Comparative study of treatment of the dry eye syndrome due to disturbances of the tear film lipid layer with lipid-containing tear substitutes

Efficacy of lipid-containing tear substitutes

Authors: Dausch Dieter [1, 2], Lee Suwan [1, 2], Dausch Sabine [1], Kim Jae Chan [2], Schwert Gregor [3], Michelson Wanda [4]

Instituts:
[1] Abteilung Augenheilkunde, Klinikum St. Marien, Amberg
[2] Department of Ophthalmology, Chung-Ang University Hospital, Seoul, Korea
[3] augenärztliche Praxis, Beckum
[4] augenärztliche Praxis, Kronshagen

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Key words
Dry eye – keratoconjunctivitis sicca – lipid layer – phospholipids – polar lipids – triglyceride – nonpolare lipids – eye spray – eye gel – carbomer

Abstract

Backround: A deficiency in the tear film lipid layer is aetiological in about 80 % of the patients suffering from dry eye, which results in excessive evaporation (so-called hyperevaporative dry eye). The treatment with conventional artificial tears did not prove to be successful here. In this study the treatment with two tear substitutes containing lipids were set in contrast with each other.

Material and Methods: The randomised, controlled, multicenter cross-over study included 74 patients suffering from dry eye caused by a deficiency of the tear film lipid layer, which were organised into two groups. Group A (n = 38) was treated for the first 6 weeks with a liposomal eye spray (Tears Again®), while the patients of the group of B (n = 36) were treated with an eye gel containing triglycerides (Liposic®) in the same period. After 6 weeks the crossover was performed. The patients were treated in the following 6 weeks with the product which was not used before. Control examinations by masked examiners took place at the beginning of the study as well as after 6 and 12 weeks, considering following parameters: eyelidedge-parallel conjunctival folds (LIPCOF), BREAK UP time (BUT), Schimer-I Test, measurement of the tear meniscus, investigation of the edges of eyelid and visual acuity. In addition the subjective feelings of the patients were also determined by means of questionnaires.

Results: At the beginning of the study both groups did not differ significantly with respect to the initial values. After the first treatment period the improvement of the examined parameters LIPCOF, BUT, Schirmer, visual acuity and inflammation of the lid margin in group A (eye spray) proved to be significant superior in comparison to group B (eye gel). The results of the second treatment period after the crossover were similar and showed an analogical supremacy of the liposomal eye spray. The interview of the patients resulted that the subjective evaluation concerning efficacy and compatibility of the eye spray turned out to be more favourable explicitly than that concerning the eye gel. 74,6 % of the patients favoured the application as an eye spray onto the closed eyelids over the eye gel into the conjunctival sac. 62,5 % of the patients rated the liposomal eye spray to be better all in all, 12,5 % rated that both preparations are equal and 25 % favoured the eye gel.

Conclusions: The treatment with phospholipid-liposomes shows statistically significant clinical advantages and proves to be favourably and explicitly superior compared to the conventional standard treatment all in all.

Introduction

The tear film is a very complex structure made up of numerous different components, such as lipids, proteins, salts, mucin and water, whose interactions are essential to its stability.

The lipid layer forms the outer layer of the tear film located at the air interface and plays a key role in the composition and functionality of the tear film.

An intact lipid layer can reduce the evaporation rate of tear fluid by 90-95% (36).

Further, it ensures a smooth surface of the tear film and is therefore critical for the quality of vision. In addition, the spreading of the lipid layer reduces free energy at the tear film surface and reduces surface tension by 25%, which is crucial for the structure of the entire tear film [63].

Disorders of this exterior lipid layer are responsible for almost 80% of tear film disorders embraced by the term "dry eye" [25, 26] and are its major etiology [33,54]. Correspondingly, patients suffering from dry eye exhibit an increased evaporation rate at the eye surface [19, 40,50,51] and a higher surface tension of the tear liquid [47,71].

A lipid layer disturbance leads to an excessive evaporation rate and damage to the eye surface due to the resulting increased osmolarity [18, 42, 39].

The use of conventional artificial tear products does not appear to be an appropriate therapy for hyperevaporative dry eye, since it has been shown to additionally increase evaporation rates [38, 66, 67].

After application of the artificial tear product, it takes approximately 40 minutes for the evaporation rate to return to the original value [68]. The application process itself creates a significant disturbance in the lipid layer [24]. Additional preservatives, especially benzalconium chloride, do lasting damage to the lipid layer [28] by dissolving it due to their detergent action [20, 21, 26]. There is a reason why 56% of patients report applying their ophthalmic agents more than 8 times daily [25].

Successful treatment of lipid phase disturbances would instead require a targeted addition of the lipids that are actually lacking.

The lipid phase itself is made up of two layers; the inner layer borders the aqueous phase, consists of polar lipids, and is surface-active. The thick outer layer is composed of neutral lipids with anti-evaporative properties [44].

Phospholipid-liposome therapy has already proven useful in several previous studies [3, 32, 52, 60].

The authors are not aware of any specific study results that would justify a recommendation of therapy with the triglyceride-containing eye gel.

Therefore, this study compared both therapy options regarding their suitability for successful treatment of lipid layer disturbances.

Methods

A randomized, controlled, multi-center cross-over study had been carried out in the participating centers beginning in December 2004, with blinded examiners and including 74 patients.

Participants suffered from dry eye due to disturbance of the lipid phase; they were assigned to treatment groups A (n= 38) or B (n= 36) and treated with the corresponding preparations.

Lipid layer disturbances were diagnosed using reduced tear break-up time and the findings of a slit-lamp examination of the lids, with particular regard to signs of chronic blepharitis [25].

Patients in both groups were treated with a lipid-containing preparation applied 3 times daily in accordance with manufacturer recommendations.

Tears Again® liposomal eye spray (Optima Pharmazeutische GmbH) contains phospholipids in liposomal form (polar lipids). Liposic® eye gel (Dr. Mann Pharma) is an artificial tear preparation based on carbomer (polyacrylic acid) containing triglycerides (neutral lipids).

Lipid layer disturbances were diagnosed using reduced tear break-up time and the findings of a slit-lamp examination of the lids, with particular regard to signs of chronic blepharitis [25].

