

Evaluation of retinoic acid ophthalmic emulsion in dry eye

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PURPOSE. *An oil in water emulsion of 0.01% all-trans-retinoic acid (tretinoin) was prepared and clinically evaluated in dry eye patients.*

METHODS. *The ophthalmic emulsion consisted of 10% of arachis oil and 90% of the hydrogel of Carbopol 940. To evaluate retinoic acid emulsion clinically, a placebo-controlled, open-labeled, randomized study was performed with 22 dry-eye patients. Symptoms were recorded before and after the treatments. The Schirmer I test, measurement of tear film break-up time (BUT), rose Bengal and fluorescein staining of cornea and conjunctiva, and mucus fern test were done.*

RESULTS. *Retinoic acid did not improve the dryness, photophobia and foreign body sensation more than placebo. Schirmer test and BUT were significantly improved by retinoic acid treatment. Corneal and conjunctival epithelium maintained their characteristics during the use of retinoic acid, as indicated by rose Bengal and fluorescein staining.*

CONCLUSIONS. *Ophthalmic emulsion of retinoic acid can be suggested as a promising approach for the treatment of dry eye. (Eur J Ophthalmol 2000; 10: 121-7)*

KEY WORDS. *All-trans-retinoic acid, Tretinoin, Dry eye, Ophthalmic emulsion, Carbopol*

Accepted: June 29, 1999

INTRODUCTION

Dry eye is the result of a deficiency in tear secretion and instability of the precorneal tear film. Normal conjunctival and corneal mucosa gradually change into a non-secretory keratinized epithelium in dry eye, and squamous metaplasia occurs. Cellular differentiation in dry eye results in poor adhesion and insufficient spreading of tear film, with corneal complications such as poor healing of epithelia (1). Conventional therapies including polymeric eye drops, osmotic systems, inserts, slow-releasing systems, and surgical procedures do not reverse keratinization (2). All-trans-retinoic acid (tretinoin) is a normal metabolite and the carboxylic form of retinol (Vitamin A). It is a hydrophobic, lipid-soluble compound. Retinoic acid has been shown to be effective in ocular surface disorders such as squamous metaplasia by reversing

the corneal and conjunctival keratinization and improving the epithelial wound healing rate (3, 4). The beneficial effect of tretinoin on dry-eye patients with squamous metaplasia was first shown by Tseng (3), who reported that conjunctival keratinization and corneal epithelial defects could be treated by topical application of retinoic acid.

The aim of the present study was to prepare a suitable delivery system for topical use of retinoic acid and to evaluate the formulation clinically in dry eye.

METHODS

Chemicals

All-trans-retinoic acid (tretinoin), 13-cis-retinoic acid, DL- α -tocopherol and arachis oil were purchased

from Sigma Chemical Co. (St. Louis, Missouri, USA). Carbopol 940 was supplied by BF Goodrich Chemical Co. (Ohio, USA). All other chemicals and reagents used were analytical grade.

Preparation of the ophthalmic emulsion

The oily phase of the emulsion consisted of 10% of arachis oil containing 0.01% all trans-retinoic acid and 0.05% α -tocopherol. The aqueous phase (90%) was the hydrogel of 0.1% Carbopol containing 5% mannitol. The formulations were prepared in a dark, cool chamber, under a nitrogen stream and laminar flow to provide an oxygen and microorganism free environment. Carbopol hydrogels were prepared by neutralizing the polymer aqueous dispersion to pH 7.05–7.65 using sodium hydroxide solution, and sterilizing by autoclaving. Retinoic acid and α -tocopherol were added to the arachis oil which had been sterilized by dry heating. The oil phase was mixed with a horizontal agitator until a complete fine dispersion of tretinoin was obtained, then the dispersion was added slowly to an appropriate amount of Carbopol 940 hydrogel. The final mixture was homogenized until a uniform dispersion was obtained. Osmolarity of the ophthalmic emulsions was determined with a cryoscopic osmometer, Osmomat 030 (Gonotec7 Berlin, Germany); pH values of the formulations were measured by a Philips pHmeter, Model PW9422 (Cambridge, UK).

