Major review

Retinoic acid and the ocular surface

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ABSTRACT

Retinoic acid is known to improve cutaneous wound healing and, in recent years, its application in ophthalmology has been investigated. This review looks at the role of retinoic acid on the ocular surface. Retinoic acid can be produced synthetically, and its mechanism of action includes both nuclear and non-nuclear receptor mediated pathways. It has been shown to improve full and partial thickness corneal lacerations as well as corneal epithelial defects. Retinoic acid plays a critical role in cell differentiation at the cornea, conjunctiva, and limbus, and may have an anti-tumor role. Its positive effect is only achieved at the correct concentration, however; excess concentrations of retinoic acid have a deleterious effect. The main limiting factor of retinoic acid use is its detrimental effect on meibomian glands, resulting in cell death, atrophy of acini, hyposecretion of oils, and altered gene expression, eventually resulting in dry eye symptoms. This effect is reversible on discontinuation of the drug.

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1. Introduction

Vitamin A has long been known to improve cutaneous wound healing. Numerous studies demonstrate that vitamin A accelerates epithelial migration, granulation tissue formation, and reversal of the retardation of healing induced by corticosteroids. This review will explore the international literature on the ophthalmic use of retinoic acid on the ocular surface.

2. Production of retinoic acid

2.1. Natural production in the human body

Retinoic acid is produced in the body by two sequential oxidation steps: first from retinol to retinaldehyde and then from retinaldehyde to retinoic acid, which is the active form of vitamin A. Retinol (vitamin A), ingested in food and absorbed by intestinal mucosal cells, is bound to the serum...
retinol-binding protein and transported into target epithelial cells by a membrane receptor encoded by the gene stimulated by retinoic acid 6. Additionally, a second enzyme, lecithin retinol acyltransferase, is required for the uptake of retinol into cells. Once inside the cell, enzymes such as alcohol dehydrogenase, short-chain dehydrogenase, and retinol dehydrogenase of the microsomal fraction catalyze the oxidation of retinol to retinaldehyde via a reversible reaction. This is followed by the non-reversible oxidation of retinaldehyde to retinoic acid by retinal dehydrogenase (RALDH 1, 2, 3 and 4). Most organs have the capacity for retinoic acid biosynthesis, including corneal epithelial cells. Vitamin A is transported to the eye via the ocular surface blood vessels and tears.

2.2. Synthetic production

Retinoic acid is produced synthetically for both therapeutic and research purposes. There are two main methods described in the literature. In one, the reaction products of β-ionone and γ-bromocrotonic acid are dehydrated and subsequently saponified to produce ionylidene crotonic acid I. Using lithium methyl, ionylidene crotonic acid III is transformed into C18 ketone IV. Subsequently, the C18 ketone IV reacts with methylbromoacetate, producing all-trans-retinoic acid.

The second method consists of the condensation of β-ionylideneacetaldehyde with an ester of β-methylglutaconic acid under alkaline conditions to form 4-carboxyvitamin A acid. This is decarboxylated by heating the solution in an organic base containing small amounts of copper powder. The resulting neovitamin A is isomerized with iodine to produce all-trans retinoic acid.

All-trans-retinoic acid is inherently unstable because it undergoes photoisomerization. As a result, synthetic retinoic acid, which remains stable to degradation, has been investigated. Though it is meant to have comparable activity, synthetic retinoic acid may not exert all the biological effects of all-trans-retinoic acid. Further studies are needed.

Retinol/retinyl palmitate, another derivative of vitamin A with increased stability compared to all-trans-retinoic acid, is the precursor and storage form of vitamin A and has similar effects to retinoic acid in certain conditions such as dry eyes. Briefly, vitamin A is dissolved in a mixture of ethylene chloride and pyridine. Palmityl chloride is then added. Finally, all unesterified vitamin A and remaining palmitic acid is removed from the resultant mixture.

3. Vitamin A deficiency

Ocular manifestations of vitamin A deficiency remain the leading cause of childhood blindness in developing countries, though these also occur in developed nations. A deficiency of this fat-soluble vitamin or its metabolites (e.g., retinoic acid) manifests in two ways—night blindness (nyctalopia) and a spectrum of ocular disease known as xerophthalmia. Retinoic acid promotes incorporation of glucosamine into specific glycoproteins, which is significantly reduced in xerophthalmia. Ocular changes include epidermal keratinization and squamous metaplasia of the cornea and conjunctiva, corneal ulceration, night blindness, and retinopathy. This spectrum of pathology provides an insight into the various mechanisms by which vitamin A and its metabolites exert their ocular effects.

The initial and most common ocular manifestation of vitamin A deficiency is nyctalopia, because the visual pigments of the photoreceptors are derived from vitamin A. When light strikes the retina, the 11-cis configuration is converted to the trans form, which is released by the photoreceptors to enter the retinal pigment epithelium. Here it is reconverted to the cis form. Throughout this process of phototransduction, some retinal (both cis and trans) is lost; therefore, a constant source of vitamin A is required for optimal photoreceptor function. Retinal electrophysiology can assist in the diagnosis and follow-up of vitamin A deficiency.

