribution of other sources such as the conjunctival vasculature also should be considered. Corneal and conjunctival epithelium need vitamin A to maintain normal epithelial differentiation.2,13,25 Because of its avascular environment, the corneal epithelium is undoubtedly dependent on the lacrimal tear fluid for vitamin A. Therefore, it is conceivable that vitamin A deficiency may affect the lacrimal gland, which in turn may lead to pathological corneal changes.

There has been no detailed morphologic study of the lacrimal gland in vitamin A deficiency. Here we report an investigation of the pathologic changes of the lacrimal gland in vitamin A deficiency and examine whether these alterations are reversible when the animals are treated with vitamin A.

Materials and Methods

Rats

Experimental animals were treated in accordance with the ARVO Resolution on the Use of Animals in Research. Twenty-eight male Sprague-Dawley rats (Charles River Breeding Laboratories, Wilmington, MA) were used. Ten rats were placed on an A- diet for 17 to 18 weeks and paired with ten control rats fed the same diet with a vitamin A supplement (700 μg retinyl acetate/week). The animals were raised until
Fig. 1. Light micrographs of lacrimal glands. (A) Pair-fed control. Acinar cells contain abundant granular cytoplasm in the central portion. Cell nuclei are located in the basal portion. (B) Vitamin A-deficient. Granular cytoplasm of acinar cells is scant, and intercellular boundaries are not evident. Cell nuclei are located predominantly in the central portion and reveal marked pleomorphism. (C) Vitamin A-supplemented. Lacrimal gland acini have recovered normal gland architecture. Granular cytoplasm is scant compared with controls. Nuclei still show mild pleomorphism. (D) Pair-fed control. Positive reaction to PAS staining is seen in the central region of lacrimal gland acini. (E) Vitamin A-deficient. No positive staining is seen in the acini. (F) Vitamin A-supplemented. Most acinar cells do not show positivity to PAS; some are faintly stained (arrows). (A, B, C; hematoxylin & eosin staining; D, E, F, alcian blue-PAS staining; all original magnification X346).

The A− rats had a 20–25% weight loss compared to pair-fed controls and revealed ocular signs of vitamin A deficiency (eg, corneal surface keratinization, stromal edema, eyelid dermatitis). The remaining eight rats were fed the A− diet for 14 weeks and thereafter 700 μg retinyl acetate was administered orally once a week for 5 weeks. At the end of this period, these supplemented rats weighed virtually the same as pair-fed controls of the same age, and ocular signs of vitamin A deficiency had disappeared.
Tissue Preparation

Rats were sacrificed by intraperitoneal injection of an overdose of sodium pentobarbital. Immediately after sacrifice, both exorbital lacrimal glands were excised and fixed in 10% neutral buffered formalin or half-strength Karnovsky's fixative (2.5% glutaraldehyde and 2% paraformaldehyde in cacodylate buffer) for light and electron microscopy.

Light Microscopy

Thirty lacrimal glands fixed by Karnovsky's fixative were embedded in methacrylate, and sections were stained with hematoxylin and eosin (H & E) for routine histological examination and quantitative analysis of the acinar area. Paraffin sections of 26 formalin-fixed glands were used for alcian blue (pH 2.5)–periodic acid-Schiff (PAS) staining to examine acid glycoproteins.

Quantitative Analysis of Glandular Area

The areas of acini in H & E-stained sections were measured using a Zeiss Videoplan II image analysis system (Rainin Instruments, Woburn, MA), which includes a computer-assisted program. In each section (pair-fed controls, ten; vitamin A-deficient, ten; vitamin A-supplemented, ten), two fields in the central portion of the glands were randomly selected. All acini in the selected area were measured, and the measurements were repeated three times. Statistical analysis of the data was performed using the student t-test.

Electron Microscopy

Lacrimal gland specimens were post-fixed in 1% osmium tetroxide, dehydrated in serial dilutions of alcohol and embedded in araldite epoxy resin. Semithin sections were cut and stained with paraphenylene-diamine and examined by phase contrast microscopy. Representative areas were cut as ultrathin sections, double-stained with uranyl acetate and lead citrate, and examined with a Philips 410 transmission electron microscope.

Tear Volume

Tears were collected from both eyes of etherized rats (pair-fed controls, 15; vitamin A-deficient, 15) according to the method of Sullivan et al.26-28 In brief, the tip of a graded capillary micropipet (Fisher, Medford, MA) was placed at the inner canthus and gently moved along the palpebral conjunctiva. This procedure was repeated twice on each eye. The tear collection typically lasted less than 1 min and appeared to drain the entire available tear content. Tear volumes were accurately measured to within 0.1 ml. Statistical analysis of the data was performed using the student t-test.