In vitro assessment of the ophthalmic emulsion

The particle size distribution of the emulsions was obtained with a Malvern MSE 02 SM laser scattering instrument (Malvern Instruments Ltd., Malvern, UK). A Brookfield viscometer, Model LVT (Stoughton, Massachusetts, USA) was used to determine the flow property and viscosity of the formulations. Retinoic acid content in the emulsions was determined by a Waters high-pressure liquid chromatograph (Waters Assoc., Milford, Massachusetts, USA) using an ODS column (15 cm, Whatman Inc., New Jersey, USA) with a particle size of 5 μ m, an UV detector and U6K injector Model 481, and a pump Model 510. The isocratic mobile phase of the chromatographic technique was a mixture of acetonitrile (70%, v/v) and water (30%, v/v) containing 0.5% (w/v) acetic acid and 0.02% (w/v) triethylamine. Formulations were followed at weekly

intervals for physical stability of the emulsion and for retinoic acid content until the active substance was reduced to 90% of its initial dose.

Preclinical evaluation

An irritation test in the rabbit eye was performed by a modification of the Draize test (5). The eyes of six albino rabbits weighing 2.0–2.5 kg were examined before the test. For the test procedure, 0.1 ml of the emulsion was instilled into the conjunctival sac of the right eye of each rabbit. The left eye served as control. The eyes were examined for the reaction of the cornea, conjunctiva and iris to the test material at intervals of 1, 24, 48, 72 h and 7 days. In the first week, placebo emulsion was administered three times daily. After a week's rest, retinoic acid emulsion was administered using the same regimen as placebo. The eyes were checked to see whether the test material produced any opacity and ulceration of the cornea, redness, chemosis or edema of the conjunctiva and inflammation of the iris.

Test results were scored in four classes for cornea (0, no reaction, 1, scattered or diffuse area of opacity; 2, translucent areas; 3, necrotic areas or complete corneal opacity), four grades for conjunctiva (0, normal vessels; 1, some vessels not easily discernible; 2, diffuse redness and edema; 3, diffuse beefy red and chemosis), and two grades for iris (0, normal; 1, congestion, swelling or hemorrhage). The criterion for irritation was grade 1 and over for cornea, 2 and over for conjunctiva, and 1 for iris. The test material was considered irritant if 4 or more, and non-irritant if none or one of the six rabbits showed irritation.

Clinical evaluation

Twenty-two dry-eye patients diagnosed at the ophthalmology out-patient clinic were chosen for clinical study. They ranged in age from 29 to 75 years. They were asked to continue their current therapy with a tear substitute containing polyvinyl alcohol. The following criteria were considered to qualify the patients for clinical trial: 1) Symptoms including burning, itching, foreign body sensation, dryness and photophobia; 2) a Schirmer test less than 5 mm/5 min; 3) a tear film breakup time (BUT) less than 10 sec; 4) a score less than 3 for rose Bengal staining. Eyes with ble-

pharitis, lid deformation and related ocular surface abnormalities were discarded. The study was conducted with the permission of the University Ethics Committee.

A placebo-controlled, open-labelled, randomized trial was performed. Initially, three times daily administration of placebo emulsions for a week was recommended. After a week's drug-free period, patients were asked to use the retinoic acid emulsion following the same dosage regimen. Clinical examinations including visual tests, slit-lamp examination, examination of the cornea after fluorescein staining, rose Bengal staining, tear film BUT, Schirmer I test, and examination of mucus morphology by the ferning test were done before and after the weekly treatments. At each visit, the following symptoms were recorded: discomfort, dryness, foreign body sensation, photophobia, burning and itching, and vision disturbance. Patients were asked to report their experience of each symptom as a two-choice answer (yes, symptom exists; no, no symptom).

The Schirmer I test was conducted according to van Bijsterveld (6), and results lower than 5.5 mm were considered eye dryness. Values from 5.5 mm to 10 mm were accepted as moderate to mild dryness.

Tear film BUT was determined by fluorescein staining and the cornea was scanned by a slit-lamp fluorophotometer (Z 2476, Bern, Switzerland). Values lower than 10 seconds were considered positive (7).

For rose Bengal staining of the cornea and conjunctiva a drop of 1% rose Bengal stain was instilled into the lower fornix of each eye (8). The eyes were examined with the slit lamp and the degree of staining of the cornea, the temporal conjunctiva and nasal conjunctiva was recorded on a scale of 0 to 3 according to van Bijsterveld (6). The sum of these three scores gave a total of 0 to 9 for each eye. The test was considered positive if the score was more than 3.

Fluorescein staining was done by instilling 1% fluorescein solution into the conjunctival sac. Any staining of the cornea was recorded as grades I, II and III corresponding to mild, moderate and severe. For no staining the grade was taken as 0. Eye dryness was defined as II and III grades (9).

