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Review paper

Therapeutic applications of retinoids in ophthalmology

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Abstract

Vitamin A was used to treat night blindness by Egyptian physicians as early as 1500 BC. All compounds that exhibit pharmacological and physiological properties of the basic vitamin A alcohol moiety are nowadays referred to as retinoids. Retinoids have been investigated for use in the treatment of various ocular diseases such as xerophthalmia, corneal wounds, dry-eye disorders and even proliferative vitreoretinopathy. In the beginning, retinoids were administered topically by mixing them with oils or through ointments. However, more recently, the emphasis has been on developing systems for controlled release of retinoids, mainly by using biopolymers. Topical application has a great advantage in that it will overcome some of the serious side effects of systemically or orally administered retinoids. Along with the development of controlled release systems, new derivatives of currently available retinoids have been developed in order to achieve a better drug. This review summarizes various publications dealing with the local administration of retinoids either by topical route or by intravitreous route in animals and humans to treat various ocular diseases. © 1997 Elsevier Science B.V.

Keywords: All-*trans*-retinoic acid; 13-*cis*-Retinoic acid; Corneal wounds; Dry-eye disorders; Proliferative vitreoretinopathy; Vitamin A deficiency

1. Introduction

Egyptian physicians described night blindness as early as 1500 BC. They may have obtained this information from medical sources from as far back as 2500 BC. Of course, the Egyptians did not know about vitamin A or that vitamin A deficiency could be responsible for night blindness. They treated the disease by instilling juice of compressed liver onto the surface of the eye. Hence, unknowingly, they treated the disease by

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topical application of vitamin A (Maumenee, 1993; Marcus and Coulston, 1995). Since this use of vitamin A, science has come a long way. Today it is well established that night blindness can be caused by vitamin A deficiency and may be treated by administering vitamin A.

Vitamin A comprises those compounds or series of β-carotene derivatives which are necessary for maintenance of vision, reproduction, and general epithelial growth and differentiation. Strictly speaking, vitamin A refers to retinol only. However, currently the term is used for any compound that exhibits pharmacological and physiological properties of the basic vitamin A alcohol moiety. All such compounds are referred to as retinoids, and include retinol, retinoic acid (RA), etc. (Chader, 1984; Marcus and Coulston, 1995).

Retinoids play an important role in vision. The retina contains retinoic acid. So far, no specific function has been determined for retinoic acid in the adult retina. However, the intraretinal synthesis of retinoic acid and the presence of its binding protein in retinal neurons and/or in Muller cells suggest that retinoic acid plays a role in the retina (Edwards et al., 1992). When humans have a vitamin A-deficient diet, their dark adaptation gradually diminishes. Insufficient amounts of vitamin A in the diet can cause keratomalacia, which is characterized by ulceration and xerosis of the cornea and the conjunctiva. If the vitamin A deficiency is not corrected, it can lead to severe visual impairment and blindness.

Vitamin A deficiency is treated with oral or systemic administration of vitamin A. When the effects of serious deficiency are observed in the eyes, as in subtropical countries, it is advisable to include local application in the treatment. Topical application of vitamin A is more effective in the ophthalmic field than oral or intravenous administration. For decades, vitamin A has shown good results when used in the treatment of different diseases of the lids such as chronic eczema, and injuries as well as different diseases of the cornea and the conjunctiva such as burns, keratoconjunctivitis sicca and cicatricial eye surface disorders. Retinoids have also shown beneficial effects on injuries and burns of the conjunctiva (De Grosz, 1939).

Retinoid deficiency may alter the pathogenicity of infectious agents. The mechanism by which this occurs is not yet clear. However, it can be presumed that a compromise in the integrity of the epithelial barrier may play a significant role in the apparent vulnerability of the ocular surface to infection. El-Ghorab et al. (1988) found out that early retinoid deficiency slowed down the healing capacity of the ocular surface epithelium. They observed that topically applied retinoids stimulated the healing rate of experimental corneal epithelial wounds in normal rabbits. Retinoids stimulate glycoprotein synthesis in the corneal epithelium as well as the attachment of epithelial cells *in vitro*. Continued glycoprotein synthesis is required for sustained corneal epithelial migration following wounding. Topically applied retinoids may exert their effect on the process of ocular surface epithelial defect closure by modulating cell membrane glycoprotein synthesis. Scientists continue to study the exact role of retinoids in the visual cycle so that they can be effectively used to treat diseases of the eye.

There has been debate about oral and systemic versus topical administration of retinoids to treat various ocular diseases. It is interesting to see how local applications of retinoids have changed over the years (Table 1). Most of the emphasis has been placed on the administration of retinoids by mixing them with oils or through ointments. However, the current strategy is moving toward the use of polymeric delivery systems and the development of systems for controlled release of retinoids. In addition, they are being used to treat complex intraocular diseases such as proliferative vitreoretinopathy. In this review, we have tried to summarize work done on local administration of retinoids to cure several ocular diseases.