Patients in group A were initially treated for 6 weeks with the liposomal eye spray, while patients in group B were treated with the triglyceride-containing eye gel for the same time period.

The crossover occurred after this first treatment period, with patients of group A being treated with the triglyceride-containing eye gel for the following 6 weeks, while patients in group B received treatment with the liposomal eye spray.

A washout phase prior to switching preparations at crossover was deemed unnecessary since both preparations do not contain pharmacologically active ingredients; therefore, long-term effects were not expected.

Examinations were carried out at the beginning of the study, at six and at twelve weeks. The following parameters were examined and documented by classification into categories: Lid parallel conjunctival folds (LIPCOF), break-up time (BUT), Schirmer-I test, tear meniscus measurement, slit-lamp examination of the lid margins, and visual acuity.

The established data were documented by assigning categories or severity levels derived from the recommended classification of stages and severities of dry eye (4, 6). The Schirmer-I test values were classified into four categories: >10mm/5 min, <10mm/5min, > 5mm/5 min, < 5 mm/5 min. The values of the tear break-up time were also classified into four categories: > 15 s, 10–15 s, 5–10 s, < 5 s. LIPCOF results were classified into degrees from 0 to 4.

In a slit-lamp exam, the lid margin was evaluated for edema, inflammation, hyperaemia, keratinization and abnormalities of the Meibomian gland orifices (centralization, blockage, scarring), the lashes (abnormal position, loss), and the lash base.

In addition, subjective patient evaluations were recorded using questionnaires administered during control examinations.

Statistical analysis was performed using the statistics software program SPSS v. 12.0.

To illustrate how rapidly the liposomal eye spray reaches the tear film after application onto closed lids, the application of liposomal eye spray mixed with 10%

fluorescein solution in a 1:1000 ratio was observed and photographed under blue light conditions in a volunteer.
The study was designed and carried out in accordance with the regulations of the declaration of Helsinki.

Results

Study population

The study population (N = 75) consisted of 28 male and 47 female patients. One patient was less than 25 years old, 9 patients were between 25 and 45 years of age, 16 patients were between 46 and 60 years old, and the remaining 49 patients were over 60 years old. A correlation between age and gender or a non-random representation was not found (χ^2-test: χ^2-value = 2.134; df = 3; p> .100; n.s.).
Regarding the comparability of the two patient groups, it was shown that the distribution of gender (χ^2-test: χ^2-value = 0.175; df = 1; p> .100; n.s.) and age categories (χ^2-test: χ^2-value = 1.815; df = 3; p> .100; n.s.) was homogenous between the two groups. The study was completed according to protocol by 74 patients. One patient chose to discontinue participation, not due to side effects or medical reasons.

Tear Break-up Time

The tear break-up time measured with the BUT test does not significantly differ between groups A and B at the beginning of therapy (Mann-Whitney U-test: $z = -.426; p > .10; n.s.$).
Over the course of the study, both groups exhibited a significant improvement of break-up time (GLM with repeated measures: Factor time: $F_{(2,122)} = 16.409; p < .001$). However, the increase in tear break-up time differs between the two groups (time by treatment interaction: $F_{(2,122)} = 2.962; p = .055$). The group that first received the liposomal eye spray exhibited a significantly greater improvement during the first six weeks and showed less improvement in the second part of the study (after crossover), when using Liposic (test of the square of the contrast of time by treatment group interaction: $F_{(1,61)} = 6.713$; $p<.05$).
Figure 1 illustrates the difference in improvement between the two groups over the course of the entire study period.

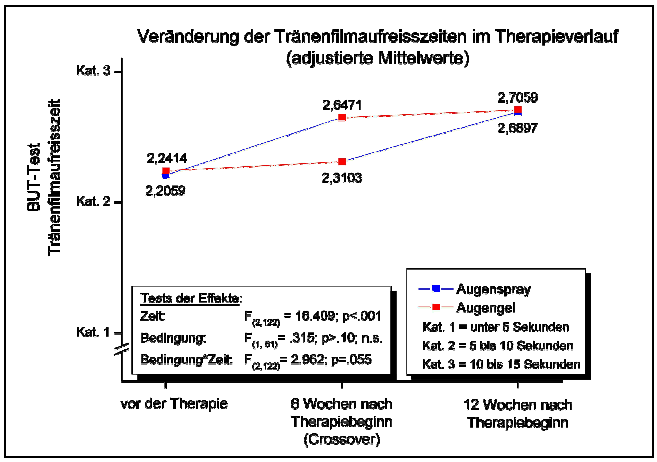

Fig. 1: Changes in Tear Break-up Time over the Course of Therapy (Adjusted Means)

Veränderung der Tränenaufreißzeiten im Therapieverlauf (adjustierte Mittelwerte)	Change in tear break-up times over the course of therapy (adjusted means)
BUT - Test Tränenaufreißzeit	BUT test tear break-up time
Vor der Therapie	Prior to therapy
6 Wochen nach Therapiebeginn (Crossover)	6 weeks after the onset of therapy (crossover)
12 Wochen nach Therapiebeginn	12 weeks after the onset of therapy
Tests der Effekte	Test of effects
Zeit	Time
Bedingung	Condition
Bedingung*Zeit	Time*condition
Augenspray	Eye spray
Augengel	Eye gel
Kat. 1 = unter 5 Sekunden	Category 1 = less than 5 seconds
Kat. 2 = 5 bis 10 Sekunden	Category 2 = 5 to 10 seconds
Kat 3 = 10 bis 15 Sekunden	Category 3 = 10 to 15 seconds

Degree of lid-parallel conjunctival folds (LIPCOF)

Prior to therapy, the two groups did not significantly differ regarding the degree of folding of the lid-parallel conjunctival folds (Mann-Whitney U-test: $z = -.838; p > .100; n.s.$).
Over the course of the study, both groups exhibited a significant reduction in the degree of folding (GLM with repeated measures: Factor time: $F_{(2,138)} = 13.076; p < .001$). As expected, the average improvement of the degree of folding over the entire study period did not differ between the two treatment groups (time by treatment interaction: $F_{(2,138)} = 1.460; p > .100; n.s.$), since both groups had applied both products due to the crossover design.
However, like in the measurement of tear break-up time, the improvement significantly differs between the liposomal eye spray and the triglyceride-containing eye gel. The group that first received the liposomal eye spray exhibited a significantly greater reduction in the degree of folding during the first six weeks, while showing less reduction the second part of the study (after the crossover) when using Liposic (Test of the square of the contrast of time by treatment group interaction: $F_{(1,69)} = 4.004; p < .05$).
Figure 2 illustrates the improvements.