 Conjunctival pathology typically follows nyctalopia, but can also occur without concurrent clinical night blindness. The first sign is xerosis (dryness) caused by a marked decrease in mucous-secreting goblet cells. Epidermal keratinization and squamous metaplasia of mucous membranes results to a degree inversely proportional to serum vitamin A levels. Clinically, the conjunctiva appears thickened and wrinkled with loss of transparency. Occasionally present are Bitot spots—triangular, perilimbal foamy gray plaques of keratinized conjunctiva overlying an area of dryness. Although Bitot spots are said to be pathognomonic of current vitamin A deficiency, they may not reverse with replacement therapy.

 As the deficiency worsens, the cornea becomes involved. Instability of the precorneal tear film leads to punctate keratopathy, which progresses to epithelial defects, keratinization, and stromal edema. Left untreated, corneal epithelial defects progress to partial or full-thickness ulceration and may develop bacterial infection. Keratomalacia is full-thickness liquefactive necrosis of the cornea and, in conjunction with vitamin A deficiency, is often associated with a preceding systemic stressor such as measles or severe protein malnutrition. The corneal stroma can slough, either leaving a descemetocoele or, in severe cases, causing corneal perforation.

 Lastly, there is an uncommon condition known as the xerophthalmic fundus, characterized by numerous small yellow dots representing loss of pigment from the retinal pigment epithelium. These may be accompanied by scotomas.

 Replenishment of vitamin A stores typically results in the reversal of night blindness and the conjunctival and retinal pathology. Keratopathy without severe ulceration also responds favorably to vitamin A replenishment, whereas severe corneal ulceration leads to permanent vision loss from corneal scarring, particularly if complicated by secondary infection.

4. Mechanism of retinoic acid action

The mechanisms by which retinoic acid produces its actions can be classified into two broad categories: nuclear receptor...
mediated and non-nuclear receptor mediated. Multiple studies have demonstrated retinoic acid’s effects via nuclear receptors, suggesting this is the primary pathway.\textsuperscript{6,107} Retinoic acid binds to nuclear receptors that act as ligand-activated transcription factors, resulting in either transcriptional activation or repression of retinoid controlled genes. In comparison, less is known about the latter non-nuclear receptor mediated pathway, the mechanism by which retinoic acid exerts its action independent of nuclear receptors. These mechanisms are summarized in Table 1.

### 4.1 Nuclear receptor-mediated mechanism

The predominant mechanism by which retinoic acid exerts its effects is by regulating the transcriptional levels of target genes. Retinoic acid binds to various nuclear receptors, causing conformational changes that increase or decrease gene transcription. Some effects of transcriptional up-regulation include the promotion of ocular surface hydration,\textsuperscript{60,61} corneal epithelial healing,\textsuperscript{6} and ocular differentiation and development.\textsuperscript{187} In contrast, the transrepressive activity of retinoic acid results in a reduction of keratinization,\textsuperscript{10} protection of the cornea from dissolution,\textsuperscript{184} and suppression of oncogenic proliferation and neoplasia.\textsuperscript{97}

#### 4.1.1. Transactivation

The actions of retinoic acid are primarily mediated by two families of nuclear receptors, the retinoic acid receptor (RAR) and retinoid X receptor (RXR). Each family comprises three isotypes: RAR\textsubscript{a}, RAR\textsubscript{b}, and RAR\textsubscript{c}; and RXR\textsubscript{a}, RXR\textsubscript{b}, and RXR\textsubscript{c}, respectively.\textsuperscript{104} RAR\textsubscript{a}, RAR\textsubscript{b}, and RXR\textsubscript{a} are expressed by the human cornea and conjunctiva.\textsuperscript{117} RARs bind to all-trans-retinoic acid and its stereoisomers 9-cis-retinoic acid and 13-cis-retinoic acid; RXRs only bind to 9-cis-retinoic acid.

Retinoic acid receptors exist in various forms, either as hetero- or homodimers. The most common form through which retinoic acid exerts its effects is mediated through the RAR/RXR heterodimer. Retinoic acid binds to the RAR/RXR heterodimer causing transconformational changes, which then allows it to bind to specific promoter regions in DNA sequences known as retinoic acid response elements.\textsuperscript{107,117} The end result is an increased transcription of target genes.

Specifically, the RAR/RXR heterodimer has been shown to be responsible for up-regulating the following:

1. secretory phospholipase A\textsubscript{2} group IIA gene, which mediates the induction of the membrane-associated mucin, MUC16, an important hydrating molecule of the ocular surface.\textsuperscript{60,61}
2. CYP4B1 gene, leading to increased production of pro-inflammatory 12-HETE and 12-HETrE by the corneal epithelium, which is important for wound healing.\textsuperscript{4}
3. AP-2 transcription factor, which is an important regulator of gene expression during morphogenesis and may be essential for ocular development.\textsuperscript{187}

In addition to these, the hyaluronic acid synthetase (HAS) gene may be up-regulated by retinoic acid receptors. Produced by skin cells, corneal epithelial cells, and keratocytes, hyaluronic acid has been shown to promote corneal epithelial healing by stimulating migration, adhesion, and proliferation of the corneal epithelium.\textsuperscript{118} Retinoic acid, through its action of increasing hyaluronic acid production via up-regulation of the HAS gene, is therefore an important factor for corneal epithelial healing.