2. Xerophthalmia

Xerophthalmia is caused by a deficiency of vitamin A. It is a major cause of childhood blindness in many developing countries. The World Health Organization has divided this disease into five main stages: night blindness, conjunctival xerosis, conjunctival xerosis with Bitot's spot,

Table 1
Examples of use of retinoids over the years

Decade	Investigators	Drugs used	Vehicles	Diseases	Model	Year
1930s	De Grosz	Vitamin A	Oil, ointment	Chronic eczema, burns of cornea and conjunctiva, keratitis, herpes of cornea, dystrophies, etc.	Humans	1939
1970s	Pirie	Retinoic acid	Oil	Xerophthalmia	Humans	1977
	Sommer et al.	Retinoic acid	Oil	Xerophthalmia	Humans	1978
	Smolin et al.	Tretinoin	Ointment	Topical wound healing	Rabbits	1979
1980s	Sommer	Retinoic acid	Oil	Xerophthalmia	Humans	1983
	Van Horn et al.	Retinoic acid	Oil	Xerophthalmia	Rabbits	1981
	Hatchell et al.	Retinol, tretinoin, etretinate	Oil	Xerophthalmia	Rabbits	1984
	Ubels et al.	Retinoic acid, 13-*cis*-retinoic acid, retinol, retinal acetate, retinyl palmitate	Oil	Corneal healing	Rabbits	1983
	Tseng et al.	Retinoic acid	Ointment	Ocular surface squamous metaplasia	Humans	1985
	Herbort et al.	Retinoic acid	Ointment	Metaplastic and dysplastic keratinization	Humans	1988
	Stonecipher et al.	All-*trans*-retinoic acid	Ointment	Corneal and limbal changes	Rabbits	1988
1990s	Driot and Bonne	Synthetic retinoic acid analog	Aqueous vehicle	Keratoconjunctivitis sicca	Rabbits	1992
	Araiz et al.	Retinoic acid	Oil	PVR	Rabbits	1993
	Giordano et al.	Retinoic acid	PLGA microspheres	PVR	Rabbits	1993

corneal xerosis, corneal xerosis with ulceration, and keratomalacia.

There has been debate regarding the primary role of vitamin A deficiency as a cause of xerophthalmia. Xerophthalmia is a disease primarily of early childhood. It is often difficult to conclude that vitamin A deficiency is the primary cause of xerophthalmia when this disease occurs in association with severe malnutrition, systemic illness or secondary, irreversible corneal changes and infections (Carter-Dawson et al., 1980; Sendele et al., 1982; Seng et al., 1982). Investigators working to cure xerophthalmia have tried to find evidence that can identify vitamin A deficiency as a primary or secondary cause of xerophthalmia (Valenton and Tan, 1975; Sommer et al., 1980, 1981; Sommer and Sugana, 1982; Brooks et al., 1990). Sommer et al. (1978) reported the development of xerophthalmia in an otherwise healthy woman who lacked vitamin A in her diet. Her disease was corrected rapidly by intramuscular and oral administration of vitamin A. Bors and Fells (1971) have reported a similar case. They reported a case of a healthy male who deliberately omitted vitamin A from his diet. He was treated with topical ophthalmic application of vitamin A as well as oral and intramuscular administration of vitamin A. A dramatic ocular improvement was observed in this patient. These studies tried to prove that xerophthalmia is primarily caused by vitamin A deficiency. Apart from such reported human cases, scientists have experimentally developed xerophthalmia in the eyes of animals by maintaining them on vitamin A-deficient diets (Van Horn et al., 1980; Sendele et al., 1982). This literature base strongly implicates vitamin A deficiency as the etiology of xerophthalmia.

Traditionally, xerophthalmia has been treated by oral and/or systemic therapy of vitamin A (Sullivan et al., 1973; Sommer et al., 1979, 1980, 1982; Sommer and Green, 1982; Djunaedi et al., 1988). Though such therapy has been proved effective in curing xerophthalmia, corneal healing was sometimes delayed when systemic therapy only was used. Many researchers have conducted trials to study if topical therapy can prevent this delay. On the basis of their results, they have recommended topical therapy along with systemic therapy.

In vitamin A-deficient rats severe edema accompanies xerosis of the cornea and corneal ulceration. This results in an increase in the weight of the affected cornea to three to four times that of the normal cornea. With recovery, the weight returns to normal as the edema subsides. Pirie in 1977 investigated the effect of locally applied retinoic acid (RA) on the weight of the xerophthalmic cornea. Xerophthalmia was developed in rats by feeding them an RA-deficient diet. The investigator compared the two corneas of a rat after applying RA in oil to one eye and oil alone to the other eye: 5 or 10 μl of oil with 1 μg/μl of RA were applied to the cornea. The cornea was judged by visual examination as well as by weight to achieve some quantitative analysis. A total of 26 rats were treated with RA in oil in one eye and only oil in the other eye. Eleven eyes treated with RA progressed to complete normality; 11 improved significantly whereas four did not respond and became opaque and perforated. In the case of eyes treated with only oil, 14 became worse, six did not show any significant change, four remained clear and two recovered their clarity. The weights of the corneas ranged from 4.6 to 13.4 mg. The average weight of the corneas treated with RA was 7.08 mg, whereas that of the corneas treated with oil was 9.0 mg. This study showed that RA-treated corneas were lower in weight than those treated with oil alone. In xerophthalmia, degeneration of the cornea is rapid and thus local treatment with RA is advantageous. As RA increases growth rate and DNA synthesis of epidermal cells, it can be speculated that locally applied RA may be more effective than parenteral or oral administration of retinol. Pirie suggested that corneal healing in xerophthalmic children treated with systemic vitamin A might be delayed due to the reduced level of circulating retinol binding protein and, therefore, that local application of RA would be effective in bridging this delayed action (Pirie, 1977). A more accurate animal model is required to reproduce and study this state in children.

