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A new Therapy Concept with a Liposome Eye Spray for the Treatment of the “Dry Eye”

Background:

Most used eye drops replace only the aqueous phase of the tear film. But due to the fact that with approximately 80% of the patients with a dry eye, a disturbance of the lipid phase is present, an approach for new treatment methods was to be found. We examined a new therapy concept with an eye spray, containing liposomes for the therapy of the “dry eye” in a long-term study.

Goal:

Examination of the effectiveness of a liposome eye spray (Tears again®, Optima Pharmaceutical GmbH, Germany) at patients with “dry eye” compared with a spray containing balanced salt solution.

Method:

Between August 2003 and May 2004 a double-blind study with 382 patients was accomplished. The treatment group (V; n=191) was analyzed with the control group (K; n=191) for a period of 6 months regarding the following criteria examined: Eyelid edge parallel conjunctival folds (LIPCOF), BREAK UP time (BUT), Schirmer I Test, best corrected visual acuity, as well as slit lamp findings of cornea and conjunctiva. Altogether 135 parameters were analyzed. Follow up was after 4 weeks and 6 months. The statistical analysis was performed with the statistical program SPSS v.11.5.

Results:

The examined parameters such as LIPCOF, BUT, Schirmer and Visual Acuity were significantly better in the treatment group than in the control group. We found likewise significant improvements of the inflammations of the edge of eyelid with a remarkable decrease around 89.5%. The questioning of the patients resulted in, among other things, that the liposome eye spray led altogether to a clear subjective improvement of the symptoms in 72% of the cases, although initial burning was indicated after the application. All patients were of the opinion, that application with a spray is more favourably and more pleasantly than teardrops.

Conclusion:

The liposome tear substitute shows statistically significant advantages against balanced salt solution. This new liposome eye spray represents a new, revolutionary and effective procedure in the therapy of the “dry eye”. Considering the disturbance of the lipid phase in 80% of the patients, Tears Again ought to be a first choice treatment.

Keywords:

Dry eye – Sicca syndrome – Phospholipid – Liposome – Inflammation of eyelid

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Bibliografie

KlinMonatsbl Augenheilkd 2004; 221: 825-836 p. c Georg Thieme Verlag KG Stuttgart • New York
DOI 10.1055/s-2004-813715
ISSN 0023-2165

Key Words

Dry eye – sicca syndrome – phospholipid – liposome – inflammation of eyelid

Introduction:

The Dry Eye has evolved to the most frequent eye disease in Germany, with a steadily increasing number of persons affected. However, the previous therapy using artificial tear eye drops was mostly unsuccessful and was equally frustrating for patients and ophthalmologists. Although there was a continuously increasing scientific interest on the research of the tear film during the last years, the exact interaction of its different components is not completely understood.

The highly complicated structure of a perfectly functioning tear film makes it easily understandable, that the conventional therapy using artificial tears cannot be successful for the majority number of cases.

According the study of Heiligenhaus et al., only 8 % of the patients show an only disturbance of the aqueous layer, whereas for 78 % thereof, a disturbance in the lipid layer could be found [4, 5]. These findings are confirmed in numerous studies whereby their results clearly demonstrate that the evaporation of the tear liquid is considerably increased in patients with Dry Eye syndrome [8, 16, 23] and the abnormal rate of evaporation is the main reason for the liquid loss [16]. The dysfunction of the meibomian glands – and the resulting disturbance of the lipid layer – must be considered as the most significant cause for the Sicca syndrome [27].

Consequently, the most frequent problem for the Dry Eye is not based on a too low quantity of tear liquid but rather caused by fast evaporation due to disturbances of the lipid phase.

In particular, the serious cases of Dry Eyes, like the Sjögren's syndrome, are also caused by dysfunctions of the meibomian glands and the resulting excessive evaporation of tear liquid [26].