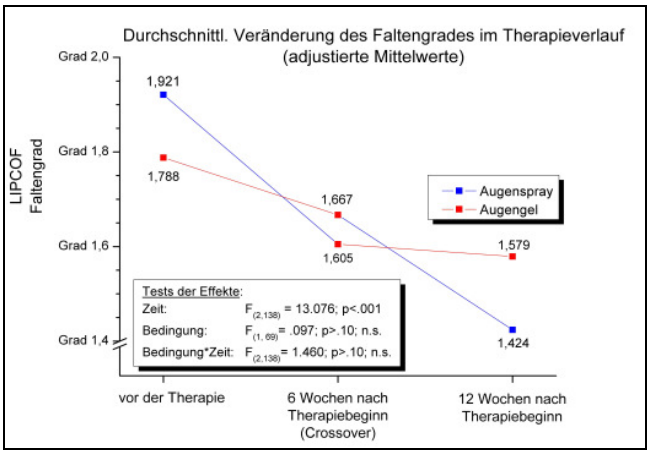

Fig. 2: Reduction of the Degree of Folding (LIPCOF) over the Course of Therapy (adjusted treatment group means)

Durchschnittliche Veränderung des Faltengrades im Therapieverlauf (adjustierte Mittelwerte)	Average Change in degree of folding over the course of therapy (adjusted means)
LIPCOF Faltengrad	LIPCOF degree
Grad 1,4	Degree 1.4
Grad 1,6	Degree 1.6
Grad 1,8	Degree 1.8
Vor der Therapie	Prior to therapy
6 Wochen nach Therapiebeginn (Crossover)	6 weeks after the onset of therapy (crossover)
12 Wochen nach Therapiebeginn	12 weeks after the onset of therapy
Tests der Effekte	Test of effects
Zeit	Time
Bedingung	Condition
Bedingung*Zeit	Time*condition
Augenspray	Eye spray
Augengel	Eye gel

Durchschnittliche Veränderung der Quantität der Tränenflüssigkeit (adjustierte Mittelwerte)	Average Change in tear fluid quantity (adjusted means)
Schirmer-I Quantität der Tränenflüssigkeit	Schirmer-I Tear fluid quantity
Kat. 1,5	Category 1.5
Kat. 2,0	Category 2.0
Kat 2,5	Category 2.5
Vor der Therapie	Prior to therapy
6 Wochen nach Therapiebeginn (Crossover)	6 weeks after the onset of therapy (crossover)
12 Wochen nach Therapiebeginn	12 weeks after the onset of therapy
Tests der Effekte	Test of effects
Zeit	Time
Bedingung	Condition
Bedingung*Zeit	Time*condition
Augenspray	Eye spray
Augengel	Eye gel

Tear quantity (Schirmer-I)

Prior to therapy, both groups exhibited equal quantities of the aqueous layer, on average approximately 5 mm in 5 minutes (Schirmer-I test values; Mann-Whitney U-test: $z = -.107; p > .100; n.s.$) and thus once again did not differ initially. At six weeks, both groups exhibited an improvement in Schirmer-I test values (GLM with repeated measures: Factor time: $F_{(2,142)} = 22.453; p < .001$; Factor condition: $F_{(1,71)} = .804; p > .100; n.s.$; time by condition interaction: $F(2,142) = 2.069$; $p<.150$). While the group initially using the liposomal eye spray demonstrated a significant average improvement of about half a category, or about 2.5 mm of moisture on the test strip at 5 minutes, the group that initially used the triglyceride-containing eye gel only exhibited an average improvement of about 1 mm at six weeks. After the crossover, this difference could also be demonstrated to a lesser degree using inference statistical methods (test of the square of the contrast of time by treatment group interaction: $F_{(1,71)} = 3.722; p = .058$). The first group, which applied the triglyceride-containing eye gel after the crossover, only improved by an average of 0.3 mm in the following (last) 6 weeks, while the second group that applied the liposomal eye spray in the latter 6 weeks improved by an average of 1 mm. Figure 3 illustrates the divergent course of improvements.

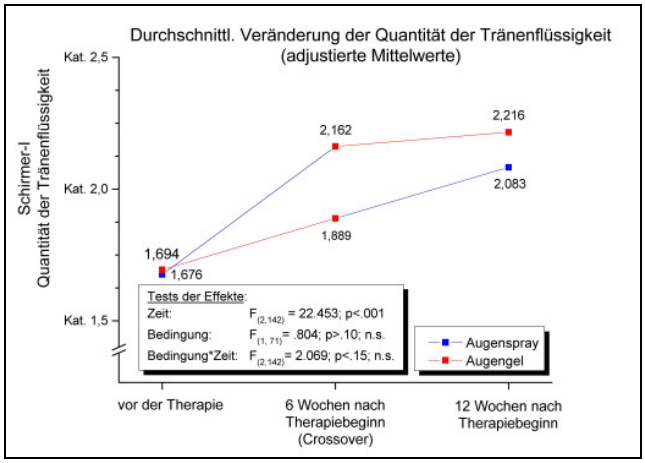

Fig. 3: Increase in Tear Fluid Quantity (Schirmer-I test) over the Course of Therapy for Both Groups (adjusted treatment group means)

Best corrected visual acuity

When examining the best corrected visual acuity prior to the study, the two study groups again do not differ (T-test for independent samples: T = -.575, df 69, p> .100; n.s.). Both groups exhibited an average best corrected visual acuity of about 0.8 (geometric mean) (eye spray group: .786, n = 37; eye gel group: .815, n = 34). The subsequent discussion of results regarding reduction of visual acuity is based on repeated measures with a slightly lower sample number due to "missing values". Thus, initial visual acuity values of the patients in this measurement of change differ descriptively from the total sample; however, while the difference is noticeable, it is not statistically significant.