Sommer and Emran (1978) observed that when they treated human subjects suffering from corneal xerophthalmia with systemic vitamin A, the clinical response was often delayed for one to

four days and, in some cases, the cornea deteriorated. To reduce this delayed effect, they conducted some trials with topical retinoic acid (RA). They treated eight children with 0.1% RA in arachis oil and placebo. Initially, children were administered systemic vitamin A. When two eyes were equally affected, RA was applied to one eye and placebo to the other. In all cases, the eye treated with topical RA healed earlier than the other eye. This difference was more pronounced in children receiving RA more frequently. It was observed that 0.1% RA in arachis oil appeared to be safe when applied to intact globes of vitamin A-deficient children. Even though local administration of RA appeared to be effective, the authors acknowledge that RA can fulfill only some functions of vitamin A and children with xerophthalmia need to be given immediate, massive and systemic vitamin A therapy.

Encouraged by preliminary trials conducted in 1978, Sommer conducted more trials in 1983 with a higher number of patients (Sommer, 1983). Retinoic acid (RA) was applied topically three or five times a day in these patients. When it was applied five times a day, increased scarring was observed and, consequently, that trial was stopped earlier. In eyes treated three times a day, some eyes started improving at least one day before those treated with placebo. In some other eyes, though improvement began on the same day in eyes treated with and without RA, improvement was more advanced in those treated with RA than those treated with the placebo. It was not certain from this trial whether or not the RA provided the additional vitamin A required for epithelial differentiation, or hastened desquamation of the abnormally keratinized surface. Nevertheless, this trial confirmed the preliminary results of the same author (Sommer and Emran, 1978) that 0.1% RA in arachis oil applied topically three times a day speeds corneal healing in a substantially larger proportion of xerophthalmic cases than systemic vitamin A alone.

Van Horn et al. (1981) evaluated the efficacy of topical retinoic acid (RA) and systemic vitamin A in the treatment of various stages of xerophthalmia in vitamin A-deficient rabbits. In rabbits, xerophthalmia develops in five stages. All rabbits received systemic vitamin A at the beginning of the treatment. The rabbits were then treated with 0.1% RA in sesame oil in one eye and only sesame oil in the other eye once a day. At the time of the treatment, the rabbits were divided into three groups based on their ocular changes. They were divided into mildly, moderately, and severely affected. In the mildly affected group, all eyes treated with or without RA responded rapidly. In the moderately affected group, eyes treated with topical RA reversed the corneal changes in three days, whereas those treated with only sesame oil took five to seven days to heal. When severely affected animals were treated with systemic as well as topical therapy, the density and size of the infiltrate was improved. Electron microscopy confirmed that the corneas returned to the normal ultrastructure in mild and moderately affected eyes by the end of either treatment, whereas in severely affected eyes, permanent irreversible changes had occurred. Thus, it can be said that topical RA is effective in mild and moderate xerophthalmia.

Hatchell et al. (1984) wanted to determine if topical retinol, tretinoin and etretinate would reverse epithelial keratinization in the corneas of vitamin A-deficient rabbits when used alone instead of in combination with systemic vitamin A. It has already been shown (Sommer and Emran, 1978; Sommer, 1983) that topical tretinoin in combination with vitamin A therapy reverses epithelial keratinization in the cornea of vitamin A-deficient rabbits more quickly than systemic vitamin A therapy. The drugs were mixed with corn or sesame oil in either 0.1 or 0.2% concentration. The rabbits were treated with these formulations and placebo for five days. The eyes treated with 0.2% retinol improved and were cleared by the third or fourth day, whereas the eyes treated with only corn oil showed no improvement for five days. When tretinoin was instilled in one eye and etretinate in the other eye, improvement was seen in both eyes by the first or the second day. In contrast, when etretinate was instilled in one eye and oil in the other eye, no improvement was seen. When tretinoin was instilled in one eye and oil in other eye, improvement was observed in both eyes. These results indicated that topical

retinol and tretinoin were able to reverse the keratinization but etretinate was not, and apparently tretinoin was able to cross over to the control eye and show the improvement. This could be due to the presence of an arterial connection between the two internal ophthalmic arteries.

3. Corneal wound healing

Corneal epithelium serves as a natural barrier to microorganisms. It also functions in wound healing and collagen formation. Recurrent erosions and delayed healing may occur in corneas in several diseases such as diabetes. Vitamin A is necessary for maintenance of all epithelial tissues. Vitamin A is also required by the cornea for maintenance of normal growth and cellular differentiation. It is taken up quickly by corneal epithelium when applied topically (Ubels and Edelhauser, 1982). When injected in the anterior chamber of the eye, vitamin A is taken up by corneal epithelium and is concentrated in cells that migrate to cover a corneal wound (Tanaka, 1980). Hence, it may be suggested that topical vitamin A may be effective in healing corneal epithelial wounds. It has also been shown that topically applied retinoic acid promotes rearrangement of rabbit corneal endothelial cells after wounding (Junquero et al., 1990; Kruse and Tseng, 1994). Retinoids inhibit the proliferation of cultured bovine corneal endothelial cells but enhance the mitogenic effect of epidermal growth factor (EGF). It has been demonstrated that retinoic acid (RA) and its synthetic analogue CBS-211A are able to potentiate the mitogenic effect of EGF at low concentrations. The cooperation between retinoids and EGF offers new insight into the pharmacological approach to corneal endothelial repair (Junquero et al., 1990).

Smolin et al. (1979) attempted to enhance the healing ability of corneal epithelium with tretinoin. They applied 0.1% tretinoin ointment and placebo ointment to rabbit eyes after removing the entire corneal epithelium. Tretinoin ointment (0.1%) seemed to be nontoxic to the rabbit eye. It also improved the healing of epithelium on each day of observation. When the researchers applied 1.0% tretinoin ointment to the rabbits' eyes, the eyes developed conjunctival hyperemia and appeared to be immediately irritated. There was a mild reaction observed in eyes treated with 0.1% tretinoin ointment.