In recent years, many highly interesting findings regarding the lipid layer of the tear film and in particular its structure and composition have been published. However, for incomprehensible reasons, these findings have unfortunately not been realized with the due attention in Germany. The current physico-chemical model of the lipid phase differentiates between an outer, thick,

non-polar layer and a thin polar layer bordering to the aqueous phase [13, 15]. The non polar layer of the lipid phase protects tear liquid from evaporation and is composed of wax-ester, sterolester, triglycerides, and further lipids [1]. About 15% of all secrets of the meibomian glands are polar lipids [1]. The polar layer enables the non-polar compounds to expand on the aqueous layer, it forms an interface layer between the non-polar lipids and the aqueous phase and is responsible for the functional stability of the lipid phase [17].

Molecules which have the property to lower the interfacial tension must have both – a hydrophilic as well as a lipophilic part. They are called molecules with amphiphilic properties. Phospholipids are amphiphilic, their non-polar fatty acids interact with the lipids, and the polar headgroups interact with the aqueous phase. 70% of the polar lipids of the tear film are phospholipids [29]. Among the lipids, the phospholipids have the highest polar character enabling them to stabilize the polar layer [15]. The Dry Eye syndrome caused by evaporation is thought to be due to an anomaly of the polar lipids because a deficient structured polar layer influences the non-polar layer of the lipid phase leading to an increased evaporation manifesting as Dry Eye syndrome [28].

Moreover, further mode of actions concerning the stability of the tear film have been identified during the last years: lipocalin the most secreted protein by the lacrimal glands is able to bind different lipids including phospholipids [6, 7]. It was demonstrated that the interaction of lipocalin with the lipids of the tear liquid can lower the surface tension of the tear film which, in turn, is in correlation with a higher stability of the tear film [20].

Considering this background, it is easily to understand that conventional therapies using artificial tears fail. As far as the liquid applied by using eye drops is not drained immediately via the tear channels, it will be evaporate freely. Only the residual ingredients of the artificial tears remain within the tear film, such as for example thickening and, if present, preservative agents. Recommendations to treat lipid dysfunction, mainly lid massage, eyelid edge care, and the application of warm compresses, do not lead to satisfactory results and are hardly practicable for normal daily use. Today, the im-

portance of the polar lipids for the tear film is generally accepted.

For this reason in particular, the research on or with solutions containing amphiphilic lipids (above all phospholipids) has been enhanced, reflected also by an increasing number of publications [3, 9, 19, 22, 30].

However, these concepts are only preliminary experiments showing a principal chemical problem: phospholipids are solid substances. They can only be transferred into a liquid form (e.g. for application as eye drops) using detergents or organic solvents. The use of organic solvents is impossible due to functional reasons. However, the use of detergents of the here required concentration for dropping into the eye is not applicable as they would inevitably destroy the natural lipid layer of the tear film and, thus, rather deteriorate than improve the treatment of the Dry Eye. Furthermore, detergents are toxic and further injure the already irritated eye.

However, this problem can be overcome when transferring the phospholipids into a liposomal form. Liposomes are vesicles (so-called phospholipid bilayer vesicles) formed by lamellar structured phospholipid double layers, enclosing an aqueous compartment and floating in water. They are only stable in aqueous solvents as they are kept together by hydrophobic interactions. Liposomes are only visible by electron microscopy due to their small size < 100 nm in diameter ($= 0.0001$ mm). In modern medicine, only a few liposomal drugs are available. The main reason for this is that the predominant number of currently developed liposomes is only stable during a relatively short period of time and, therefore, their pharmacological use was strongly limited.

The use of liposomes is mostly known within the field of oncology where they are considered to be a vehicle for active substances (like e.g. cytostatic substances). Special preparation processes are required to prepare homogeneous liposomes, size tailored for their individual application. The physico-chemical characteristics of liposomes depend on many other factors, which must act synergetically. Therefore it is principally wrong to talk about liposomes in general because one must differentiate between different liposome types.