Retinoic acid also stimulates the DNA-binding activity of NF-\kappaB,\textsuperscript{188} a DNA transcription factor involved in the synthesis of pro-inflammatory cytokines such as tumor necrosis factor \(\alpha\) (TNF-\(\alpha\)) and interleukin 1\(\beta\) (IL-1\(\beta\)), in addition to being required for corneal healing.\textsuperscript{72} Also, retinoic acid induces the expression of genes required for differentiation of embryonal carcinoma cells by activation of NF-\kappaB.\textsuperscript{137} Retinoic acid therefore may play an important role in corneal healing and differentiation through the actions of NF-\kappaB.

RXRs can act as a homodimer on transcription activity via the retinoid X response element or form heterodimers with several nuclear receptors to exert its effects on thyroid.

### Table 1 – Summary of the mechanism of retinoic acid action

<table>
<thead>
<tr>
<th>Nuclear receptor mediated</th>
<th>a. Transactivation of genes</th>
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<tbody>
<tr>
<td>1. Secretory phospholipase A\textsubscript{2} group IIA\textsuperscript{39,40}</td>
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</tr>
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<td>2. CYP4B1 gene\textsuperscript{42}</td>
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<td></td>
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</tr>
<tr>
<td>7. Hyaluronan synthase 2\textsuperscript{52}</td>
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<tr>
<td>8. Lipocalin 2\textsuperscript{52}</td>
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<tr>
<td>9. G protein-coupled estrogen receptor 1\textsuperscript{12}</td>
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<tr>
<td>10. Angiopoietin-like 4\textsuperscript{52}</td>
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<tr>
<td>11. Insulin-like growth factor binding protein 3\textsuperscript{52}</td>
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<td>12. Matrix metalloproteinase 9 in meibomian gland cells\textsuperscript{72}</td>
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<td>11. Centromere protein M\textsuperscript{12}</td>
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<tr>
<td>12. Meiosis-specific nuclear structural 1\textsuperscript{52}</td>
</tr>
<tr>
<td>13. Nicotinamide N-methyltransferase\textsuperscript{52}</td>
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<th>Non-nuclear receptor mediated</th>
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AP, activator protein; cAMP, cyclic adenosine monophosphate; CYP4B1, cytochrome P450 4B1; ERK, extracellular signal regulated kinase; HAS, hyaluronan synthase; NF-\kappaB, nuclear factor kappa light chain enhancer of activated B cells; TNF, tumor necrosis factor.
hormone receptors, peroxisome-proliferator activated receptors, and vitamin D receptors.107 Because of the potential to form varying heterodimers in addition to RAR/RXR, retinoic acid appears to affect signaling pathways independent of RAR signaling. Some examples include the induction of apoptosis and differentiation of cancer cells in the presence of cAMP.9,60

Ding et al28 recently explored the role of retinoic acid on gene expression in immortalized human meibomian gland epithelial cells. They discovered that retinoic acid affected a large number of genes either through transactivation or transrepression (Table 2). Retinoic acid increased the expression of genes encoding inflammatory mediators such as interleukin 1,8,15, and 36Y that could promote conjunctival inflammation. Additionally, genes involved in the regulation of cell death such as matrix metalloproteinase 9 and interleukin 1β were also up-regulated. Conversely, there was decreased expression of gene-associated cell proliferation such as exonuclease 1 and centromere protein M.28

4.1.2. Transrepression

Besides increasing gene transcription, RARs also have transrepressive activity, including:

1) reduction of keratin production by binding to the receptor response elements of keratin gene promoters.207
2) formation of an inactive complex with c-Jun, an activator of the collagenase gene, inhibiting collagenase production. A lack of retinoic acid therefore results in excess collagenase activity, causing enzymatic dissolution of corneal stromal collagen.184
3) possible down-regulation of matrix metalloproteinase 13 production, which plays a role in the pathogenesis of ocular surface squamous neoplasia.89
4) down-regulation of activator protein 1 (AP1) transcriptional activity, which is responsible for genes involved in oncogenic proliferation and cellular proliferation. Several explanations have been put forth to explain this, one of which is the interaction of RAR with c-Jun, preventing it from binding to AP1 binding sites.95 This relationship between retinoic acid and AP1 is of particular interest because of its use as adjuvant chemotherapy for conjunctival neoplasia.

4.2. Non-nuclear receptor mediated mechanism

More recently, it has been found that retinoic acid also appears to mediate its effects through non-nuclear mechanisms. These include binding to extra-nuclear retinoid receptors, retinoylation (modifications to proteins after binding to retinoic acid), activation of or interaction with other signaling molecules, and the mediation of effects via its metabolites.

Multiple studies have demonstrated the effects of retinoic acid in binding to extra-nuclear retinoid receptors.10,22,99 Some of these extra-nuclear receptors have been localized to axonal and dendritic neuronal membranes and membrane lipid rafts.126 Some of these actions include induction of rapid dendritic growth and increased surface expression of glutamate receptor 122 and an increase in frequency of spontaneous neurotransmitter release.22

In retinoylation, the formation of covalent bonds with retinoic acid modifies biochemical properties of target proteins and influences their activity.107 Multiple retinoylated proteins have been detected, including the RII and RII regulatory subunits of cAMP-dependent protein kinase. This may explain why retinoic acid increases cAMP-dependent protein kinase activity. The RII subunit is associated with regulatory proteins, which may explain retinoic acid’s effect on cell proliferation.142 Additionally, retinoylation reduces available retinoic acid for nuclear-receptor mediated action.