Ubels et al. (1983) studied the effect of several retinoids on the corneal healing rate and corneal thickness. They studied the effect of all-*trans*-retinoic acid (tretinoin), 13-*cis*-retinoic acid (isotretinoin), retinol, retinyl palmitate and retinal acetate. Epithelial wounds were made on the corneas of rabbits. The retinoids were dissolved in ethanol and the solution was mixed with corn oil. One eye of each animal was treated three to five times daily with corn oil containing the retinoids. It was observed that three times daily application of retinyl esters and tretinoin had no significant effect on corneal healing. However, treatment with tretinoin three times a day significantly increased the healing rate. Similarly, application of tretinoin five times a day promoted corneal healing. These investigators have shown that tretinoin at a concentration of 0.1% applied three to five times a day promoted the healing of corneal epithelial wounds. Corneal thickness was rapidly brought to normal with tretinoin, retinol and retinyl palmitate. The investigators have stipulated that the promotion of corneal wound healing may take place in two steps in which retinoic acid increases epidermal growth factor receptor sites on epithelial cells, followed by stimulation of healing by increased binding of endogenous epidermal growth factor. The mechanism by which recovery of normal corneal thickness occurs in the presence of retinoids is uncertain. These investigators have suggested that as retinoic acid does not appear to be toxic to the cornea, it can be used as an ophthalmic drug in low and controlled doses.

Recurrent erosions occasionally occur in corneas of diabetic patients and need extensive epithelial turnover before slowly healing. Hatchell et al. (1985) wanted to compare the rate of wound healing and increase in corneal thickness in normal and diabetic rabbits and to determine if topical treatment with tretinoin would accelerate the rate of epithelial healing in either normal or diabetic rabbits after wounding with heptanol. No statistically significant difference was observed in

wound healing rates of normal and diabetic untreated eyes. Tretinoin significantly increased the epithelial healing rate in corneas of normal rabbits but showed no effect on corneas of diabetic rabbits. Also, there was no significant difference in the corneal thickness between the normal and diabetic rabbits. Based on these mixed results, the authors have indicated that retinoids may be effective in promoting normal corneal wound healing. Since retinoic acid did not appear to be toxic to the cornea at low doses, they have suggested that clinical trials should be considered.

Even though wound healing rates of corneal epithelium have been calculated (Moses et al., 1979; Jumblatt et al., 1980), the endothelial healing rate has not been calculated. Therefore, Matsuda et al. (1986) have attempted to develop a method to calculate the endothelial healing rate in rabbits and to evaluate the effect of topical RA on the endothelial healing process. For the experiment, petrolatum ointment containing 0.1% all-*trans*-retinoic acid (tretinoin) was used. It was observed that the ointment vehicle had no effect on healing. The healing rate in tretinoin-treated corneas was greater than in the control corneas. Most of the wounded area is covered during the first rapid healing phase. Since topically applied tretinoin promoted healing during the first six hours of application, it may be beneficial in promoting the re-establishment of the barrier function of the endothelium by rapidly restoring a continuous monolayer of cells in rabbits. This hypothesis is supported by data showing a decrease in endothelial permeability to insulin and dextran with the re-establishment of an endothelial monolayer following wounding.

Retinoids alter epithelial cellular proliferation and differentiation both *in vitro* and *in vivo*. They play an important role in modulating ocular surface epithelial differentiation. Thus, Huang et al. (1991) studied the effect of topical retinoid application on the paracellular permeability of the normal cornea and conjunctiva. They prepared tretinoin ointment in mineral oil and petrolatum base and applied it to the lower conjunctival sac of one eye of the normal rabbits. The other eye received ointment base as a control. *In vitro* perfusion studies on whole corneal buttons and conjunctival tissues showed that there was no difference in corneal permeability between the experimental and control eyes. This indicated that topically applied tretinoin did not alter the paracellular permeability of the ocular surface epithelia.

4. Conjunctival transdifferentiation inhibition/squamous metaplasia

Dry-eye disorders could be divided into four categories: (1) aqueous deficiency; (2) mucin deficiency; (3) lipid deficiency; and (4) a combination of (1), (2) and/or (3). The ocular surface epithelia in these diseases are characterized by indications such as the loss of goblet cells, increase in cellular stratification and keratinization. This abnormality in epithelial differentiation is known as squamous metaplasia. In squamous metaplasia, the normal secretory conjunctival mucosa gradually develops into a nonsecretory keratinized epithelium. Squamous metaplasia of the ocular surface epithelium is responsible for ocular dryness, irritation and photophobia. It can also result in complications such as recurrent erosion, ulceration, scarring and perforation. Though the exact mechanism for squamous metaplasia is not known, it is hypothesized that loss of vascularization due to scar formation in the chronic cicatricial stage of the conjunctiva and intense inflammation in the acute inflammatory stage are the two main pathogenic processes in the development of squamous metaplasia. It has been found (Tseng et al., 1984a) that in the rabbit model, goblet cell density correlates well with vascularization. These results indicate that factors in the blood circulation are crucial for the normal epithelial differentiation. As vitamin A is known to be useful for epithelial growth and differentiation, its deficiency can cause squamous metaplasia. Various researchers have studied the effect of topical retinoids in the treatment of humans with ocular squamous metaplasia.

