Increased biosynthesis of specific glycoconjugates in rat corneal epithelium following treatment with vitamin A

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The biosynthesis of corneal epithelial proteins and glycoconjugates and their response to vitamin A were measured in vitamin A–deficient and normal rats. The synthesis of specific high-molecular-weight epithelial glycoconjugates was directly related to the levels of vitamin A administered. Protein synthesis, however, remained unaltered. Since vitamin A is known to reverse the keratinization process, the vitamin A–regulated high-molecular-weight glycoconjugates may play a role in modulating the expression of the keratinizing phenotype.

Key words: epithelium, vitamin A, glycoproteins, keratinization

Xerophthalmia, which can progress to keratomalacia and lead to blindness, is a human disease characterized by the loss of goblet cells from the conjunctival epithelium and keratinization of the corneal epithelium.1–3 Vitamin A deficiency and protein malnutrition seem to be key factors in producing this spectrum of diseases. In experimentally produced vitamin A deficiency alone, animals may exhibit a similar loss of conjunctival goblet cells as well as corneal keratinization.4, 5

The mechanism by which vitamin A reverses epithelial keratinization is not understood. The transition of keratinized epithelium to nonkeratinized epithelium may represent a phenotypic change resulting from alterations in protein synthesis. Cellular vitamin A–binding proteins have been isolated from a number of tissues, and it has been suggested that they act, in a manner similar to cellular steroid-binding proteins, to regulate the synthesis of unidentified differentiation proteins.6–8 Alternatively, the cellular vitamin A–binding proteins may merely serve in intracellular transport or as a protective agent9 to ensure the availability of the vitamin for its metabolism to an active derivative such as mannosyl retinyl phosphate (MRP). MRP has also been isolated from a number of tissues, and it may mediate the glycosylation of glycoproteins.10, 11 Several studies have shown that vitamin A–deficient animals exhibit reduced incorporation of radiolabeled sugars into glycopeptides of a variety of tissues, including epithelia.12 However, since these glycopeptides were obtained by pronase treatment, it is not clear whether the glycosylation of many or only a few glycoproteins was affected. In the present study we have examined the synthesis of intact proteins and glycoproteins in the corneal epithelium of vitamin A–deficient rats and normal rats treated with vitamin A.
Table I. Specific activity of corneal epithelial proteins and glycoproteins of corneas from vitamin A–deficient rat repleted with increasing levels of retinoic acid

<table>
<thead>
<tr>
<th></th>
<th>[14C] leucine (cpm/μg of protein)</th>
<th>[3H] mannose (cpm/μg of protein)</th>
<th>[3H] glucosamine (cpm/μg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deficient</td>
<td>405</td>
<td>495</td>
<td>2018</td>
</tr>
<tr>
<td>Repleted</td>
<td>429</td>
<td>796</td>
<td>2487</td>
</tr>
<tr>
<td>Excess repleted</td>
<td>436</td>
<td>940</td>
<td>2690</td>
</tr>
</tbody>
</table>

Materials and methods

Weanling Osborne Mendel rats (NIH stock) were fed vitamin A–deficient feed (Brosero, Inc.). After 7 to 9 weeks, when the weights of the animals had reached a plateau stage due to the deficiency, the rats were divided into three groups of nine rats each: (1) a “deficient group” which received 0.25 ml of dimethyl sulfoxide (DMSO), (2) a “repleted group” which received 300 μg of retinoic acid in 0.25 ml of DMSO per animal, and (3) an “excess-repleted group” which received 3 mg of retinoic acid in 0.25 ml of DMSO per animal, all by intraperitoneal injection. This protocol of acute vitamin A repletion produces no immediate weight gain and thereby minimizes the possibility that any biochemical differences found between deficient and repleted rats are due to differences in eating habits or in the weights of the animals. These two levels of vitamin A repletion were chosen to determine whether the biochemical responses were dose-dependent.