For the mucus ferning test tear samples were collected in the lower fornix using a thin glass tube. Local anesthesia was not used. The tear samples were allowed to dry at room temperature and examined by

light microscopy. Four types of mucus crystallization patterns were graded according to the classification of Rolando (10): types I and II were normal mucus morphology and types III and IV eye dryness.

The chi-square test was used for statistical evaluation of subjective symptoms, fluorescein staining and mucus ferning test. The Schirmer I test, BUT and rose Bengal staining were examined statistically by the difference between means for paired observations.

RESULTS

In vitro assessment

The mean diameter of the inner phase particles of retinoic acid and placebo emulsions was 2.34 μm . The highest viscosity of the emulsion system was 232 ± 12.22 cps at 60 rpm angular velocity of viscometer. Retinoic acid content decreased to 90% of the initial dose at the end of two months' storage at 4°C. The emulsion showed a pseudoplastic flow, meaning that an increase in shear stress will result in a decrease in viscosity. The pH of the formulations was 7.4 on average and the osmolarity was measured in a range from 291 to 308 m Osm/L.

Preclinical evaluation

In the modified Draize test, none of the eyes of six rabbits reacted to the placebo. With retinoic acid, two eyes of one rabbit were graded as 1 for conjunctiva and 1 for iris, and the formulations were considered non-irritant.

Clinical evaluation

Two of the 24 patients withdrew from the trial because of discomfort related to side effects such as pain, foreign body sensation and sticky eyelid, so the study continued with 22 subjects. One patient's preference for retinoic acid emulsion and two patients' preferences for placebo emulsion were recorded. One of the 22 patients complained of pain with retinoic acid but did not need to stop treatment. Symptoms reported by the 22 patients are shown in Table I. Table II sets out the results of statistical analysis of symptoms. Significant relief ($p < 0.05$) of dryness and pho-

tophobia was observed with retinoic acid and placebo. The difference between treatments was not significant. Foreign body sensation was improved significantly by the use of placebo ($p < 0.05$) but not retinoic acid emulsion. Burning sensation did not change with

placebo and retinoic acid.

The results of Schirmer I test, BUT and rose Bengal staining are presented in Figure 1 and the statistical data are in Table III. The mean Schirmer value (6.32 ± 3.30 mm) improved respectively to 7.32 ± 4.16

TABLE I - SUBJECTIVE SYMPTOMS IN DRY EYE PATIENTS

Symptom	Response	Baseline		Placebo		Retinoic acid	
		N ^a	%	N	%	N	%
Dryness	+	18	40.9	4	9.1	4	9.1
	-	26	59.1	40	90.9	40	90.9
Photophobia	+	25	56.8	12	27.3	10	22.7
	-	19	43.2	32	72.7	34	77.3
Foreign body sensation	+	11	25.0	4	9.1	24	54.5
	-	33	75.0	40	90.9	20	45.5
Burning	+	13	29.5	7	15.9	14	31.8
	-	31	70.5	37	84.1	30	68.2

^aNumber of eyes; + Symptoms present; - No symptom

TABLE II - STATISTICAL DATA OF SYMPTOMS (CHI SQUARE TEST)

Symptom	Baseline-Placebo	Baseline-Retinoic acid	Placebo-Retinoic acid
Dryness	S (R)	S (R)	NS
Photophobia	S (R)	S (R)	NS
Foreign body sensation	S (R)	S (R)	S
Burning	NS	NS	NS

S (R): Significant relief ($p < 0.05$)

NS: Not significant

S: Significant, opposite result

TABLE III - STATISTICAL DATA OF CLINICAL TESTS

	Baseline-Placebo	Baseline-Retinoic acid	Placebo-Retinoic acid
Schirmer I (mm/5 min)	NS	S (I)	S (I)
BUT (Sec)	S (I)	S (I)	S (I)
Rose Bengal (score)	NS	S (I)	NS
Fluorescein (grade)	NS	NS	NS
Mucus ferning (grade)	S (I)	S (I)	NS

NS: Not significant

S (I): Significant improvement ($p < 0.05$)

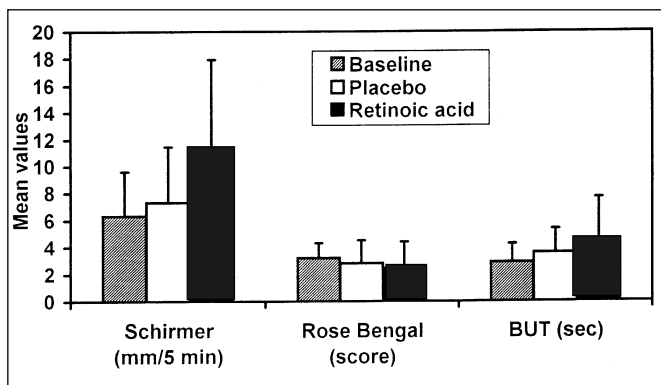


Fig. 1 - Results of Schirmer I Test, BUT measurements and rose Bengal staining. (Error bars are standard deviations for n=44.)