Short-term Reduction of Best Corrected Visual Acuity

As expected, a reduction of visual acuity was demonstrated for the application of the triglyceride-containing eye gel, since gel application is known to significantly reduce visual quality for a certain time period (GLM with repeated measures: Factor time: $F_{(4,220)} = 39.758; p < .001$; Factor condition: $F_{(1,55)} = 356; p > .100; n.s.$; time by condition interaction: $F_{(4,220)} = 26.497; p < .001$). Figure 4 illustrates the short-term reductions of visual acuity for both preparations. As shown, the application of the eye spray was hardly associated with any average reductions (significance of higher-order contrasts). Rather, a slight increase of visual acuity occurred within 10 minutes, while initial visual acuity had not yet been restored in the eye gel application during the same time period. Both preparations together average an increase of visual acuity within 60 minutes ($F_{(1,55)} = 10.614; p < .010$); however, the improvement was significantly greater for the liposomal eye spray application (time by condition interaction: $F_{(1,55)} = 4.112; p < .05$).

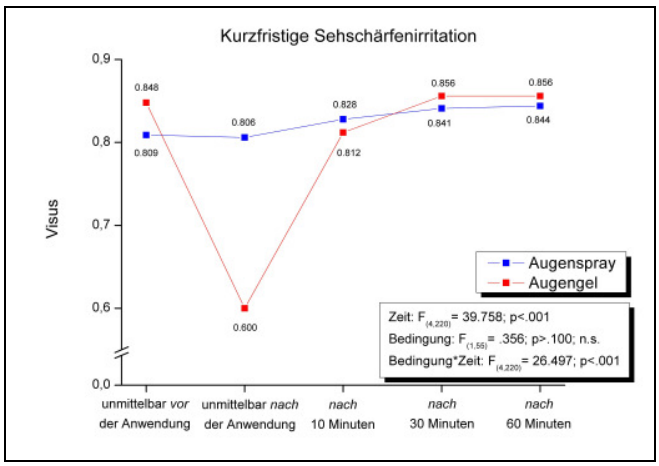

Fig. 4: Short-term Reduction of Visual Acuity after Application of Eye Gel Compared to Eye Spray Application

Kurzfristige Sehschärfenirritation	Short term reduction of visual acuity
Visus	Visual acuity
Unmittelbar *vor* der Anwendung	Immediately *prior to* application
Unmittelbar *nach* der Anwendung	Immediately *after* application
Nach 10 Minuten	*After* 10 minutes
Nach 30 Minuten	*After* 30 minutes
Nach 60 Minuten	*After* 60 minutes
Zeit	Time
Bedingung	Condition
Bedingung*Zeit	Time*condition
Augenspray	Eye spray
Augengel	Eye gel

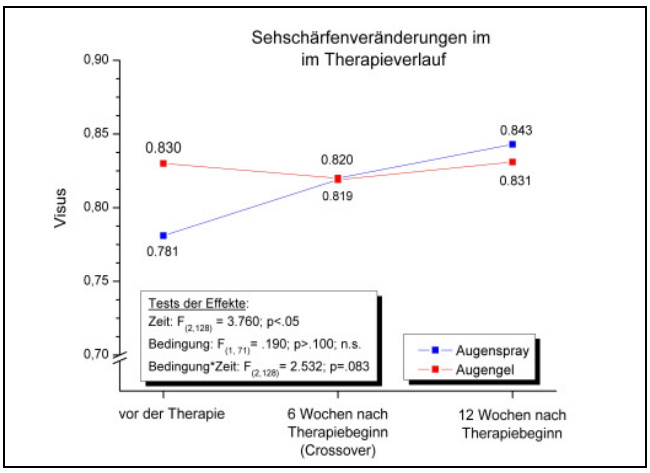

Fig. 5: Long-term Changes in Visual Acuity over the Course of Therapy

Kurzfristige Sehschärfenirritation	Short term reduction of visual acuity
Visus	Visual acuity
Unmittelbar *vor* der Anwendung	Immediately *prior to* application
Unmittelbar *nach* der Anwendung	Immediately *after* application
Nach 10 Minuten	*After* 10 minutes
Nach 30 Minuten	*After* 30 minutes
Nach 60 Minuten	*After* 60 minutes
Zeit	Time
Bedingung	Condition
Bedingung*Zeit	Time*condition
Augenspray	Eye spray
Augengel	Eye gel

Long-term Changes in Best Corrected Visual Acuity

Both groups experienced increased visual acuity after the application of both preparations over the course of the entire study (GLM with repeated measures: Factor time: $F_{(2,128)} = 3.932; p < .05$). However, the improvement was heterogeneous (time by condition interaction: $F_{(2,128)} = 2.532; p = .083$). While an increase in visual acuity was found in the group that had initially applied the eye spray, prior to crossover, a drop in visual function was measured for the group applying the eye gel ($F_{(2,68)} = 4.277; p < .05$). Figure 5 illustrates the long-term changes in visual acuity.

Visual function improved over the first treatment interval in the group applying the liposomal eye spray. In contrast, visual function declined slightly compared to the initial measurements in patients who used the triglyceride-containing gel first.

As evident in the illustration (Fig. 5), both groups experienced an increase in visual acuity after crossover, with the increase again being greater for eye spray application than for use of the eye gel.

Abnormalities in the Lid Margin Examination

Prior to therapy, the two groups did not differ regarding abnormalities in the lid margin examination (T-test for independent samples: T = .180; df = 72; p> .100; n.s.). Both groups exhibited approximately the same number of symptoms, an average of about 2.5 symptoms per person.

While both groups' symptoms improved over the course of the entire study (GLM with repeated measures: Factor time: $F_{(2,144)} = 44.720; p < .001$; Factor condition: $F_{(1,72)} = .563; p > .100; n.s.$), improvements differed during therapy, exhibiting as a decrease of symptoms in both groups (time by condition interaction: $F_{(2,144)} = 3.589; p < .05$). While the average number of abnormalities decreased by about 1 symptom in 6 weeks for the group using the liposomal eye spray, the decrease in the group using the triglyceride-containing eye gel was less pronounced with about 0.5 symptoms on average. During the rest of the study, after crossover, the advantage of the eye spray continued (square contrast of time by condition interaction: $F_{(1,72)} = 6.725; p < .05$), although the effects were only about half as pronounced for both groups. Figure 6 illustrates these effects.