By using a rabbit model of conjunctival transdifferentiation, Tseng et al. (1984b) and Farazdaghi et al. (1984) have previously shown that goblet cells can be maintained on the avascular corneal

surface with topical administration of several retinoids. In 1985, they went one step further and used topical retinoids in the treatment of humans with ocular surface squamous metaplasia (Tseng et al., 1985). They also investigated whether this therapy can eliminate keratinization, facilitate regeneration of goblet cells and promote tear production. They studied 22 patients. The patients were divided into four groups. They were: Group I, keratoconjunctivitis sicca; Group II, Stevens–Johnson syndrome; Group III, drug-induced pseudopemphigoid and ocular pemphigoid; and Group IV, surgery- or radiation-induced dry eye. Topical all-*trans*-retinoic acid (tretinoin) ointment in doses of 0.01 or 0.1% w/w was applied to the involved eyes one to three times daily. The application of a particular dose was based on the severity of the disease. The investigators evaluated clinical efficacy of this treatment based on changes in the symptoms, visual acuity, dependence on previous artificial tears and/or lubricants, and improvement of Schirmer test and rose Bengal staining. To avoid the subjective variability in reports of symptomatic improvement, they also used the impression cytology technique to assess hystological changes in conjunctival epithelial differentiation. All patients in Group I became asymptomatic while receiving vitamin A ointment treatment. The frequency of tretinoin ointment application was reduced as patients improved during the treatment. Before treatment, a total loss of goblet cells and abnormally enlarged epithelial cells were disclosed by the conjunctival impression cytology. Normal small epithelial cells with moderate goblet cell density returned after the treatment. In patients from Group II, clinical severity was largely reduced due to the tretinoin treatment. The impression cytology revealed return of normal epithelium after the treatment. In Group III and IV patients, the areas of keratinization disappeared and the symptoms of irritation and dryness were eliminated or reduced. When the patients from all the groups were followed after the treatment, no ocular side effects involving visual acuity, anterior segment, intraocular pressure, and retina were observed. This study has demonstrated the clinical efficacy of vitamin A in terms of its capability to reduce clinical symptoms and signs and reversing the process of squamous metaplasia. The results from this study have shown that squamous metaplasia of the ocular surface epithelium is a factor in poor epithelial wound healing and reversal of that process can facilitate healing. The researchers have suggested that before using the topical RA treatment it is important to confirm the diagnosis of ocular surface squamous metaplasia. It is also important to understand that all surface squamous metaplasia do not have the same pathogenesis as proposed in this particular study and so all ocular surface disorders will not respond to this treatment.

The study conducted by Tseng et al. (1985) had two main drawbacks, viz. the study was not double-masked and placebo-controlled and the total number of patients enrolled in the study was limited as it was conducted at only one medical center. Soong et al. (1988) tried to overcome these drawbacks by conducting a randomized, double-masked, multicenter, placebo-controlled study to evaluate the efficacy and safety of topical tretinoin in the treatment of dry eyes. They divided the patients into two categories, viz. keratoconjunctivitis sicca (KCS) and nonkeratoconjunctivitis sicca (nonKCS). Tretinoin was applied at 0.01% concentration in petrolatum and mineral oil base. In the KCS patients, both the active as well as placebo-treated cases showed improvements. Even though there were no statistically significant differences between tretinoin- and placebo-treated patients, improvements in symptoms and signs tended to favor the placebo over tretinoin. In the nonKCS category, no statistically significant difference was observed between the tretinoin-treated and the placebo-treated eyes with respect to signs and symptoms. However, analysis of each cytology site revealed significant reversal of keratinization at the temporal bulbar site. This study showed contradictory results compared to a previous study by Tseng et al. (1985). They showed that topical tretinoin therapy did not improve the symptoms and clinical signs of noncicatricial dry eyes and also did not significantly improve aqueous tear production. However, the authors felt that tretinoin may be useful in reversing ocular keratinization in cicatricial diseases of the ocular surface.

Even though Soong et al. (1988) found that tretinoin did not improve the symptoms of keratinization and squamous metaplasia, Nelson (1988) did find beneficial effects of tretinoin in one patient. The patient responded wonderfully to topically applied tretinoin, recovered from long-standing squamous metaplasia, and had no recurrence even nine months after the treatment.

Another study was conducted by Herbort et al. (1988) These investigators also studied the effect of topical RA on keratinizing diseases and added more clinical evidence on the efficacy of RA. Keratinization of the corneoconjunctival epithelium may be the result of dysplastic changes of the epithelium or squamous metaplasia. The authors report four cases of keratinizing diseases, two metaplastic and two dysplastic in origin, treated with topical tretinoin. Topical tretinoin ointment in a 0.05% concentration or a 0.01% concentration was used. The authors were able to achieve with topical application of tretinoin a clinical reversal of squamous metaplasia which resulted in the disappearance of keratinization and restoration of normal tear function. Similar beneficial effects were achieved after topical application of tretinoin in cases with conjunctival neoplasia. In all of these cases, the leukoplastic lesion regrew after discontinuation of the treatment and disappeared when the treatment was resumed. This finding supports the *in vitro* finding that tretinoin is tumorostatic rather than tumoricidal. The authors have suggested that long-term administration of tretinoin is necessary and, in their patients, treatment for up to 12 months was well tolerated. Based on their results, these researchers feel that tretinoin is a valuable supplementary therapy to surgery in suspected neoplastic lesions of corneoconjunctival epithelium. The authors warn that since RA is a relatively new drug in ophthalmology, it should be used carefully. As tretinoin is known to irritate the conjunctiva, they recommend starting the therapy with 0.5% tretinoin ointment three times a day for both metaplastic and neoplastic conditions. The dose should be then reduced to the minimal effective dose in metaplastic keratinization but maintained at the highest tolerable dose in neoplastic conditions.