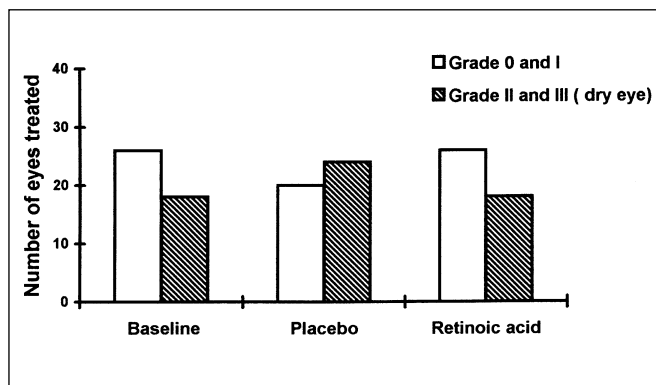


Fig. 2 - Results of fluorescein staining.

mm and 11.35 ± 6.59 mm for placebo and retinoic acid emulsion. The improvement was significant ($p < 0.05$). Placebo treatment increased tear film BUT. The baseline mean BUT was 2.89 ± 1.38 sec and rose to 3.59 ± 1.78 sec after placebo. An increase was obtained to 4.60 ± 3.09 sec with retinoic acid. Rose Bengal staining of mucus in the test population changed from 2.23 ± 1.09 to 2.82 ± 1.71 and 2.45 ± 1.97 respectively after placebo and retinoic acid. Differences between baseline and retinoic acid scores were significant. There was no significant change in corneal and conjunctival fluorescein staining after retinoic acid. Fluorescein staining did not change in corneal and conjunctival epithelium after retinoic acid (Fig. 2). The morphological characteristics of mucus improved significantly with the retinoic acid emulsion ($p < 0.05$) (Fig. 3). Clearing of mucus debris was observed.

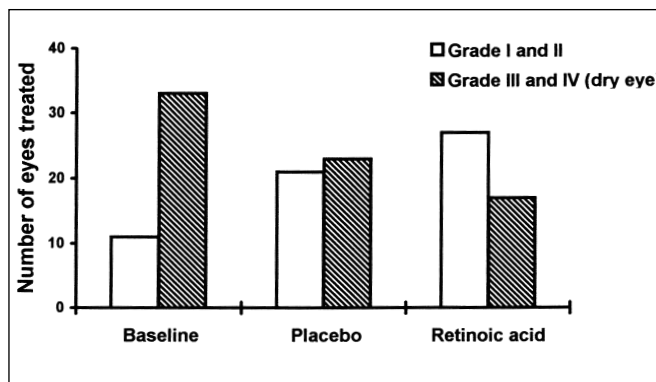


Fig. 3 - Results of mucus ferning test.

DISCUSSION

Although the symptoms in dry eye can sometimes be misleading because patients' complaints are subjective, they can help physicians evaluate clinical tests and findings. Some of the symptoms and signs may not disappear even where the patients are treated with an effective tear substitute. In the present study, there was significant relief in dryness, photophobia and foreign body sensation after the placebo treatment and no real difference was obtained for the first two between placebo and retinoic acid. For burning sensation, there was no difference between the treatments. For the foreign body sensation, however, a signifi-

cant increase was recorded with retinoic acid.

The symptomatic improvement with placebo can be explained by a better lubricating effect on the ocular surface. The increase in foreign body sensation with retinoic acid may reflect an adverse effect. Irritation is still the main problem with retinoic acid and a common finding of earlier studies was a mild to moderate irritating effect. The adverse effects of retinoic acid have been attributed to a higher drug concentration (0.1%) and a mild, not clinically significant reaction was reported with lower concentrations (4). Smolin (11) reported an irritant effect after administration of 1% retinoic acid ointment, and minimal or no conjunctival hyperemia after 0.1% retinoic acid ointment. Three of the 22 patients in the present study complained of blurring of vision.