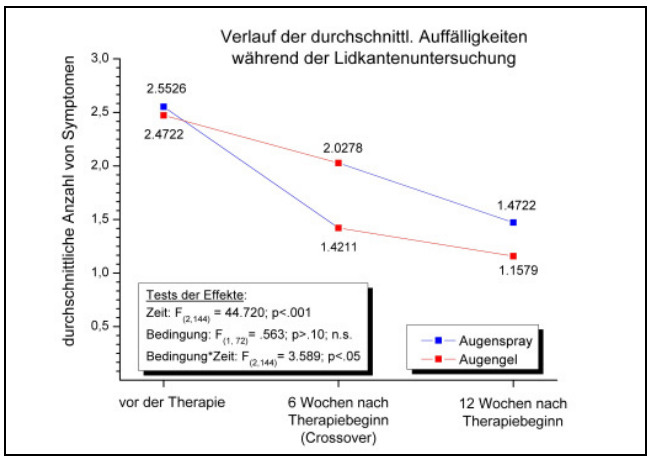

Fig. 6: Change in Lid Margin Examination Abnormalities

Verlauf der durchschnittl. Auffälligkeiten während der Lidkantenuntersuchung	Course of average number of abnormalities found in lid margin examination
Durchschnittliche Anzahl von Symptomen	Average number of symptoms
Vor der Therapie	Prior to therapy
6 Wochen nach Therapiebeginn (Crossover)	6 weeks after the onset of therapy (crossover)
12 Wochen nach Therapiebeginn	12 weeks after the onset of therapy
Zeit	Time
Bedingung	Condition
Bedingung*Zeit	Time*condition
Augenspray	Eye spray
Augengel	Eye gel

Subjective Assessments

Relief of Symptoms

The question "Did you experience relief of your symptoms when using the preparation" was answered differently by patients of the two groups (T-test for independent samples: T = -3.165; df = 54; p< .01). On average, patients felt the greatest relief when applying the liposomal eye spray (expressed on a scale of 1 to 6, 1 being most relief) (M = 2.10; SD = .908; eye gel: M = 2.92; SD = 1.038). The difference between the ratings on perceived relief was about 0.8 on the scale of 1 to 6. Eight of 31 patients (25.8%) in the eye spray group reported that their symptoms disappeared for an extended time period, compared to two of 25 patients (8%) in the eye gel group.

Evaluation of Effectiveness

The subjective evaluation of the preparations' effectiveness shows similar results (t-test for independent samples: T = -3.358; df = 55; p < .01). Again, patients who initially used the eye spray responded with a higher rating on a scale from 1 to 6, 1 being the highest (M = 1.88; SD = 1.1) than those who initially used the eye gel (M = 2.94; SD = 1.294). The difference was greater than one point. Figures 7a and 7b illustrate the divergent evaluations.

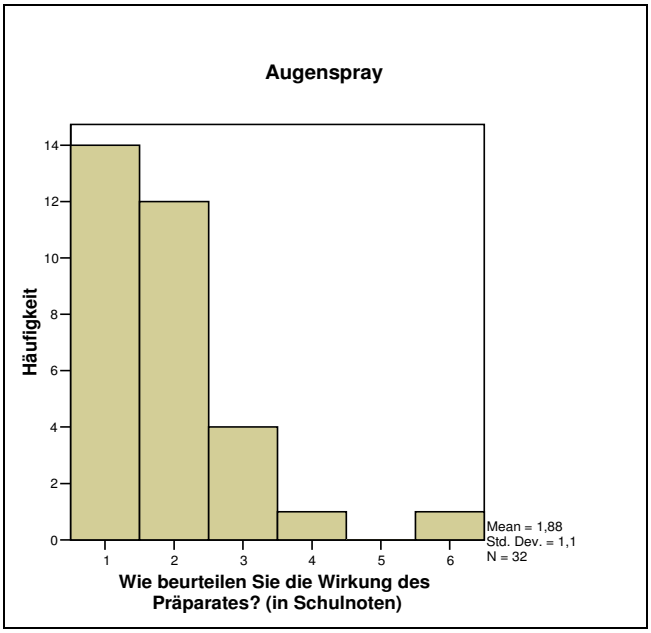

Fig. 7a: Subjective Assessment of Efficacy on a scale of 1 to 6 (1 being best)

Augenspray	Eye spray
Häufigkeit	Frequency
Wie beurteilen Sie die Wirkung des Präparates? (in Schulnoten)	How effective was the preparation? (on a scale of 1 to 6, 1 being the best)
Mean	Mean
Std. Dev.	Std. Dev.
N	N

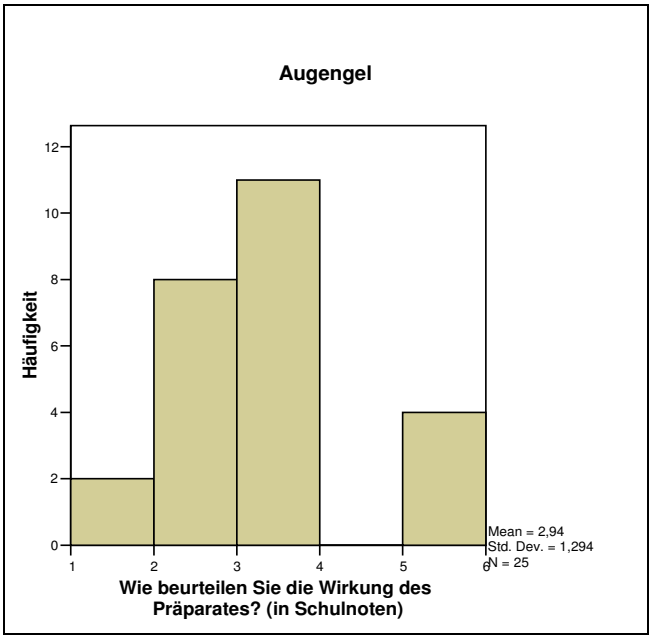

Fig. 7b: Subjective Assessment of Efficacy on a scale of 1 to 6 (1 being best)