Since Tseng et al. (1985) reported that patients with KCS who were treated with topical tretinoin became asymptomatic and about half of the treated eyes experienced increased tear production, Gilbard et al. (1989) conducted an open label crossover study to compare vitamin A ointment with its placebo base in patients with (KCS). They studied 11 patients. A 0.01% vitamin A ointment was used for the study. Initially one eye was treated with the vitamin A ointment and the other with the placebo. After five weeks, the treatments were switched. It was observed that 0.01% vitamin A ointment was no more effective than the placebo in patients with KCS. This was indicated by no increase in tear secretion and no decrease in ocular surface disease. These authors do not agree with the results from Tseng et al. (1985). They hypothesize that the squamous metaplasia change in the ocular surface can be a protective response of the tissue that inhibits further fluid loss from the ocular surface and does not actually cure the disease. The authors believe that in this case vitamin A ointment did not work due to removal of the cells that have undergone squamous metaplasia as a protective mechanism without the normalization of tear film osmolarity on the ocular surface. This study had the advantage over the earlier study of being a placebo-controlled, crossover design (Tseng et al., 1985) and both studies had the disadvantage of being open labeled.

After completing the first study, Herbort et al. (1989) have reported results from another small trial. They conducted a randomized, double-masked, placebo-controlled, crossover trial to study the effect of topical retinoic acid (RA) on ocular surface keratinization. They found that ocular surface keratinization was responsive to topical RA treatment. They also observed that the improvements in the ocular surface after topical RA therapy were proportional to the extent of pretreatment keratinization, and thus they recommend a thorough examination of patients before treatment.

During past few years, retinoic acid has been studied with controversial results. In spite of this, Driot and Bonne (1992) reported the effects of a synthetic retinoic acid analog which was specially

designed for topical eye administration. In rabbits, KCS was induced by surgical closure of the lacrimal gland excretory duct and removal of the nictitating membrane and the harderian gland. Impression cytology and histology revealed that the conjunctival lesions involved a drastic enlargement of epithelial cells and a typical loss of goblet cells. These alterations could be reversed by a topical treatment for nine weeks with the synthetic analog of RA. It was shown that an RA analog was able to restore goblet cell density and to improve corneoconjunctival surface cytology in KCS, and thus can be used to treat dry-eye diseases induced by means other than a general vitamin A deficiency.

Many researchers have studied the cornea and associated structures in a vitamin A-deficient state and for corneal wound healing. Some have suggested that topical RA is useful in reversing squamous metaplasia and some have shown contradictory results. Hence, in order to understand this behavior in more details Stonecipher et al. (1988) studied the corneal and limbal changes associated with topical tretinoin application over a limited time interval in normal rabbit eyes. They selected these structures for evaluation as they had been followed before for studies on diseases such as xerophthalmia, dry-eye syndromes and others. Tretinoin was used as the drug. It was emulsified in a petrolatum ophthalmic base and mineral oil base. This emulsion was placed in the right eye of eight rabbits and the base alone was placed in the right eye of two rabbits as a control. In the case of all rabbits, no treatment was instilled in the left eye. The cytological changes in corneas treated with tretinoin showed mucin-like metaplastic changes in the corneal structures. A crossover effect of tretinoin was observed in the left eyes when the right eyes were treated with tretinoin. This could be due to a systemic absorption of the drug which passes through the nasolacrimal system. The authors proposed a mucin-like metaplasia as a mechanism for the effect of tretinoin in dry-eye disorders. As this mucous metaplasia plays an important role in the maintenance of corneal and conjunctival epithelial function, it is thought that topical application of RA will be advantageous to treat mucin-deficient dry-eye disorder.

Another important disease that shows complications of dry-eye disorders such as secondary corneal ulceration and opacification is ocular cicatricial pemphigoid (OCP). It is a rare disease with late age onset. It affects women 1.5–3 times more often than men. It has been observed following the topical use of epinephrine, echothiopate and pilocarpine. Herbort et al. (1986) have reported a case of OCP with topical RA. They treated a woman with OCP by applying 0.05% all-*trans*-retinoic acid (tretinoin) ointment three times a day to her eyes. They observed a rapid improvement with disappearance of all corneal keratin and most of the conjunctival keratinization within seven days. The patient was prescribed 0.01% tretinoin ointment three times a day as a follow-up treatment. It helped in bringing the situation under control. Squamous metaplasia is generally seen in advanced OCP. It is thought that it occurs due to vitamin A deficiency. Tretinoin in this case acted mainly on squamous metaplasia and was also successful in halting the progression of the cicatricial process. Tretinoin thus appears to be useful in treating OCP either as a main treatment or as a complementary treatment in patients under immunosuppressive therapy.

Conjunctival transdifferentiation occurs when the cornea involved has minimal vascularization. This conjunctival transdifferentiation can be inhibited by maintaining the presence of goblet cells. Retinoids are believed to play an important role in the presence of goblet cells as they are essential for epithelial growth and differentiation. Tseng et al. (1987) tested the hypothesis that retinoids can inhibit conjunctival transdifferentiation in nonvascularized corneas by maintaining goblet cell differentiation. 13-*cis*-Retinoic acid (isotretinoin) and etretinate were used as the drugs. They were mixed in corn oil and applied topically in the eyes. The rabbits were divided into nonvascularized (nonV) and vascularized (Vas) groups. It was observed that goblet cell densities of nonV corneas with etretinate and isotretinoin were higher than their controls but were similar to Vas controls. The distribution of goblet cell densities was similar in the Vas control group and those receiving retinoids, but those receiving retinoids showed more goblet cells. In the nonV

groups, retinoids maintained a high plateau density and higher densities were observed in Vas groups receiving topical retinoids. This study has demonstrated that when either isotretinoin or etretinate were applied topically, the nonV corneas could maintain the conjunctiva-like epithelium containing goblet cells, thus supporting the hypothesis that conjunctival transdifferentiation can be inhibited by maintaining goblet cell differentiation, through effective concentration of vitamin A or retinoids at the site.