The preparation used for this study contains phospholipid liposomes with a stability of 3 years and thus appropriate for therapy. The required special preparation as well as the composition of the liposomes are subject to international patent protection. During the past years, the effectiveness of the preparation has been discussed several times [24, 31]. However, in previous publications, the main focus was the fact that due to the application of the spray, the increased eyelid temperature could be reduced to a normal value. From the current point of view, this observation can be rather considered

as side effect, although the application, considered as pleasant by all patients, is in direct relation with this fact. On the background of the already mentioned new scientific findings, it becomes clear that the observed and tested effect of the preparation is based on the phospholipids.

Highly purified soy lecithin is used for the production of liposomes, consisting at least at 94 % of phosphatidylcholine. With 38 %, phosphatidylcholine represents also the highest amount of phospholipids within the tear film [29]. Korb et al. reported, that the administration of phosphatidylcholine did not increase the thickness of the lipid layer [13] so that there is no danger of an undesired destabilisation of the lipid layer or an optical impairment. Phosphatidylcholine is furthermore of high importance, because it favours the formation of a monomolecular layer [15]. Moreover the liposomes also contain other phospholipids, i. e. lysophosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol, which have also been detected to be secreted by the meibomian glands [10, 11].

The liposomes are sprayed onto the closed eyelid. It is generally accepted that lipids, which are applied onto the outer lid close to the lid edges, reach the tear film. Norn already reported in 1980, that an ointment applied to the outer skin at the edge of the lid with the closed eye is carried onto the tear film due by blinking of the eye [21]. In this context, it was reported several times that active substances by means of paraffin oil containing ointment are released when the ointment was applied onto the lower outer eyelid [18, 34].

In theory, the number of liposomes of one spray-push could cover a surface of approx. 50 cm², if one could place the liposomes in one layer onto a surface and press them flatly against each other. The surface of the open tear film at the eye is only about 2 cm² [2]. Therefore, it can be expected that already a small number of liposomes (phospholipid bilayer vesicle) can stabilise the lipid layer. We took this as the reason to examine the effectiveness of the preparation TEARS AGAIN® liposomal eye spray within a double-blind study.

Method

A double-blind study with 382 patients (Table 1) was carried out between August 2003 and May 2004 at the „Laserklinik B. Dausch“, Nürnberg, Germany, and in the medical practice of Prof. Dr. med. Dieter Dausch, Amberg, Germany. Patients suffering from Sicca syndrome were treated on both eyes either with TEARS AGAIN®, a liposomal eye spray, or with a physiological sodium chloride solution. Both preparations were applied as a spray. Neither examiner nor patient knew which preparation was used. The eyes of the treatment group (V; n = 191) were treated with TEARS AGAIN®,

the eyes of the control group (K; n = 191) were treated with a physiological sodium chloride solution. The patients should apply the spray three times a day onto the closed eye. Comparison between both groups were carried out after 10 minutes, 4 weeks and 6 months, in particular with respect to the following criteria or changes: lid parallel conjunctival folds (LPCOFS), tear film Break-up time (BUT), Schirmer I Test, best corrected visual acuity, slit lamp findings of the cornea and conjunctiva, subjective discomfort, only to name the most important examined parameter. The testing physician was informed about the results only after the evaluation of the questionnaires.

Table 1: Age and sex of 382 patients (65,2 % of the patients with Dry Eyes were female)

Age (years)	female	male	total
< 25	22	11	33
26 - 45	69	29	98
46 - 60	62	47	109
> 60	96	46	142
	249	133	382

Statistical analysis was performed using the statistical program SPSS v.11.5. All data were tested for normal distribution using the Kolmogorov-Smirnov adaptation test. Parametric t-test was used to evaluate statistical significance in case of normal distribution. Otherwise non-parametric tests such as Wilcoxon test for paired data, Mann-Whitney-U test for unpaired data with 2 variables, and Kruskal-Wallis test for unpaired data with more than 2 variables were applied to carry out statistical significance.

The aim of this study was to find out whether, and if so, which advantages were obtained by TEARS AGAIN® liposomal eye spray when using for treatment of Dry Eyes.