Augengel	Eye gel
Häufigkeit	Frequency
Wie beurteilen Sie die Wirkung des Präparates? (in Schulnoten)	How effective was the preparation? (on a scale of 1 to 6, 1 being the best)
Mean	Mean
Std. Dev.	Std. Dev.
N	N

Evaluation of Tolerability

The evaluation of tolerability of the preparations was consistent with the previous results (non-parametric testing due to non-random distribution: Mann-Whitney U-Test: Z =

-2.531; $p < .05$). Again, patients who initially used the eye spray responded with a higher rating on a scale from 1 to 6, 1 being the highest (M = 1.88; SD = 1.185) than those who initially used the eye gel (M = 2.40; SD = 1.032). The difference between the groups was one half point. Figures 8a and 8b illustrate the divergent tolerabilities. As shown, 15 of 32 patients (about 46.9%) give the highest rating of "one" to the tolerability of the liposomal eye spray, while this is the case for only two of 24 cases (about 8.3%) using the eye gel.

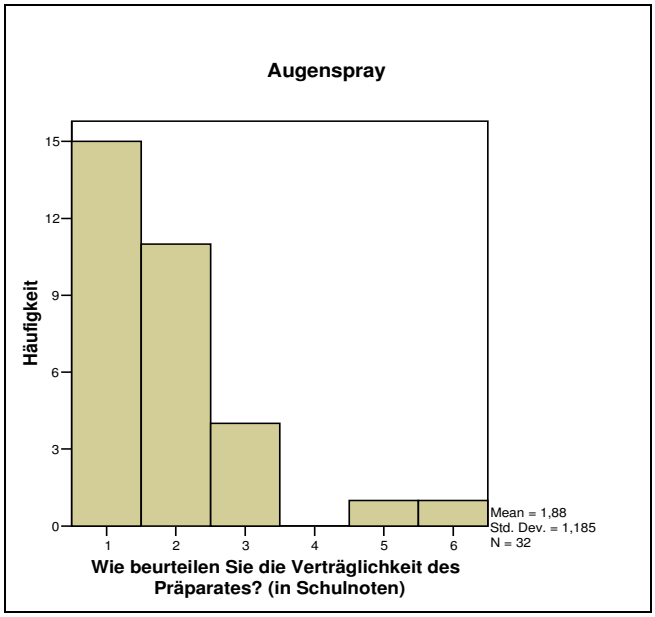

Fig. 8a: Subjective Assessment of Tolerability on a scale of 1 to 6 (1 being best)

Augenspray	Eye spray
Häufigkeit	Frequency
Wie beurteilen Sie die Verträglichkeit des Präparates? (in Schulnoten)	How tolerable was the preparation? (on a scale of 1 to 6, 1 being the best)
Mean	Mean
Std. Dev.	Std. Dev.
N	N

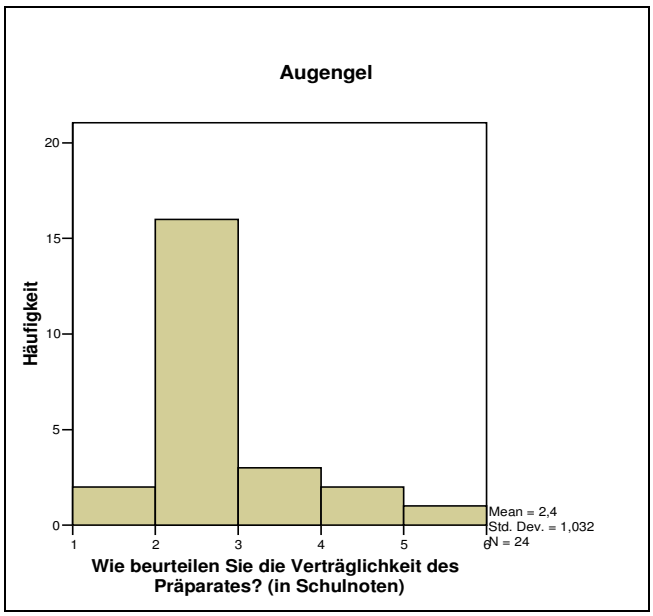

Fig. 8b: Subjective Assessment of Tolerability on a scale of 1 to 6 (1 being best)

Augengel	Eye gel
Häufigkeit	Frequency
Wie beurteilen Sie die Verträglichkeit des Präparates? (in Schulnoten)	How tolerable was the preparation? (on a scale of 1 to 6, 1 being the best)
Mean	Mean
Std. Dev.	Std. Dev.
N	N

Preference of Application Type and Preparation

In the final questions about their preferred application type and preparation, patients clearly favored the liposomal eye spray.

The eye spray was the preferred application method of 74.6 % of patients (53 of 71). Only 18 of 72 patients (25.4%) found the application of the gel more comfortable.

62.5 % of patients (45 of 72) altogether preferred the liposomal eye spray over the triglyceride-containing eye gel (preferred by 25.0%; 18 of 72 patients), while 12.5 % (9 of 72) could not decide. Figures 9a and 9b illustrate patients' preferences.

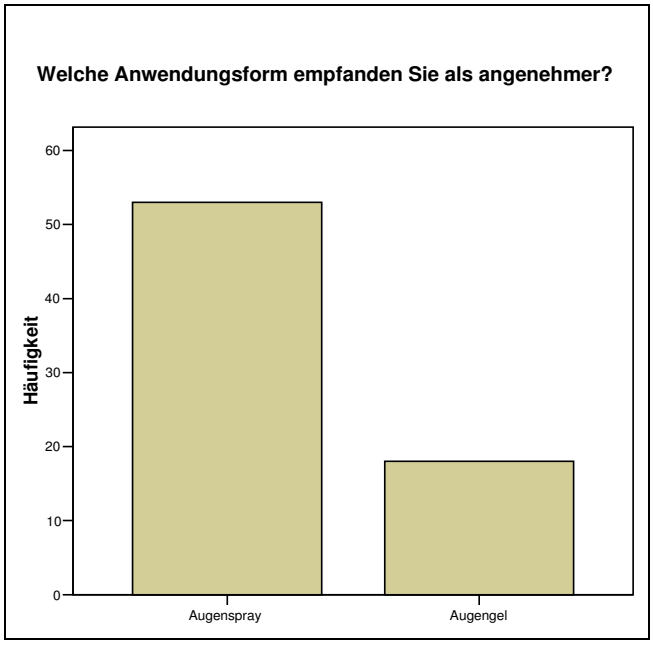

Fig. 9a: Subjective Evaluation – Favored Application Type

Welche Anwendungsform empfanden Sie als angenehmer?	Which application type do you consider more comfortable?
Häufigkeit	Frequency
Augenspray	Eye spray
Augengel	Eye gel