Our placebo-controlled study found that retinoic acid gave no improvement over placebo, in conflict with Tseng's findings (3). In summary, an improvement was

documented in the placebo control group but there was no further improvement in the retinoic acid group. Tseng (3) observed symptomatic improvement in the patients treated with retinoic acid ointment. However, that study had no placebo control group and used a different vehicle for retinoic acid. Our symptomatic findings confirm two earlier studies (12, 13) which found no difference in symptoms (foreign body sensation, burning, dryness and photophobia) between the placebo (petrolatum, mineral oil) and 0.01% retinoic acid in Keratoconjunctivitis sicca (KCS) and non-KCS patients.

The Schirmer test showed that retinoic acid did promote tear production, unlike an earlier report (12). Retinoic acid has been reported to improve tear secretion in dry eye (3). The significant improvement of BUT by retinoic acid in the present study, supported by the Schirmer test, suggests the treatment did help the tears spread better over the eye surface. Two previous studies (12, 13) indicated that 0.01% retinoic acid ointment and oily drops were no more effective than placebo in KCS, and did not promote aqueous tear production. In the current study, the Schirmer test and BUT gave similar results. Although BUT never reached 10 sec, the increases with placebo and retinoic acid were significant. Fluorescein staining indicated the corneal and conjunctival epithelium maintained their characteristics during the retinoic acid treatment.

Retinoic acid modified rose Bengal scores, with improved staining of the epithelial surface of the eye. The difference between baseline and retinoic acid scores, although small, was significant. The average baseline score was slightly higher than 3, and both placebo and retinoic acid modified the scores that were lower than the average. Only retinoic acid reduced the rose Bengal score significantly. This may reflect the fact that retinoic acid reduces the keratinized and devitalized cells in the cornea and conjunctiva.

With retinoic acid treatment, a common report is that the drug reversed squamous metaplasia and keratinization in the dry eye (2, 3, 12, 14). Clinical tests including Schirmer 1, BUT measurement and the mucus ferning test combined are reportedly the most sensitive approach in dry eye patients (personal communication from Prof. Murat Irkeç). We included the mucus ferning test in the present study because it is easy to perform, better tolerated and gives higher sensitivity and specificity.

Oily solutions and ointments of retinoic acid are the usual formulation for ocular delivery of the drug. Since retinoic acid has poor stability in the presence of light and oxygen, and is insoluble in water, its formulations and clinical use are limited. The emulsion prepared in the present study maintained the chemical stability of retinoic acid for two months when it was stored at 4°C in tightly closed amber glass bottles. Ointment dosage forms of retinoic acid have been found more stable than oily solutions (4). Four weeks at 4°C is still a short time for the clinical use of the drug and attempts must be made to extend the shelf-life in future retinoic acid studies. The more probable advantage of the emulsion used in the current study is the presence of polyacrylic acid polymer in the formulation which makes the rheological behavior of the system better and improves local tolerability.

Ointments and oily eye drops are less acceptable to patients because they cause blurred vision and an unpleasant feeling in the eye. Another problem with oily vehicles in ophthalmic use is poor mixing with tears (15). Polyacrylic acid (Carbopol) adheres to conjunctival and corneal epithelial cells and forms a stable tear film as a result of the mucoadhesive characteristics (16). Non-Newtonian fluids such as Carbopol hydrogels show a decrease in viscosity under a shearing effect such as blinking (17). This provides good tear film spreading over the ocular surface even at high viscosities of the vehicle. The pseudoplastic behavior of the emulsion in the present study was obtained with Carbopol 940 in the formulation. The significant improvement in BUT confirmed the utility of the vehicle in tear film spreading.

In conclusion, retinoic acid in an emulsion was effective in promoting aqueous tear production, and also in improving tear film spreading over the ocular surface. The current study appears to confirm the keratolytic effect of retinoic acid, as shown in earlier studies. A suitable vehicle for the retinoic acid preparation is an important factor for enhancing the effectiveness and reducing the adverse effects of the drug. The present results suggest that an ophthalmic emulsion of retinoic acid may be a promising pharmaceutical approach for the treatment of dry eye. This formulation requires further evaluation of retinoic acid in reversing ocular surface keratinization.

ACKNOWLEDGEMENTS

The Authors wish to thank B.F. Goodrich, Cleveland, Ohio, and Mrs. Laura J. Waldeck for providing Carbopol 940®.

This study was supported by Eczacibaşı Scientific Research and Award Fund, Turkey.

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