A second set of experiments was conducted on 9-week-old Osborne Mendel rats (here termed “normal” rats) raised on standard feed. The rats were divided into four groups of three rats each. One group received 3 mg of retinoic acid in 0.25 ml of DMSO per animal, and the remaining three groups received just the 0.25 ml of DMSO per animal, all by intraperitoneal injection. The animals were sacrificed 18 hr after injection.

The whole corneas were excised, pooled by group, and divided into three groups of three corneas each. One group received 3 mg of retinoic acid in 0.25 ml of DMSO per animal, and the remaining three groups received just the 0.25 ml of DMSO per animal, all by intraperitoneal injection. The animals were sacrificed 18 hr after injection.

The epithelia were scraped off the corneas in phosphate-buffered saline, harvested by centrifugation, solubilized in 2% sodium dodecyl sulfate (SDS) (containing 10 mM sodium phosphate, pH 7.0) by sonication, boiled for 5 min, and stored frozen. Incorporation was determined by measuring radioactivity in material precipitated by 5% trichloroacetic acid (TCA) in the presence of carrier bovine serum albumin, solubilizing the precipitate in NCS (Amersham Corp., Arlington Heights, Ill.), and counting in a liquid scintillation counter. Protein measurements were made according to the method of Lowry et al. A mean value, the specific activity, was obtained by averaging duplicate measurements of epithelia pooled from six corneas.

Polyacrylamide gels were constructed to contain a 3.0% stacking gel and a 7.5% running gel (except where noted), all containing 0.1% SDS. The solubilized epithelium was reduced with 500 mM dithiothreitol at 37° for 10 min prior to electrophoresis. An equivalent amount of radioactivity from each sample within a group was applied to each track. The radioactivity represents a portion of the pooled epithelia from six corneas. After electrophoresis the gel was fixed in 50% TCA, stained with Coomassie blue, destained with 7% acetic acid, dried, and exposed to Kodak X-Omat film at room temperature for 14C-labeled material. The gel was embedded with 2,5-diphenyloxazole before drying, and the dried gel was exposed to the film at −76° for 3H-labeled samples.

Liquid scintillation spectrophotometry of acrylamide gels was accomplished by cutting the gel in 1 mm slices and soaking them overnight in 0.5 ml of NCS prior to the addition of 10 ml of scintillant (Amersham).

Results and discussion

Gross examination of the exterior ocular surface of the vitamin A–deficient rats by reflected light indicated that the epithelial surface was abnormally irregular and pitted. Histological examination showed no indication of extreme keratinization, but the early stages of keratinization (desquamation and cellular enucleation) were evident.
Fig. 1. Autoradiogram of [14C]leucine-labeled proteins of corneal epithelium from vitamin A-deficient (D) rats and vitamin A-deficient rats repleted with retinoic acid (R) or excess retinoic acid (XR).

The incorporation of radioactive precursors into corneal epithelial proteins and glycoconjugates of deficient rats repleted with retinoic acid is shown in Table I. Retinoic acid administration produced only small increases in [14C]leucine incorporation into protein. Increasing levels of retinoic acid repletion did stimulate [3H]glucosamine incorporation by 23% and 33%. However, [3H]mannose incorporation increased by 61% and 90% after repletion with increasing doses of retinoic acid. Similar increases in mannose incorporation into glycopeptides have been reported in other systems.15-18

Autoradiographs of SDS polyacrylamide gels after electrophoresis of equivalent amounts of incorporated [14C]leucine or equivalent amounts of protein showed no major differences between corneal epithelia of vitamin A-deficient rats and the epithelia of deficient rats repleted with either level of retinoic acid (Fig. 1). It is of particular interest to note that no major changes could be detected in the synthesis of proteins between 40,000 and 70,000 molecular weight (MW) which would contain the class of proteins termed "keratins."20 These proteins are capable of self-assembly to form the 7 to 8 nm electron-dense cytoplasmic filaments characteristic of epithelium.21 Epidermal cells treated in culture for 2 weeks with vitamin A exhibited a reduced incidence of these filaments, and the reduction is considered ultrastructural evidence that vitamin A inhibits keratinization.22 Thus, although vitamin A is known to ultimately reverse the keratinization process, we found little immediate effect on the synthesis of the keratin proteins.