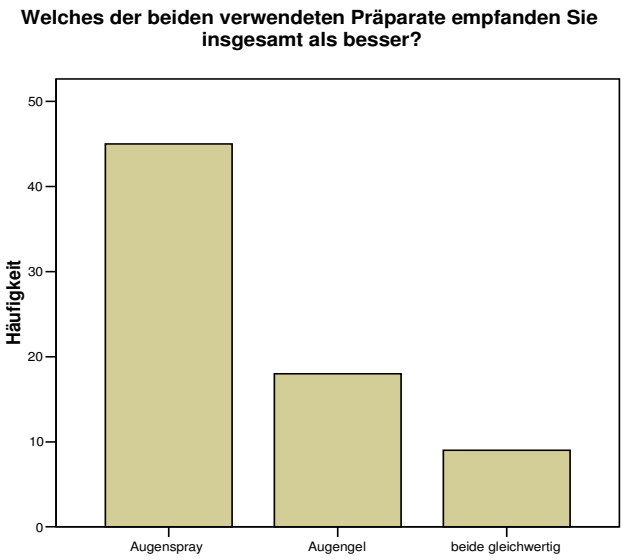

Fig. 9b: Subjective Evaluation - Favored Preparation

Welches der beiden verwendeten Präparate empfanden Sie insgesamt als besser?	Which of the two preparations do you consider better overall?
Häufigkeit	Frequency
Augenspray	Eye spray
Augengel	Eye gel
Beide gleichwertig	Both are equivalent

Photographic Documentation of the New Liposomal Eye Spray Application Type Using Fluorescein

After the application of a liposomal eye spray mixed with a fluorescein solution onto closed lids, the photo series documented how the sprayed-on solution reaches the lid margins and the tear film within a few minutes. Figures 10 to 12 show the pictures prior to application, after application with closed lids, and after subsequent opening of the eye lids. Fluorescein is visible in the tear meniscus after only a few blinks (compare Fig. 12).

Fig. 10: Prior to Application

Fig. 11: After Application onto the Closed Eye

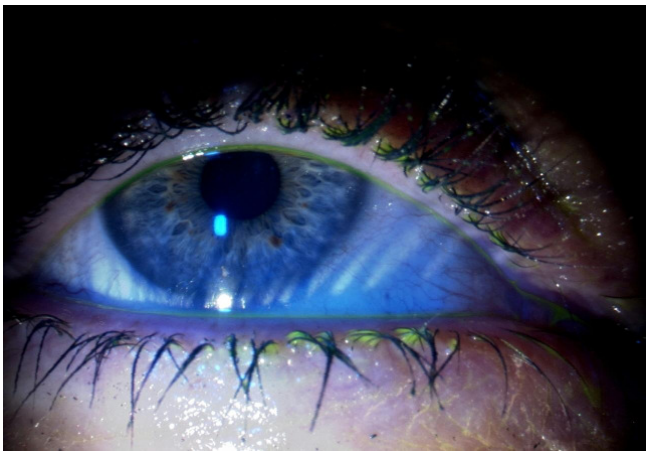

Fig. 12: After Application, Eye Reopened: The mixture of fluorescein and liposomal eye spray is visible in the upper and lower tear meniscus.

Discussion

To better understand the distinct difference between the treatment results with the two preparations, the lipids contained in each one must be compared regarding lipid class, molecular structure and properties.

The current chemical-physical model divides the lipid layer into two phases:

The thicker, neutral layer is located at the interface between the tear film and the air. It is composed of long chain, non-polar, neutral lipids, particularly wax and sterol esters, and is largely responsible for reducing evaporation.

The other is a thin interface layer consisting of polar lipids (especially phospholipids) that forms a stable interface between the aqueous layer and the outer layer of non-polar lipids (22, 23, 29, 44).

When opening the eye lids, the lipids spread from the lid margin onto the tear film, forming the lipid phase. The phospholipids spread first; then the non-polar lipids can follow (61). The surface-active phospholipid molecules carry the hydrophobic non-polar lipids onto the aqueous layer (11, 62), enabling them to spread. Non-polar lipids can only spread out on top of the layer composed of polar lipids (44).

The triglyceride-containing eye gel is applied in the

traditional manner directly onto the eye's conjunctival sac. The application interferes with the tear film by significantly damaging the already disturbed lipid phase, causing its rupture (24, 68). Eye gels and salves in particular lead to long-term disturbance of the entire tear film (24). Their application causes a 7-fold increase in the liquid volume at the eye surface when compared with the normal volume (68). Further, it has been shown that the use of eye gels also increases the evaporation rate (38).

The applied eye gel is based on the thickening agent carbomer (polyacrylic acid).

Carbomer preparations, both with and without preservatives, have a toxic effect on corneal cells *in vitro*, causing severe damage after more than 30 minutes of exposure (14).

It has been shown that carbomer gels remain on the cornea for more than 35 minutes (70), raising concerns about *in vivo* corneal damage due to these toxic effects, at least with long-term use.

The lipid components of the eye gel are non-polar triglycerides, which make up only 3.7 % of the lipid secretions of the Meibomian glands (8) and thus are likely to play a minor role in the stability of the lipid layer. There is no conceivable triglyceride-deficiency in the lipid secretions either, particularly not related to dry eye.

Even among non-polar lipids, triglycerides play a subordinate role, whereas sterol and wax esters together total almost 60% of Meibomian secretions (8).

While the use of lipid-containing artificial tear products has been shown to thicken the lipid layer (30), one can not necessarily deduce that patients are free of symptoms and that treatment was successful (15).