Another non-genomic rapid mechanism is the activation of extracellular signal-regulated kinase (ERK1/2), causing a cascade of events that culminates in the transcription of late genes involved in growth arrest and differentiation.19 This was confirmed by two recent studies: A mouse model confirmed that retinoic acid increases ERK1/2 phosphorylation22 and an in vitro study demonstrated that retinoic acid inhibits human scleral fibroblast proliferation by a mechanism associated with modulation of ERK1/2.150

Lastly, retinoic acid has the potential to act via its metabolites, for example, retinoyl β-glucuronide and 4-hydroxyphenyl retinamide. In animal models, these metabolites inhibit mammary cell proliferation, promote differentiation of vaginal epithelium, and stimulate apoptosis of tumor cell lines.36,131

| Table 2 – Summary of the effects of vitamin A on the ocular surface |
|-----------------------------|------------------|
| **Ocular structure**       | **Effect**       |
| Cornea                     | Required for correct differentiation of epithelial cells80,4 |
|                           | Reverse corneal keratinization32,70 |
|                           | Speeds up re-epithelization of cornea76 |
|                           | Helps preserve epithelial barrier function93 |
|                           | Increases stromal keratocyte numbers in vitro, potentially increasing corneal wound healing and strength54 |
| Conjunctiva                | Reduction of conjunctival keratinization and reversal of squamous metaplasia25,83 |
|                           | Inhibits conjunctival fibroblast proliferation74,75 |
|                           | Increases goblet cell numbers (dose >1,000 IU/ml)76 |
| Limbus                     | Maintenance of goblets cells and reversal of conjunctival surface keratinization (high concentrations)98 |
|                           | Conversion of secretory epithelium into squamous epithelium (low concentrations)98 |
| Meibomian gland            | Decrease meibomian gland function, hyposcretion of meibomian lipids, acini atrophy, disruption of tear film osmolality and increased tear evaporation.32,132–343 |

a Excess concentration results in abnormal differentiation.81,82
In summary, retinoic acid modulates its effects through either nuclear-receptor or non-nuclear receptor mediated mechanisms. Understanding this has allowed better characterization of the actions of topical retinoic acid, which include:

1) corneal epithelial cell repair via increasing production of hyaluronic acid and pro-inflammatory cytokines.
2) maintenance of ocular surface hydration via production of MUC16.
3) apoptosis, cellular differentiation, and repression of oncogenic proliferation.
4) reduction of keratinization of ocular surface epithelium.

5. Wound healing

5.1. Full thickness penetrating corneal wounds

Studies on rabbit eyes show that the application of retinoic acid increases the tensile strength of full thickness corneal wounds. Mehra’s group created a central corneal incision and compared the use of placebo drops against six variations of treatment: dexamethasone drops with and without vitamin A, subconjunctival dexamethasone with and without subconjunctival vitamin A, and dexamethasone drops and subconjunctival injection with and without subconjunctival vitamin A. All variations showed that the addition of vitamin A significantly increased tensile strength (P < 0.01). Kasti et al. created a corneoscleral incision and assessed tensile strength between placebo and wounds treated with retinol palmitate in two doses, 0.1% and 0.5%. In both the open application and the double-blinded study, a significant increase in tensile strength was demonstrated with the use of the 0.1% preparation (P < 0.05). Interestingly, the 0.5% application (n = 5) did not show any benefit to wound healing and tensile strength, and it was postulated that the higher dose may have an inhibitory effect. More recently, experiments on stromal keratocytes in serum-free medium have shown that retinoic acid at an ideal concentration of 10 × 10^{-6} M (equivalent to 33%) increased keratocyte numbers, which may account for the clinical findings of improved corneal healing and tensile strength. At higher concentrations, retinoic acid became toxic to keratocyte proliferation, mirroring the decreased wound healing noted previously.

5.2. Non-penetrating corneal wounds

Retinoic acid increases the rate of corneal epithelial wound healing. This was consistently demonstrated with the use of all-trans-retinoic acid, though variable results have been reported with retinol palmitate. Interestingly, higher doses of retinoic acid actually have an inhibitory effect.

As early as 1954, Agarwal et al. demonstrated in rabbits that the addition of intramuscular vitamin A accelerated the healing time for both superficial and deep non-penetrating corneal wounds while also decreasing the density of scar formation. This led the group to use intramuscular vitamin A in three groups of human patients: those with non-sloughing, sloughing, and hypopyon-associated corneal ulcers. All three groups, compared with the standard treatment, demonstrated faster healing time and reduced scar formation.

5.3. Corneal epithelial defects

Topical application of retinoic acid in animal studies benefits epithelial healing time. Of note, Ubels et al. compared the healing rate of epithelial defects with a placebo against five variations of topical retinoic acid: all-trans-retinoic acid, all-trans-retinol, 13-cis-retinoic acid, retinyl palmitate, and retinyl acetate. A benefit was only found with the use of all-trans-retinoic acid at a concentration of 0.1%. This effect increased with frequency of application; however, like previous results reported by Mehra et al., when a 0.25% concentration was used no benefit was seen.