Patients

382 patients suffering from Sicca syndrome were included into this study. 65.2 % of the patients were female, 34.8 % of patients were male. The patients were divided into 4 groups by age: group 1 (< 26 y), group 2 (26-45 y), group 3 (46-60 y), and group 4 (> 60 y). Age and sex are shown in Table 1. Only those patients were included into the treatment group that suffer from Sicca syndrome (table 2) for at least one year and have been treated with conventional therapy without success (table 3). Artificial tear substitutes are aqueous eye drops composed mainly of water and a thickening agent (like e.g. polyvinyl alcohol, different methylcelluloses, carbomere, hyaluronic acid, or polyvidone).

Table 2: Duration of disease in years (for 84.6 % of patients, the symptoms have persisted for at least 1 year)

Duration of Disease (Years)	Number
1	59 (15,4 %)
1 - 5	180 (47,1 %)
5 - 10	97 (25,4 %)
> 10	46 (12 %)
	382 (100 %)

Table 3: Number of drugs used before (83.6 % of patients used at least 1 to 5 other tear substitutes)

Drugs (Number)	Frequency
0	63 (16,4 %)
1 - 5	203 (53,2 %)
> 5	116 (30,4 %)
	382 (100 %)

Results

Tear Film Break-up time

When observing the tear film Break-up time measured with the BUT test, both groups (treatment vs. control group) do not differ significantly before the start of the therapy (Mann-Whitney-U test: $z = -0,173$; $p > 0,05$). After the application of TEARS AGAIN® or the placebo, a significant prolongation of the tear film Break-up time was obtained for all patients (anova with repeated measurements: factor time: $F_{(2,720)} = 402.133$; $p < 0.001$). This prolongation is considerably longer for the treatment group (interaction effect of course and of treatment groups: $F_{(2,720)} = 285.372$; $p < 0.001$). Table 4 shows a descriptive overview of the course of the therapy. Fig. 1 demonstrates it as a graph.

Inflammations of the Eyelid Edges

Regarding the inflammations of the eyelid edges, both groups do not significantly differ before the start of the therapy ($\chi^2_{(df=1)} = 3.060$; $p > 0.05$). Both groups show near the same number of patients with and without inflammations of the eyelid edges. The observed as well as the relative frequencies (in %) of inflammations of the eyelid edges at different times are summarized in table 5. 4 weeks after beginning the therapy, the number of inflammations of the eyelid edges decreased significantly more in the treatment group (application of TEARS AGAIN®) than in the control group (factor time: $F_{(1,380)} = 164.373$; $p < 0.001$; interaction: $F_{(1,380)} = 53.422$; $p < 0.001$). Whereas the number of inflammations in the control group decreased from 50.8 % to 38.7 %, the decrease of inflammations in the treatment group was significantly higher: 59.7 % to 15.7 %. So, out of 114 pa-

		longer than 15 seconds	10-15 seconds	5-10 seconds	below 5 seconds	total
before starting the therapy	control group	5 (2,6 %)	30 (15,7 %)	128 (67,0 %)	28 (14,7 %)	191 (100%)
	treatment group	5 (2,6 %)	30 (15,7 %)	128 (66,0 %)	30 (15,7 %)	191 (100%)
4 weeks after starting the therapy	control group	6 (3,1 %)	40 (20,9 %)	123 (64,4 %)	22 (11,5 %)	191 (100%)
	treatment group	11 (5,8 %)	96 (50,3 %)	84 (44,0 %)	0 (0,0 %)	191 (100%)
6 months after starting the therapy	control group	10 (5,2 %)	41 (21,5 %)	114 (59,7 %)	26 (13,6 %)	191 (100%)
	treatment group	89 (46,6 %)	90 (47,1 %)	12 (6,3 %)	0 (0,0 %)	191 (100%)