5. Proliferative vitreoretinopathy

Proliferative vitreoretinopathy (PVR) is the main cause of failure following rhegmatogenous retinal detachment surgery (Araiz et al., 1993). PVR is an abnormality in which cellular membranes are formed within the vitreous cavity as well as on both surfaces of the detached retina. During the formation of these cellular membranes, glial and retinal pigment epithelial (RPE) cells undergo a migration and cellular differentiation into fibroblast-like cells. In the pathogenesis of PVR, the disruption of the internal limiting membrane and the blood–retinal barrier are considered to be the first important steps. This breaking of the blood–retinal barrier allows serum to accumulate in the vitreous cavity. Serum is a stimulus to the migration of the cells. The most common cells involved in PVR are RPE cells, glial cells, fibroblasts, myofibroblasts and macrophages. Fibronectin, an important modulator of cellular adhesion and proliferation, along with platelet growth factors, promotes cellular adhesion to vitreous collagen and promotes proliferation and membrane formation. Fibroblasts, glial cells and RPE cells can contract collagen. By doing so, they can collapse the vitreous gel and exert tractional force on the retina (Peyman, 1996). During treatment of PVR, even though surgical membranes are meticulously removed and silicone oil is used as a long-term tamponade, failure occurs in a number of cases. This failure is due to difficulty with complete removal and continuous growth of the membranes. To overcome this problem, many antiproliferative agents have been experimented with either alone or in combination with vitreoretinal surgery. These agents have major drawbacks. Most of them have narrow safety margins and can produce severe toxic reactions. In addition, most of them are water soluble and so cannot be used with silicone oil. Thus, scientists are always looking for new antiproliferative agents. Retinoids can be used as powerful antiproliferative agents. Retinoids are implicated in cellular differentiation and are normally present in high levels in RPE *in vivo*. They are known to have an antiproliferative effect on epithelial, mesenchymal and neoplastic cells. A study by Campochiaro et al. (1991) showed that RA prevents outgrowth of human RPE cells, resulting in a morphologic appearance characteristic of mature RPE cells in vivo, and suggested that retinoids may enhance density-dependent growth regulation in RPE. Retinoids play an important role in visual transduction and therefore their recycling is needed for normal visual function. This recycling occurs through an intimate relationship between the photoreceptors and the RPE. Disruption of this intimate relationship during retinal detachment prevents recycling of retinoids and may be one reason for outer segment degeneration, but it may also have a significant impact on the RPE. Lack of retinoid input through outer segment phagocytosis could lead to depletion of retinoid stores if transport out of the RPE continues as occurs in cultured cells. This could contribute to RPE dedifferentiation, migration, and proliferation processes that occur after retinal detachment and have been implicated in PVR and, consequently, in the poor return of vision after retinal reattachment (Campochiaro et al., 1991). Hence, it is felt that retinoids would be good candidates against PVR and studies have recently been conducted with them.

Araiz et al. (1993) evaluated the ability of all-*trans*-retinoic acid (tretinoin) dissolved in silicone oil (SiO) to inhibit membrane proliferation in an animal model of PVR based on the intravitreal injection of homologous subconjunctival fibroblasts. After preparing a solution of tretinoin in SiO, its in vitro stability was measured. All rabbits underwent unilateral gas compression vitrectomy. After four days, gas/SiO with tretinoin

exchange took place so that at the end of the procedure, about 70–80% of the vitreous cavity was filled with SiO containing tretinoin. In controls, the vitreous cavity was filled with only SiO. The size of the SiO bubble was estimated and recorded, and the extent of membrane formation and retinal traction was graded. In addition, the release of tretinoin from SiO and tretinoin retinal toxicity were also determined *in vivo*. It was observed that tretinoin was stable when protected from light. The incidence of tractional retinal detachment (TRD) in the groups treated with 10 and 5 μg of tretinoin was significantly lower than that in the control group after 2 and 3 weeks, respectively. In the control group, extensive multilayered fibroblast-rich epiretinal and vitreous tractional membranes were seen, whereas in animals treated with tretinoin, much thinner and less extensive membranes were seen. Only traces of tretinoin were detected by high-performance liquid chromatography (HPLC) in the SiO extracted from the eyes 1 week after the gas/SiO exchange. No evidence of retinal toxicity was observed. In this study, it was observed that the rates of TRD due to membrane formation in eyes treated with 10 and 5 μg of tretinoin were significantly lower than in eyes from the control group and no significant differences were found between the two doses. From the vitreous cavity, tretinoin was cleared from the SiO in the first week. This suggests that tretinoin influences the cell proliferation at an early stage, which is in accordance with previously conducted studies stating that the early period at the beginning of the disorder is crucial for the initiation of a proliferative response. This study has shown that tretinoin when administered in solution with intravitreal SiO produces a significant and lasting reduction in cellular proliferation in an experimental model of PVR and may be useful in humans with severe cases of PVR requiring the use of SiO.