More recently Vetrugno et al. examined the role of supplementary oral vitamin A and vitamin E in re-epithelialization time and corneal haze formation at 1 year post photorefractive keratectomy. Corneal re-epithelialization rates were significantly improved (P = 0.029) with the addition of the oral vitamin supplements, and there was a reduction in the formation of corneal haze (P = 0.035). This was more pronounced in the higher myopic correction group (P < 6 diopters, P = 0.043). Interestingly, there was a trend towards better uncorrected visual acuity with oral vitamin A and E supplementation (P = 0.043), and this may be secondary to the faster epithelialization and healing leading to less oxidant-inflicted damage and subsequent lower corneal haze formation.

The evidence that retinoic acid increases corneal epithelial healing has led in recent years to the development of novel methods of delivery of all-trans-retinoic acid, including egg-shaped nanoparticles. Preliminary results in rabbits indicate that there is significantly faster wound healing with exposure to nanoparticle retinoic acid compared with placebo or hyaluronic acid 0.1% (P < 0.05 for both). Concurrent studies by the same group using human corneal epithelial cell culture showed earlier wound healing, but suppressed cellular proliferation, with nanoparticle retinoic acid. Higher concentrations of the nanoparticle delivered retinoic acid was both cytostatic and cytotoxic, which was not demonstrated at lower doses and is in keeping with earlier findings that higher doses of retinoic acid did not aid corneal epithelial healing.

6. Cell differentiation

6.1. Cornea

Consistently, both animal and human studies indicate that the use of retinoic acid improves corneal keratinization and the histological appearance of corneas that have undergone hyperkeratotic differentiation; however, excess retinoic acid may induce abnormal differentiation.

Early animal studies showed that retinoic acid 0.1% reverses corneal keratinization and improves the histological appearance of the cornea. Further, a lower concentration of 0.01% was equally efficacious. Interestingly, in a small proportion of animals, a crossover effect was noted, with improvement of the surface keratinization in the un-treated contralateral eye. This was thought to be...
secondary to systemic absorption. Studies using retinol palmitate (1,500 IU/mL) also found that the corneal surface re-epithelialized faster than placebo. 120

In his case series, Wright showed an improvement in persistent epithelial defects with the use of 0.1% retinoic acid at bedtime. As a benefit, while treating conjunctival keratinization, he noticed that the corneal surface became flatter, more wettable, and regular. Herbert et al.156 demonstrated an improvement in surface keratinization with 0.05% retinoic acid that was maintained with a 0.01% ointment. He argued that the degree of surface keratinization can be used as a guide to the effectiveness of retinoic acid.156 Similar beneficial results were seen in a larger cross-over trial (n = 55) with the use of 0.01% all-trans-retinoic acid at bedtime.551

Recent studies indicate that not only does retinoic acid need to be present for correct corneal epithelial differentiation, but also it has to be at the correct concentration. Kim et al.158 demonstrated that an absence of all-trans-retinoic acid on human corneal limbal epithelial cell culture resulted in excess surface keratinization, whereas concentrations of 10^{-8} M (equivalent to 0.03%) resulted in a normal appearance, preserving cell morphogenesis, polarity, and membrane-associated mucin expression (a marker of functional differentiation). Excess retinoic acid at a concentration greater than 10^{-6} M (equivalent to 3.3%), however, induced abnormal differentiation, poor polarity, and increased mucin staining.391 Further, epithelial barrier function was preserved in the presence of retinoic acid.820 Thus, vitamin A plays a pivotal role in the differentiation of corneal epithelial cells.

6.2. Conjunctiva

Retinoic acid helps improve conjunctival keratinization and may play a key role in controlling conjunctival fibroblast activity, which has implications for cicatrizing conjunctival diseases.

Animal studies have demonstrated an improvement in conjunctival histology with the reappearance of goblet cells, a reduction in surface keratinization, and even a reversal in squamous metaplasia with the use of topical retinoic acid.165,170 In humans, Tseng published a small case series of patients with keratoconjunctivitis sicca, Stevens-Johnson syndrome, pseudo-pemphigoid, and surgically induced dry eye whose conjunctival impression cytology improved with the use of retinoic acid.166 In the same year, Wright185 published a small case series where the application of topical retinoic acid 0.1% at bedtime reversed the conjunctival keratinization associated with Stevens-Johnson syndrome. More frequent applications (four times daily) were quickly shown to be toxic to the ocular surface. Best results were noted in patients with focal areas of metaplasia. The greatest factor in restoring the ocular surface was thought to be the effect of retinoic acid on cornea and conjunctival glycoprotein synthesis. Since then, many case reports have supported the efficacy of retinoic acid in controlling and even reversing squamous metaplasia while also increasing the number of conjunctival goblet cells.54–56,86,185

Animal studies have also showed that retinoic acid inhibits conjunctival fibroblast proliferation in a cytostatic rather than a cytotoxic way, significant for cicatrizing diseases.172,176

Ubels and colleagues attempted to show an inhibitory effect using human conjunctival fibroblasts and were able to demonstrate this on patients with ocular cicatricial pemphigoid and Stevens-Johnson syndrome, but not in normal eyes.172 These results, however, are not consistent across the literature.132

Studies using retinol palmitate show that higher concentrations (1,500 IU/mL) are able to improve goblet cell numbers compared with placebo in a dose-dependent manner. This was not demonstrated at 500 IU/mL, but was seen to a lesser extent using 1,000 IU/mL.120

6.3. Limbus

The role of retinoic acid at the limbus is complex. Not only is the presence of retinoic acid vital for correct limbal differentiation, but also it has to be at the correct concentration. When injury has denuded the limbal stem cells, conjunctival cells grow across the limbus onto the cornea to heal the epithelial defect. The cell composition in this situation is to a great part controlled by retinoic acid.