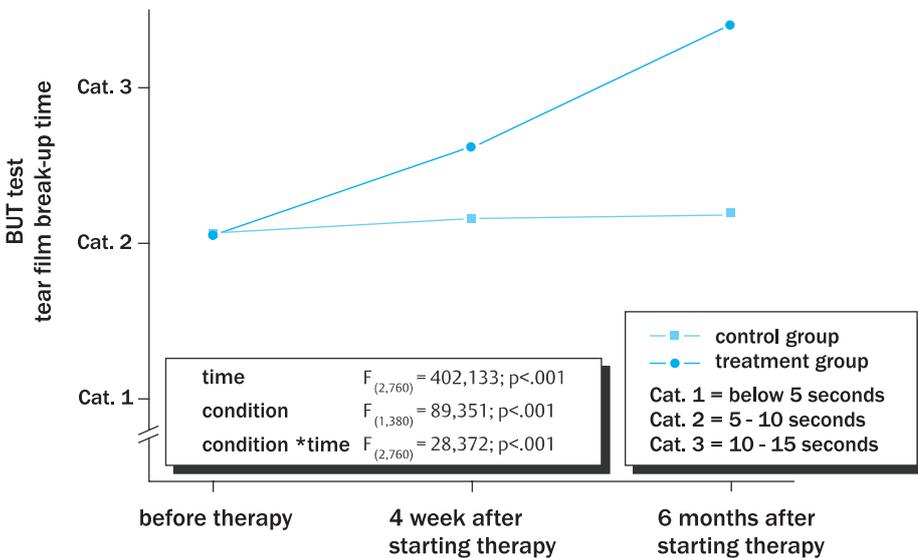


Table 4: BUT test: Tear film Break-up time frequencies (the tear film break-up time of the treatment group already extends significantly after 4 weeks of treatment: after 6 months, 93.7 % show a BUT of more than 10 seconds [pre-operative: 18.3 %], none of the patients show a BUT below 5 seconds. In the control group, 26.7 % show a BUT of more than 10 seconds (pre-operative: 18.3 %).

Fig. 1: Changes in tear film break-up time during therapy (adjusted treatment group mean values). Already 4 weeks after beginning the treatment, a significant improvement in the tear film break-up time can be observed for the treatment group, even further increasing after 6 months. There are no differences to be observed within the control group, neither after 4 weeks, nor after 6 months.

		eyelid edge		total
		without result	inflamed	
before therapy time T1	control group	94 (49,2 %)	97 (50,8 %)	191 (100%)
	treatment group	77 (40,3 %)	114 (59,7 %)	191 (100%)
10 minutes after application: time T2	control group	104 (54,5 %)	87 (45,4 %)	191 (100%)
	treatment group	77 (40,3 %)	114 (59,7 %)	191 (100%)
4 weeks after start of therapy: time T3	control group	117 (61,3 %)	74 (38,7 %)	191 (100%)
	treatment group	161 (84,3 %)	30 (15,7 %)	191 (100%)
6 months after start of therapy: time T4	control group	113 (59,2 %)	78 (40,8 %)	191 (100%)
	treatment group	179 (93,7 %)	12 (6,3 %)	191 (100%)

Table 5: Cross table for inflammations of eyelid edges (the treatment group shows a significant improvement in reduction of the inflammations of eyelid edges after 6 months [59.7 % of cases suffering from inflammation before the therapy down to 6.3 % after 6 months]. The control group shows a slight improvement from 50.8 % to 40.8 %).

tients with inflammations of the eyelid edges only 30 suffered from inflammation after 4 weeks of treatment using TEARS AGAIN® (26.3 %). After six months, an increase of inflammations of the eyelid edges could be observed for the control group (to 40.8 % with reference to the total group), whereas only residual 6.3 % of the initial 59.7 % (114) suffered from an inflammation within the treatment group. With reference to 114 patients with initial diagnosis of inflammations of the eyelid edges, this corresponds to a decrease by 89.5 %. Fig. 2 demonstrates the course of the inflammations of the eyelid edges throughout the total course with reference to the total group.