Results

Light Microscopy

The acinar cells of lacrimal glands of pair-fed control rats had abundant granular cytoplasm and clear intercellular boundaries (Fig. 1A). The nuclei were located basally and were fairly uniform in size and shape. Secretory lumina were small but evidently open in the center of each acinus. A few inflammatory cells, predominantly plasma cells, were seen in the interstitial tissue.

Lacrimal gland acini of A− rats were smaller than those of pair-fed controls. The granular cytoplasm was scant compared with that of controls, and the boundaries between acinar cells appeared obscure (Fig. 1B). The nuclei were located centrally and were markedly hyperchromatic. Secretory lumina were narrow. Stromal tissues were basically unchanged, but showed mild inflammatory cell infiltration. The epithelial cells lining ducts appeared unchanged.

Lacrimal glands of vitamin A-supplemented rats appeared to be recovering normal architecture but acini were still small compared with those of pair-fed controls (Fig. 1C). Granular cytoplasm, although increased from A− tissue, was less than that of controls. Nuclei began to relocate in the basal region but showed mild hyperchromatism.

With alcian blue (pH 2.5)–PAS (pair-fed controls, ten; vitamin A-deficient, ten; vitamin A-supplemented, six), the lacrimal glands of pair-fed controls exhibited PAS-positive reactions in the central portion of the acinus, although they were unreactive with alcian blue (Fig. 1D). Some acinar cells were intensely stained and were thought to selectively secrete acid mucopolysaccharide. In contrast, no positive staining was seen in lacrimal gland acini of A− rats (Fig. 1E). Most acinar cells of vitamin A-supplemented rats were not positive to PAS staining, but some stained weakly (Fig. 1F).

<table>
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<th>Table 1. Area of lacrimal gland acini</th>
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* The differences between pair-fed controls and vitamin A-supplemented rats, between pair-fed controls and vitamin A-deficient rats, and between vitamin A-supplemented and vitamin A-deficient rats are significant (all P < 0.001).
Fig. 2. Lacrimal gland of pair-fed control rat. Inset (top): Phase contrast micrograph shows regularly arranged glandular acini with abundant granular cytoplasm in the center and nuclei in the periphery (paraphenylenediamine, PPDA, original magnification \( \times 270 \)). Main figure: Transmission electron micrograph shows acinar cells containing many secretory granules (sg) in the apical and central portions. Nuclei and rough endoplasmic reticulum (rer) are located basally. Secretory lumen (L) is open in the center of an acinus (original magnification \( \times 4000 \)).

Inset (bottom): Many secretory granules (sg) are bound to the Golgi complex (G) in the apical portion (original magnification \( \times 11,000 \)).
Fig. 3. Lacrimal gland of A− rat. Inset: Phase contrast micrograph reveals small gland acini with scant granular cytoplasm and pleomorphic nuclei. Some acinar cells have retained secretory vacuoles. Residual bodies are scattered throughout the cytoplasm (PPDA, original magnification X270). Main figure: Transmission electron micrograph shows regression of cell organelles of acinar cells. Secretory granules are hardly seen and the secretory lumina (arrows) are almost obstructed. The increase in chromatin is prominent in the nucleus. Residual bodies are scattered in the basal portion of the cytoplasm. A plasma cell (Pl) with dilated endoplasmic reticulum is also noted. rer, rough endoplasmic reticulum (original magnification X4000).
Fig. 4. Acinar cells of lacrimal gland of A- rat. There is infolding of the nuclear membrane and increase and clumping of chromatin. The cisternae of rough endoplasmic reticulum (rer) are deformed to small tubular profiles. Golgi complex (G) is also reduced in size. Secretory lumina (arrow, L) are almost obstructed. Residual bodies with electron-dense complex lipid are seen in the cytoplasm (original magnification ×11,000).

Quantitative Analysis of Glandular Area

The areas of lacrimal gland acini are presented in Table 1. Analysis using the unpaired student t-test confirmed that the acinar areas of A- lacrimal glands were significantly smaller than those of pair-fed controls ($P < 0.001$). The acinar areas of lacrimal glands of vitamin A-supplemented rats were larger than those of vitamin A-deficient rats ($P < 0.001$) but smaller than those of pair-fed controls ($P < 0.001$).

Electron Microscopy

In lacrimal glands of pair-fed control rats, acinar cells contained many secretory granules in the apical and middle portions, which were associated with Golgi complex (Fig. 2). Secretory granules were predominantly light in electron density and granular. Nuclei, which were uniform in shape and size and contained one or two nucleoli, were located basally. Rough endoplasmic reticulum was well developed,
Fig. 5. Lacrimal gland of vitamin A-supplemented rat. Inset: Phase contrast micrograph. Basic architecture of lacrimal gland acini is recovered but granular cytoplasm is still scant. Residual bodies are scattered within the cytoplasm (PPDA, original magnification X270). Main figure: Transmission electron micrograph resolves the recovery of cell organelles. Secretory granules (sg) of varied electron density and size are found adjacent to the central secretory lumen (L). The cisternae of rough endoplasmic reticulum (rer) are greatly widened and intricate (original magnification X4000).
formed flat cisternae and was mainly located basally (Fig. 2, inset). Each secretory lumen appeared to open in the center of an acinus, and microvilli of acinar cells were visible within the lumen.