Conical re-epithelialization primarily occurs from the limbal stem cells. Retinoic acid preferentially differentiates limbal stem cells into transient amplifying cells that go on to epithelialize the cornea.15,91,92,141,157 Recent studies have shown that retinoic acid is needed at an ideal concentration (10^{-9} to 10^{-7} M; equivalent to 0.003% to 0.3%) for normal expression of limbal progenitor cell markers.80,91 Because one of the primary sources of retinoic acid is the limbal vasculature, in a normal eye the limbal vessels play an important role in providing the concentration of retinoic acid that allows normal differentiation of limbal stem cells into terminally differentiated epithelial cells.43,64,91,129,167

In cases of injury where the limbal stem cells are irreparably damaged, a process of transdifferentiation occurs. In these cases, conjunctival cells undergo a differentiation process whereby goblet cells are lost and a cornea-like morphology is adopted.40,90,139,168,169 Retinoic acid, derived from the limbal blood vessels, plays a pivotal role in this transdifferentiation process.100,160,166,170 Higher concentrations of retinoic acid (>0.01%) favor the maintenance of goblet cells (and hence the reversal of conjunctival surface keratinization), whereas relatively low concentrations result in the conversion of secretory epithelium into squamous epithelium (resulting in the squamous metaplasia seen in xerophthalmia).34,102,130,163,168–170 The avascularity of the cornea results in a relatively low retinoic acid microenvironment that allows the transdifferentiation of the conjunctival epithelium into cornea-like epithelium; this transdifferentiated cornea-like epithelium is not true corneal epithelium, however, as there are differences in histological and ultrastructural appearance, as well as in its metabolism and composition of proteins and keratins.17,48,82,90,155 Tseng et al.165 showed in a rabbit model that 13-cis retinoic acid prevents the normal transdifferentiation process across the limbus, resulting in persistent goblet cells on the corneal surface. These findings were confirmed with the use of 0.1% all-trans-retinoic acid, with similar staining patterns between the conjunctiva and cornea.41,83,84,139,156 Later, Tseng et al.166 demonstrated a reversal of the transdifferentiation process.
using 0.1% all-trans-retinoic acid, confirming the link between retinoic acid and transdifferentiation across the limbus.

In the presence of viable limbal stem cells, retinoic acid acts to encourage corneal regeneration from this source; all the stem cells are destroyed, however, re-epithelialization occurs across the limbus from conjunctival cells and is once again influenced by retinoic acid.

6.4. Anti-tumor effect

Studies have shown a positive effect of all-trans-retinoic acid in reversing squamous metaplasia.

Early animal studies demonstrated that retinoic acid was active against epithelial neoplasms.\textsuperscript{12,13,103} Wright\textsuperscript{135} was the first to show in humans that retinoic acid 0.1% once daily decreased surface keratin and caused a limbal conjunctival intraepithelial neoplasia with moderate to severe dysplasia to become flatter and more regular in contour. It was postulated that retinoic acid changes keratinocyte membrane glycoconjugates and that this may alter intracellular adhesions that control growth. Epidemiological data suggest that retinoids reduce the chances of cutaneous neoplasia.\textsuperscript{71}

Herbert et al\textsuperscript{56} also noted an improvement in the keratinization and a reduction in size of a conjunctival intraepithelial neoplasia lesion with the use of 0.05% retinoic acid. After cessation of drops at 8 weeks, the lesion began to recur. With an intraepithelial neoplasia with moderate to severe dysplasia, Herbert et al\textsuperscript{56} used 0.05% retinoic acid indefinitely to control recurrences. These applications indicate that retinoic acid has the potential to contain, but not cure, neoplastic lesions.\textsuperscript{153}

Since then, many authors have demonstrated the ability of retinoic acid, in synergistic combination with other agents such as interferon, to treat and sometimes even reverse squamous metaplasia in the eye\textsuperscript{10,152,171,172,185} and other areas of the body, including the skin, cervix, and breast cancer cell lines.\textsuperscript{3,5,9,78,113,127} Currently, interferon alpha-2b has been combined with retinoic acid 0.01% twice daily to treat ocular surface dysplasias.\textsuperscript{143}

6.5. Meibomian glands

The main limiting factor to the use of retinoic acid is its dramatic effect on meibomian glands. Animal, human, and immortalized human cell studies have shown a direct effect in inhibiting cell proliferation, inducing cell death, and altering the expression of thousands of genes, including those for keratinization and inflammation. Oral retinoic acid was first used for the dermatological treatment of severe acne because it decreases the growth and function of skin sebaceous glands.\textsuperscript{114,188} Given that meibomian glands are large sebaceous glands, these effects are not surprising.