„Lid Parallel Conjunctival Folds“ (LIPCOF)

The LIPCOF degrees are shown in Table 9. When looking at the „LIPCOF values“, the control group (M = 1.801; SE = 0.070) and the treatment group (M = 1.787; SE = 0.070) do not differ in the degree of conjunctival folds prior to starting the treatment (t-test: $T_{(380)} = 0.212; p > 0.05$). However, after six months, the degree of conjunctival folds is considerably reduced for the treatment group by one degree in average (M = 0.793; SE = 0.067), whereas there is virtually no change for the control group (M = 1.749; SE = 0.067). Fig. 3 shows the course of changes.

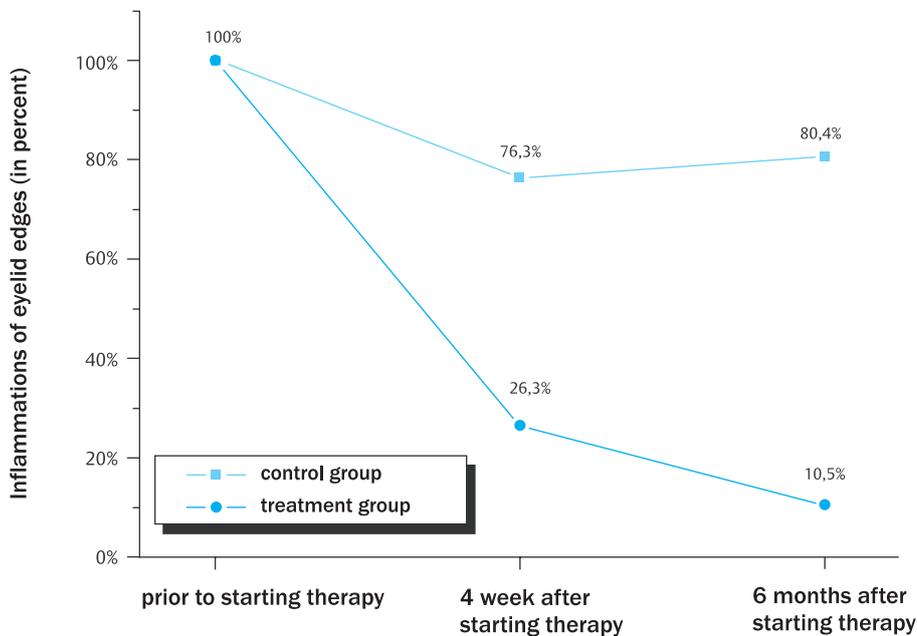


Fig. 2: Course of inflammations of eyelid edges as relative frequency in %. After 6 months there is a significant improvement by 89.5 % in the inflammations of eyelid edges for the treatment group, whereas the improvement for the control group is only 19.6 %. There is a significant difference between both groups.

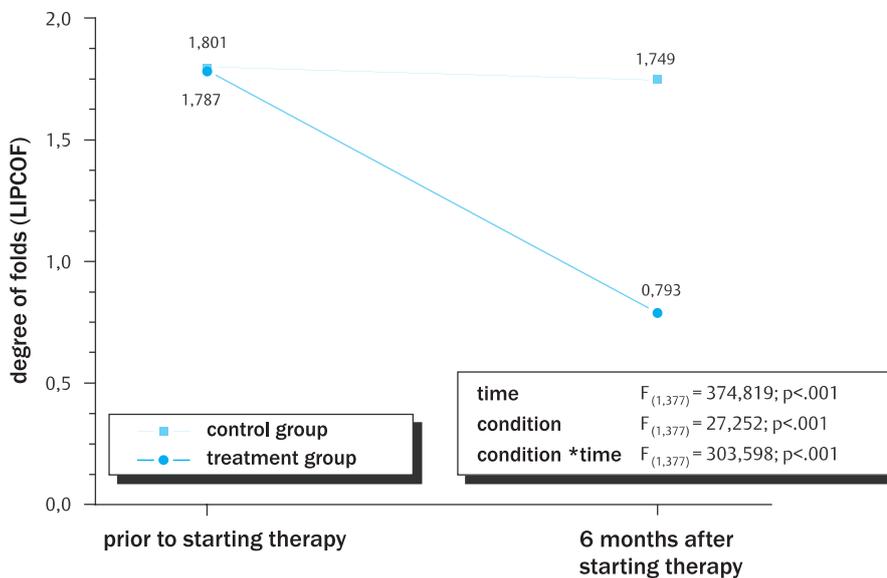


Fig. 3: Average degree of LIPCOF conjunctival folds (adjusted average value). The degree of LIPCOF folds for the treatment group has considerably decreased after 6 months; no significant difference could be observed for the control group.