Giordano et al. (1993) reported on the kinetics of all-*trans*-retinoic acid (tretinoin) release from poly(lactic-co-glycolic)acid (PLGA) microspheres *in vitro* and the antiproliferative effects of tretinoin from a single injection of tretinoin-loaded microspheres in the rabbit model. The microspheres were prepared by a modified solvent evaporation method using a single emulsion. *In vitro* studies were conducted by suspending microspheres in distilled water. Due to insolubility of tretinoin in water, its content left in the microspheres was determined to study the kinetics of its release. The microspheres were uniformly round with smooth surfaces. Some of the microspheres disintegrated after being in water for 21 days and some remained intact with porous surfaces. A nearly constant release rate of tretinoin from PLGA microspheres was observed for 40 days. There was a constant daily release of tretinoin without any significant burst effect. After 40 days, 83% of the drug was released. *In vivo* studies showed that after the intravitreous injection, the microspheres were uniformly distributed in the vitreous cavity and after four days they settled on the inferior retina in small clusters of fine powder. By the end of three weeks, TRD developed in four out of 11 eyes in the experimental group and all eyes from the control group. No further changes were observed in these eyes for up to eight weeks. The investigators used 50:50 PLGA copolymer, which has the shortest half-life, in order to achieve fast clearance of the microspheres from the vitreous cavity. However, the microspheres were still visible in the vitreous cavity after two months in 73% of the rabbits. A foreign body reaction around the microspheres and their fragments was observed at three weeks and a mild inflammatory reaction was seen even at the end of two months. This observation contradicts the observation by Wise (1984) and Gresser and Sanderson (1984) that the PLGA microspheres are inert in nature. These researchers suggest that the long-term reaction of the eye to the polymer microspheres needs to be evaluated. On the positive side, this study has shown that PLGA microspheres can significantly reduce the incidence of TRD by 64% at the end of eight weeks when used as a system for delivering tretinoin.

Nakagawa et al. (1995) described a new animal model for PVR and evaluated all-*trans*-retinoic acid (tretinoin) dissolved in SiO and silicone–fluorosilicone copolymer oil (SiFO) for retinal toxicity and its role in preventing PVR. Araiz et al. (1993) and Giordano et al. (1993) used a fibroblast rabbit model of PVR, whereas these

researchers produced PVR by intravitreous injection of homologous subconjunctival fibroblasts and platelets. Rabbits were divided into five groups. Group 1 was administered 1.2 ml SiFO with tretinoin, group 2 with SiFO as a control for group 1, group 3 with 1.2 ml SiO with tretinoin, group 4 with only SiO as a control for group 3, and group 5 with only balanced salt solution (BSS). It was observed that in group 1, 71% of eyes did not have retinal detachment and 29% of eyes had tractional retinal detachment (TRD). In group 2, 81% of eyes showed TRD. In group 3, TRD occurred in 33% of the eyes. In groups 4 and 5 TRD developed in all the eyes. This shows that the incidence of retinal detachment resulting from PVR was significantly lower in tretinoin-treated eyes than in the controls. This study has demonstrated a rapid, effective and reliable method of producing PVR in rabbit eyes. This study has indicated that tretinoin at concentrations up to 10 μg/ml in SiO or SiFO did not cause retinal histologic toxicity and had an inhibitory effect on PVR. It has been shown that RA, a naturally occurring oxidative product of vitamin A, affects cell differentiation and proliferation. At the same time, it is soluble in SiO and SiFO and can thus be used effectively to prevent PVR recurrence.

Fekrat et al. (1995) determined if postoperative oral 13-*cis*-retinoic acid (isotretinoin) alters the rate of recurrent retinal detachment in eyes undergoing surgery for PVR. Similar to tretinoin, isotretinoin is a potent inhibitor of retinal pigment epithelial (RPE) cell proliferation *in vitro* (Araiz et al., 1993). It is commonly administered orally and used to treat cystic acne. Therefore, these authors used oral isotretinoin to treat patients with PVR. The clinical evaluation showed that the eyes of patients treated with isotretinoin demonstrated less evidence of inflammation and reproliferation. They then decided to review the clinical course of a group of patients to assess patient tolerance to this drug and to find out if there was any evidence to support their clinical impression. They compared the group of patients that received oral isotretinoin to the control group to determine the effect of isotretinoin, the frequency of recurrent retinal detachment, the final anatomic status, and the final visual acuity. They did not encounter any systemic side effects of the drug. It was seen that postoperative administration of oral isotretinoin decreased the rate of recurrent retinal detachment in eyes undergoing surgery for PVR.

6. Conclusion

Retinoids have a great potential as a drug to treat several ophthalmic diseases. It appears that local or topical application will be of great significance to treat these conditions efficiently. Topical application will overcome some of the serious side effects of systemically or orally administered retinoids (Fraunfelder et al., 1985; Kaiser-Kupfer et al., 1986; Weleber et al., 1986; Brown and Grattan, 1989; Evans and Hickey-Dwyer, 1991). Even though retinoids have been around for a long time, their full potential as a topical cure in ophthalmic diseases has not been fully discovered. Further research needs to be conducted to synthesize derivatives which will be less dangerous than their current siblings. A significant amount of research also needs to be conducted to develop drug delivery systems for retinoids which will be safer than those currently available. Steps are being taken in that direction and it will not be long before retinoids can be effectively and safely used topically to cure or mitigate many ocular diseases.

7. Names and synonyms for retinoid analogs

RA	Retinoic acid
Tretinoin	All-*trans*-retinoic acid
Isotretinoin	13-*cis*-Retinoic acid

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