Studies in animals and humans have shown that systemic use of retinoic acid decreases meibomian gland function causing atrophy of the acini and hyposcretion of oil, affecting tear osmolarity and evaporation, leading to significant dry eye symptoms.\textsuperscript{11,14,16,25,28,32,38,39,46,59,88,90,94,110,124} Studies in immortalized human meibomian gland epithelial cells have shown dose-dependent effects, with up-regulation of genes for inflammatory mediators (e.g., interleukin 8 and 1β) and proteases (e.g. matrix metallopeptidase 9) and down-regulation of the genes for tyrosine kinase signaling and cell division.\textsuperscript{28} These findings were reversed with cessation of retinoic acid.\textsuperscript{14,35}

Interesting, certain genes (e.g., small proline-rich proteins 2F, 2D, and 1B)\textsuperscript{28} that are known to promote keratinization,\textsuperscript{07,67,68,74,98} the primary cause of meibomian gland disease,\textsuperscript{47,119} were up-regulated by 13-cis retinoic acid, in contrast to the findings in corneal and conjunctival cells.

7. Dry eye

Dry eye disorders can be categorized as either arising from aqueous deficiency, mucin deficiency, meibomian gland dysfunction, or a combination of these.\textsuperscript{27} More recently, it has been realized that meibomian gland dysfunction is likely to be the most frequent cause of dry eye secondary to increased evaporation of the aqueous layer.\textsuperscript{36,30,62,85,95,108,142,158}

Early studies in dry eye focused on conjunctival changes, where a loss of conjunctival goblet cells, abnormally enlarged non-goblet cell epithelium, increased cellular stratification and keratinization was noted.\textsuperscript{14,15,115,116} These changes are referred to as squamous metaplasia and represent abnormal differentiation of the normal secretory conjunctival epithelium into a keratinized, non-secretory epithelium. Ocular symptoms of dry eyes may in part be attributed to squamous metaplasia, which also causes tear film instability.

Initially, it was thought that squamous metaplasia resulted from damage to local blood vessels from scar formation or inflammation, resulting in a vitamin A deficient local micro-environment.\textsuperscript{164} By replacing vitamin A it was hoped that the signs and symptoms of dry eye would improve. Early case reports using all-trans-retinoic acid 0.01–0.1% documented success in improving symptoms in keratoconjunctivitis sicca, Stevens-Johnson syndrome, pemphigoid, pseudopemphigoid, and surgery- or radiation-induced dry eye.\textsuperscript{164,165,171} On impression cytology there was a reversal of squamous metaplasia along with an increase in goblet cell density, critical for the improved comfort and wetting of the ocular surface. One study reported no symptomatic relief with the use of retinoic acid for 1 week in 22 subjects; however, clinical signs of dry eyes (Schirmer test and tear break-up time) were improved.\textsuperscript{38} Not all studies using all-trans retinoic acid have been able to reproduce these positive results. Two studies reported no benefit of 0.01% all-trans-retinoic acid compared to placebo in patients with keratoconjunctivitis sicca, and even reported a patient preference for the placebo, highlighting the potential ocular irritation of retinoic acid.\textsuperscript{42,151} Studies have also compared retinol palmitate 0.05% with cyclopentolate A 0.05% for treating the inflammatory component of dry eye disease.\textsuperscript{79} In a study of 150 patients, an equally significant improvement was noted in dry eye symptoms, tear break-up time, Schirmer test values, corneal staining, cytology grading, and goblet cell densities between retinyl palmitate and cyclopentolate compared with placebo. A similar positive result was documented for the synthetic retinoic acid analog CBS-211 A.\textsuperscript{29}
The recent emphasis in dry eye disease is meibomian gland dysfunction. Hyperkeratinization of the orifice and ductal epithelium, leading to meibomian gland obstruction, is considered the primary cause of meibomian gland disease.\textsuperscript{37,44,47,58,85,87,109,122,142} This directly results in decreased meibomian lipids on the ocular surface, resulting in an unstable tear film and evaporative dry eye conditions. Further, the increased internal pressure from the obstruction leads to atrophic changes and squamous metaplasia within the meibomian acini, causing a secondary hyposecretion. This was discussed in detail in the 2011 International Workshop on Meibomian Gland Dysfunction.\textsuperscript{35} As discussed earlier, retinoic acid severely exacerbates meibomian gland dysfunction. The mixed results seen in earlier studies that focused on squamous metaplasia may be a result of these negative effects.

Thus, when treating patients with dry eye, the clinician should first identify whether the cause is meibomian gland dysfunction. In such cases, retinoic acid will likely exacerbate symptoms; in cases where ocular surface keratinization is the predominant mechanism, however, there may be a role for retinoic acid. Continued monitoring of dry eye symptoms and examination of the meibomian glands are recommended to try to balance the positive effects of vitamin A on surface keratinization against its negative effects on the meibomian glands.