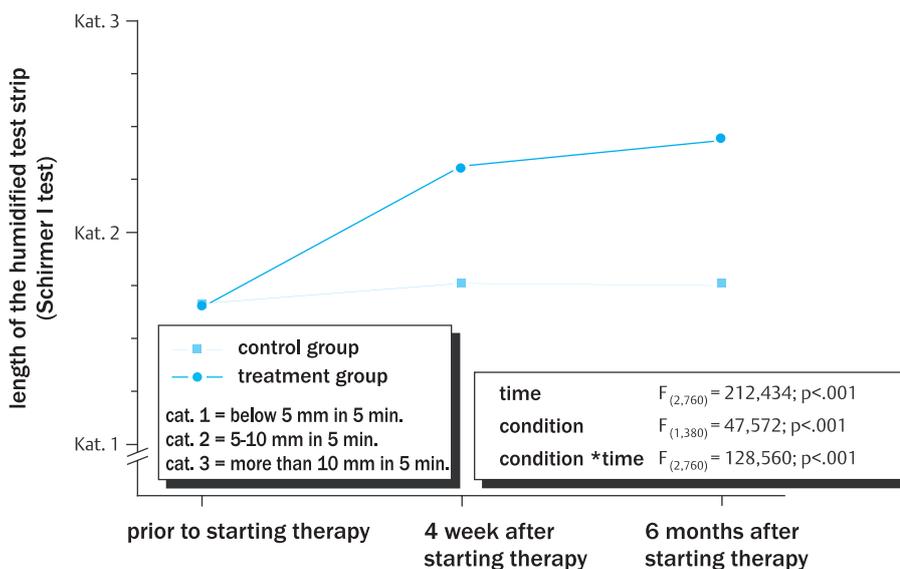


Fig. 4: Volume changes of the aqueous phase for the Schirmer I test during therapy (adjusted average values). The volume of aqueous phase of the tear film has not changed significantly for the control group during the total course of therapy. In contrary, for the treatment group the increase in tear secretion volume stabilises at approx. 5 mm with reference to the initial volume.

Determination of the Volume of the Aqueous Phase of the Tear Film (Schirmer I)

Before starting the therapy, both groups show the same volumes of the aqueous phase of an average of approx. 5 mm in 5 min. (t-Test: $T_{(380)} = 0.238$; $p > 0.05$) during the Schirmer I test. **Table 6** supplies an overview on the Schirmer test values and their changes. During the course of the therapy, a significant mean improvement by $\frac{2}{3}$ rd of one category can be already observed for the treatment group after four weeks, i.e. 3.3 mm test strip humidification after 5 minutes (factor time: $F_{(1,380)} = 241.114$; $p > 0.001$; factor condition: $F_{(1,380)} = 20.446$; $p > 0.001$; interaction: $F_{(1,380)} = 140.089$; $p > 0.001$). Whereas the volume of the aqueous phase of the tear film does not change significantly during the total course of therapy for the control group, a stabilisation of the increase in the humidification path of the test strip to approx. 5 mm with reference to the initial volume can

be detected. This means that during the total course of the therapy the humidification path for the treatment group increased from 5 mm to an average of 10 mm. Fig. 4 shows the course as a graph.

Correction of the Visual Acuity

When observing the visual acuity before starting therapy, there is no difference between both groups (t-Test: $T_{(380)} = 0.387$; $p > 0.05$); comp. table 7). The control group shows an average visual acuity of 0.942 and the treatment group a visual acuity of 0.947. Ten minutes after the application of TEARS AGAIN® or of the placebo, an improvement of the visual acuity can be observed for both groups (control group: 0.968, treatment group: 0.981). However, the improvement does not differ significantly between both groups (factor time: $F_{(1,380)} = 70.118$; $p > 0.001$; factor condition: $F_{(1,380)} = 0.620$; $p > 0.05$; interaction: $F_{(1,380)} = 1.532$; $p < 0.05$).