In contrast to the gel, the liposomal eye spray is simply applied to the closed eye. It has been established for a long time that lipids applied to the outer skin of the lid near the lid margins reach the lid margins and thus the tear film during blinking (37, 48).

Intraocular penetration has even been demonstrated for aqueous eye drops applied onto the inner canthus (1, 35, 58).

Although the application onto the closed eye lids may transfer resident bacteria onto the eye surface, there is no risk of infection since the bacterial florae on the eye lids and on the eye surface are necessarily identical (34).

Endogenous lipid secretions of the Meibomian glands are secreted onto the lid margin, forming a reservoir (8). Chew *et al.* calculated that the lipid volume in this reservoir is probably 40 times greater than the amount that actually spreads onto the tear film to form the lipid layer (12).

In the closed eye, the lipid layer is compressed between the lid margins. The lipids that were part of the tear film mix once again with the lipid reservoir on the lid margin. During the opening of the lids, some of the lipids from this reservoir spread onto the tear film, forming a lipid layer (9).

The sprayed-on phospholipid liposomes reach the lid margins via the same effect and mix with the endogenous lipids located there.

The lipid phase is stabilized by the added phospholipids.

Polar lipids comprise 15% of the total Meibomian secretions and play a key role in the stability of the lipid layer due to their surface-active properties described earlier (29, 44). Phospholipids make up 70% of the polar lipids (57).

The liposomes (vesicles made of a phospholipid bilayer) contained in the eye spray are made from highly purified soy lecithin, consisting of 94% phosphatidylcholine and a small amount of other phospholipids (32) that have also been shown to be present in the tear film (22, 23).

At 38%, phosphatidylcholine makes up the largest amount of phospholipids in the tear film (57).

Lipid phase instability is not caused by a quantitative deficiency of total lipid secretions (43) but by their inadequate composition.

This is also supported by the fact that effective evaporation protection is primarily determined by the stability, not the thickness, of the lipid layer. (13, 41).

Lipid phase disturbances and the resulting hyperevaporative dry eye are traced back to a phospholipid deficiency (55, 56), particularly in chronic blepharitis (45, 56, 59).

Observations of an increased surface tension in dry eye patients support an incorrect composition of lipid secretions as well (47, 71).

Surface-active polar lipids are very important for the maintenance of tear film stability:

The spreading of the tear film is thought to take place in a two-step process. In the first step, the (upward) movement of the upper lid draws a tear film layer over the cornea via capillary action. In the second step, an upward flow of the outer lipid layer increases the thickness of the tear film significantly, because the spreading of the lipid layer draws the tear fluid into the tear film (10).

The second step is based on a so-called boundary-layer phenomenon:

Langmuir has demonstrated that the behavior of fluid films of a thickness less than 100 micrometers is completely controlled by surface or boundary forces (31), and is not affected by gravity (27, 53).

The spreading of the lipid layer reduces the surface tension of the tear film, causing a flow of tear fluid from the upper and lower tear meniscus onto the tear film at the eye surface (known as Marangoni effect or Marangoni flow) (2, 36).

The development of a surface film – such as the lipid phase of the tear film – significantly alters the surface properties of the liquid substrate (the tear fluid), such as surface tension, viscosity, and elasticity, as well as light reflection.

The film formation of the lipids largely depends on the chemical makeup of the molecules. Lipid molecules forming a monolayer – a monomolecular film – must have a bipolar molecular structure, consisting of a hydrophilic polar head and a hydrophobic (fatty acid) chain.

During spreading onto the aqueous phase, hydrogen bonding occurs between the polar hydrophilic heads and the water molecules of the aqueous phase, "anchoring" the monolayer on the aqueous phase (16). Phospholipids are amphiphilic, their fatty acid chains interacting with the non-polar part of the lipid phase, while their polar heads interact with the molecules of the aqueous phase.

It is widely known that neutral lipids and water do not mix; therefore, neutral lipids alone could only partially cover the

aqueous layer of the tear film, which would result in the formation of a lens (16) similar to the fat droplets in a soup.
The formation of a monolayer at the surface of the aqueous layer results in decreased surface tension (16). The biggest decrease in surface tension is caused by the phospholipids, especially by phosphatidylcholine (47).
However, in dry eye, a significantly increased surface tension was demonstrated (65).
Therefore, these results support a phospholipid deficit as a cause of dry eye.
It was shown that phosphatidylcholine results in greater tear film stability and significantly increases tear break-up time (49).
The significant improvement in break-up time during use of the liposomal eye spray also supports the results of the previous studies (3, 32, 52, 60).
The slight improvement of tear film break-up time observed when using the eye gel is consistent with previous reports (5).
Tear film stability is essential for visual function. The lipid layer forms the outer layer of the tear film and is therefore the first structure of the visual system that is hit by entering light (64).
The lipid layer provides a smooth tear film surface. The results of this study indicate that the polar lipids (phospholipids) play a key role in the optical quality of the tear film as well.
In agreement with other reports, the use of the eye gel in this study resulted in a significant disturbance of visual acuity (14, 17). Even 10 minutes after application, the original value had not yet been restored. On the other hand, use of the liposomal eye spray did not decrease, but slightly improved visual acuity, also in agreement with prior results (32).
The significant improvement of Schirmer-I test values when using the liposomal eye spray confirms the results of previous studies (3, 32, 52), while the slight improvements during eye gel use correspond to the observations of other studies as well (7).
When evaluating the subjective assessments, it must be noted that besides adding triglycerdies, the eye gel also initially moistens the eye surface, which is perceived as a relief. However, its use significantly disturbs the natural lipid layer (24) and increases the tear fluid evaporation rate (38), potentially resulting in a so-called rebound-effect (68).

Conclusion

The liposomal eye spray shows statistically significant clinical advantages compared to the triglyceride-containing eye gel. The patients' subjective direct comparisons of the two preparations in the crossover study are particularly enlightening and demonstrate a clear preference for the liposomal eye spray regarding its application type onto the closed eye as well as for its effectiveness and tolerability.
Direct comparison reveals that the phospholipid-liposome therapy is altogether advantageous and distinctly superior to conventional standard therapy.^

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