8. Recent developments

Various studies have shown that retinoic acid is involved in the regulation of photoreceptor differentiation and development,\textsuperscript{26,155} lens development and regeneration,\textsuperscript{108,163} barrier function and transdifferentiation of retinal pigment epithelial cells,\textsuperscript{50,26,105,134,180} prevention of microphthalmia,\textsuperscript{96} and possibly in the establishment of immune tolerance in the eye.\textsuperscript{75} Other studies have explored potential new applications for retinoic acid. In mouse models, retinoic acid decreased the severity of optic neuritis and autoimmune uveoretinitis.\textsuperscript{76,77} In vitro, retinoic acid administration inhibited conjunctival scarring through attenuation of the contractility of Tenon fibroblasts\textsuperscript{101}; retinoic acid also inhibited human lens epithelial cell proliferation, raising the possibility of its use for posterior capsular opacification.\textsuperscript{106}

9. Dosage of retinoic acid

Successful treatments used all-trans-retinoic acid 0.01–0.1% (Table 3).\textsuperscript{5,12,13,35,41,50–56,70,80,83,84,91,105,111,125,138,139,143,145,146,151,153,156,168,171–174,176,178,185} Higher doses (0.25%) were found to be ineffective.\textsuperscript{5,91,120,173} As the dosage and frequency of application increased, the likelihood of developing ocular irritation in the form of meibomian gland disease and blepharoconjunctivitis increased, though this was reversible with discontinuation of the retinoic acid. Subsequent studies set out to use the lowest concentration and dosage regime of all-trans-retinoic acid while maintaining its effect. Studies initially began with three times a day to four times a day dosage frequencies, which was decreased to once daily or even every second day as symptoms improved (typically after 2–3 weeks).\textsuperscript{164,165,171}

| Table 3 – Therapeutic indications/effects of topical vitamin A in the eye |
|------------------------|------------------|
| Therapeutic indication | Dose of all-trans-retinoic acid |
| Ocular surface wound healing\textsuperscript{56,60–63,65} | 0.1%\textsuperscript{a,b} |
| Decrease surface keratinization\textsuperscript{56,70,72} | 0.01–0.1%\textsuperscript{b} |
| Moderate to severe dry eye\textsuperscript{76,77} | 0.01–0.1%\textsuperscript{b} |
| Potential anti-tumor effect\textsuperscript{96,96} | 0.01–0.1%\textsuperscript{b} (currently used alternate days) |

\textsuperscript{a} Early studies used a higher dose (0.1%) with no recent studies using a lower dose. It is possible that a lower dose may be equally efficacious.

\textsuperscript{b} The lower the dose the lower the side effect profile.

Fewer studies have used retinol palmitate. Early results indicate that it is effective only within a certain concentration, with low doses (500 IU/mL up to 1,000 IU/mL)\textsuperscript{120} and high doses (0.5%)\textsuperscript{3} being ineffective. Studies used retinol palmitate 1,000 IU/mL four times a day for 4 weeks with good results and fewer side effects, though a dose response was noted.\textsuperscript{25,79,86,120,121}

10. Conclusion

Vitamin A plays an important role in ocular surface maintenance. Vitamin A deficiency leads to ocular surface keratinization and squamous metaplasia, which can lead to vision loss unless reversed with systemic vitamin A supplementation.

Recent investigations into the role of topical vitamin A drops to improve a damaged ocular surface, in subjects who are not vitamin A deficient, have generally shown a positive result. In both animal and human models, most studies demonstrate that vitamin A supplementation can improve the rate of corneal wound healing, and reverse corneal and conjunctival keratinization and squamous metaplasia. Vitamin A has a role in encouraging limbal stem cells to preferentially differentiate to corneal epithelial cells, and can be used in clinical situations of limbal stem cell failure. Vitamin A may also have an anti-tumor effect. Finally, vitamin A has generally been shown to play a role in treating ocular surface keratinization, being able to reverse keratinization and improve goblet cell density.

Retinoic acid severely negatively affects meibomian gland function, however, and can worsen dry eye disease. Retinoic acid is contraindicated in meibomian gland disease causing dry eye. Taken together, clinicians must balance the positive effect on corneal and conjunctival cell differentiation against the negative effect on meibomian glands. Thus, there seems to be a specific place for retinoic acid use, especially if surface keratinization is present, with the risk of exacerbating dry eye symptoms. Continual monitoring of patient dry eye symptoms and meibomian gland function is recommended while on retinoic acid.

The method of drug delivery has been an area of contention. Most studies used all-trans-retinoic acid, the active form of vitamin A, to show the positive results described herein.
This form, however, is relatively unstable, difficult to make and store, and prone to ocular surface side effects, mainly blepharoconjunctivitis. In an attempt to reduce side effects, the lowest dose and frequency is used, typically 0.01% as a daily or twice-daily dose.

More recently, retinol palmitate, which is a precursor of all-trans-retinoic acid, has been investigated. Retinol palmitate is more stable and gentler to the ocular surface than all-trans-retinoic acid. Early reports from the few studies conducted utilizing retinol palmitate indicate that it is as effective in reversing squamous metaplasia and improving dry eye if used at the lowest dose and frequency is used, typically 0.01% as a daily or twice-daily dose.

11. Methods of literature search

A search of the MEDLINE and Cochrane Library databases were conducted independently by two authors (CS, SC) during August 2014 using the following key words: retinoid acid, vitamin A, retinoids, retinyl palmitate, eye, ocular surface, meibomian gland, ophthalmic use. The search covered all published literature until July 2014. The review was limited to peer-reviewed papers published in English including all age groups. The two reviewers also hand-searched bibliographies of identified studies for further references. Search results were collected and abstracts screened independently by both authors to remove any articles clearly not relevant to the topic. Total references for the review is 187.

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