Fluororadioautographs of acrylamide gels after electrophoresis of equivalent amounts of incorporated [3H]mannose radioactivity also showed no major differences in the pattern of labeled glycoconjugates between the corneal epithelia of deficient and repleted rats. However, [3H]mannose incorporation per microgram of protein was stimulated by as much as 90% with repletion (Table I). It thus appears that retinoic acid increased mannose incorporation into a wide spectrum of glycoconjugates rather than a select few. These findings suggest that the mannosylated derivative of vitamin A (MRP) may be involved in adding mannosyl residues common to many or all glycoproteins or that it may merely have a transporting function for the mannose to be used in the synthesis of glycoproteins.

In contrast to those results obtained with [3H]mannose and [14C]leucine, fluororadioautographs of polyacrylamide gels after electrophoresis of equivalent amounts of incorporated [3H]glucosamine showed striking changes in the pattern of incorporation into high-MW glycoconjugates of the epithelium (Fig. 2). These changes could be most clearly demonstrated by varying the length of exposure of the gel to the film. A 1-day exposure showed that repletion with retinoic acid greatly increased [3H]glucosamine incorporation into two glycoconjugates greater than 250,000 MW and that excess repletion in-
increased incorporation into these components slightly more. A 4-day exposure showed that repletion increased [3H]glucosamine incorporation into a 220,000 MW glycoconjugate, and excess repletion still further increased incorporation into this component. Thus the synthesis of these particular high-MW glycoconjugates is dose-dependent.

The synthesis of these [3H]glucosamine-labeled high-MW glycoconjugates in corneal epithelia of rats raised on standard laboratory feed was also evaluated. On a fluororadiogram of intermediate exposure, both the 220,000 MW and the two higher-MW glycoconjugates were found to be synthesized by the corneal epithelium of the normal rats (Fig. 3, track A). The labeling intensity of the higher-MW bands suggests that they are major components of the normal corneal epithelium. When normal rats received retinoic acid 18 hr prior to sacrifice and labeling, the synthesis of these high-MW glycoconjugates was increased (Fig. 3, track B). These observations indicate that the enhanced synthesis of these epithelial glycoconjugates is not limited to vitamin A-deficient conditions. Normal corneas labeled in medium containing either retinoic acid (Fig. 3, track C) or retinol (Fig. 3, track D) also showed increased incorporation of [3H]glucosamine into these high-MW glycoconjugates. Quantitation of the radioactivity (Fig. 4) in the affected high-MW components showed that this short (6 hr) in vitro incubation with retinoic acid resulted in substantial increases in [3H]glucosamine incorporation into these high-MW glycoconjugates.

General discussion

The results of this study show that vitamin A increases the synthesis of three high-MW glycoconjugates in three different conditions: (1) vitamin A-deficient rats repleted with retinoic acid, (2) normal rats administered retinoic acid, and (3) in vitro incubation of corneas with either retinoic acid or retinol. The rapid response seen by in vitro exposure to vitamin A and the dose-dependent response observed in the vitamin A deficiency experiments suggest that the vitamin or a derivative
of the vitamin may be directly involved in regulating the biosynthesis of these high-MW glycoconjugates.

It is not clear from these data whether vitamin A increases the synthesis of the entire glycoconjugate or just its glycosylation. Vitamin A repletion produced little changes in the incorporation of \(^{14}C\)leucine into proteins with MWs comparable to the high-MW \(^3\)H]glucosamine-labeled glycoconjugates. But, these glycoconjugates may contain only small amounts of protein or leucine and therefore may not show a corresponding stimulation by autoradiography. The organ culture system should, with the appropriate radioisotope labeling, prove useful in resolving this point.

The results of this study do show, however, that vitamin A increases the synthesis of several high-MW glycoconjugates which are major components of the corneal epithelium, with little effect on the synthesis of proteins. These high-MW glycoconjugates may have an important function in maintaining normal epithelial morphology, perhaps by
inhibiting some process or processes involved in epithelial keratinization.

REFERENCES