In lacrimal glands of A− rats, acinar secretory granules were strikingly diminished (Fig. 3). Nuclei frequently showed infolding of the nuclear membrane. Increase and clumping of nuclear chromatin were also prominent. Nucleoli were well developed in size and number. The rough endoplasmic reticulum and Golgi complex were disarranged and reduced in size. Numerous residual bodies were scattered in the cytoplasm of the acinar cells (Fig. 4). The secretory lumen appeared obstructed, but junctional complex components (zonula occludens and zonula adherens) were observed adjacent to the lumen. Many residual bodies were scattered in the cytoplasm of acinar cells and interacinar connective tissue (Figs. 3, inset, 4). No marked changes were noted in the epithelium of the duct system.

Lacrimal gland acini of vitamin A-supplemented rats contained many secretory granules, which varied in size and shape (Fig. 5). Nuclei had not settled in the basal portion and were still mildly hyperchromatic and pleomorphic. The cisternae of rough endoplasmic reticulum were strikingly widened and intricate, and the transition from Golgi vacuoles to secretory granules was apparent (Fig. 6). In addition, a marked increase in the number of mitochondria was noted. These findings indicated that the acinar cells in vitamin A-treated rats were metabolically active and recovering secretory activity. Many residual bodies were still present in the cytoplasm of acinar cells (Fig. 5).

**Tear Volume**

The mean tear volume of pair-fed control rats was 6.0 ± 0.7 μl (mean ± standard error) and of A− rats 8.0 ± 1.4 μl; the difference was not statistically significant.

**Discussion**

The present study shows disturbed epithelial differentiation in the lacrimal gland of A− rats. The...
acinar area of A− lacrimal gland was significantly smaller than that of pair-fed controls (Table 1), and secretory granules, which directly contribute to protein secretion, were strikingly diminished in the acinar cells. In addition, A− acinar cells did not stain with PAS, whereas PAS-positive reactions were intensely evident in controls, indicating decreased secretion of mucosubstances. These findings suggest that glandular secretory functions of lacrimal tissues are disturbed in vitamin A deficiency. Furthermore, the nuclei of acinar cells were hyperchromatic, and the rough endoplasmic reticulum and Golgi complex were involuted, indicating that A− acinar cells appeared to be immature. Thus, lacrimal tissues of A− rats were poorly differentiated as a glandular epithelium in terms of both secretory function and the structure of their nuclei and cellular organelles.

After treatment with vitamin A for 5 weeks, lacrimal tissues seemed to be recovering some normal architecture and functions. The acinar area of lacrimal glands of vitamin A-supplemented rats was larger than that in vitamin A deficiency. Mitochondria were increased in number. The cisternae of rough endoplasmic reticulum were greatly widened, and many secretory granules of various size and shape could be found, indicating accelerated secretory activity. These findings suggest that lacrimal tissues of vitamin A-supplemented rats were recovering glandular structure and secretory function. However, prominent PAS-positive staining was not seen and nuclei still showed mild hyperchromatism, indicating that this tissue had not returned to normal. Nevertheless, our results suggest that the resupply of vitamin A can reversibly induce the differentiation of lacrimal gland tissue.

We found that the tear volume in A− rats was not decreased compared with pair-fed controls. This is in agreement with other clinical and experimental studies.1,2 Our finding of regression of secretory cell organelles suggests that proteins in tears may be decreased. In addition, acini of A− lacrimal glands were not stained with PAS, indicating decreased mucoprotein production by A− lacrimal tissues and altered tear composition from that of pair-fed controls and vitamin A-supplemented rats. It is conceivable that alteration of tear composition affects the ocular surface. For example, the frequent presence of bacteria on the ocular surface of A− animals2,29 might be attributed to decreased peroxidase secretion, which is in proportion to the total protein production of lacrimal tissues. In addition to the decreased number of goblet cells of the conjunctiva,25−30 impaired mucin secretion from A− lacrimal tissues may also contribute to the breakup of the tear film and subsequent exposure of the ocular surface.31 Our study suggests that atrophy of secretory cell organelles in A− lacrimal tissues may lead to a decrease of proteins and mucopolypeptides in tears of A− rats and subsequent changes of the ocular surface epithelium.

Key words: lacrimal gland, vitamin A deficiency, epithelial differentiation, secretory granules, retinol secretion

Acknowledgment

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