Table 6: Schirmer I test cross at different times (after 6 months only 3.1 % of the treatment group had a Schirmer value of less than 5 mm after 5 minutes [pre-operative 44 %], whereas no difference could be detected in the control group).

		Schirmer I without Anaesthetisation			total
		more than 10 mm	5-10 mm in 5 minutes	below 5 mm in 5 minutes	
prior to starting therapy	control group	19 (9,9%)	89 (46,6%)	83 (43,5%)	191 (100%)
	treatment group	17 (8,9%)	90 (47,1%)	84 (44,0%)	191 (100%)
4 weeks after starting therapy	control group	20 (10,5%)	88 (46,1%)	83 (43,5%)	191 (100%)
	treatment group	65 (34,0%)	120 (62,8%)	6 (3,1%)	191 (100%)
6 months after starting therapy	control group	18 (9,4%)	90 (47,1%)	83 (43,5%)	191 (100%)
	treatment group	89 (46,6%)	96 (50,3%)	6 (3,1%)	191 (100%)

Table 7: Changes of the visual acuity during therapy (after 6 months, there was a clear improvement of the visual acuity [from 0.94 to 0.98], whereas there is no significant difference for the control group [from 0.94 to 0.95]).

testgroup	time	mean value	standard error
control group	before starting the therapy	0,942	0,009
	10 min. after application	0,968	0,009
	4 weeks after starting therapy	0,955	0,009
	6 months after starting therapy	0,956	0,009
treatment group	before starting the therapy	0,947	0,009
	10 min. after application	0,981	0,009
	4 weeks after starting therapy	0,983	0,009
	6 months after starting therapy	0,983	0,009

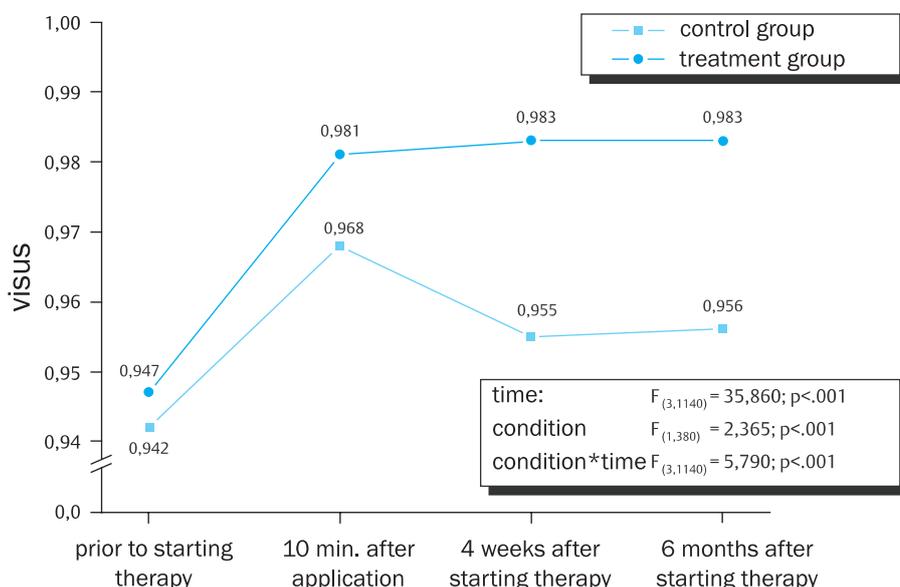


Fig. 5: Changes of the visual acuity during therapy. 10 minutes after application there was a clear improvement of the visual acuity in both groups. After 4 weeks and 6 months, this improvement difference can only be shown for the treatment group. Regarding the initial values, the average improvement of visual acuity for the treatment group was 3.6 %.

When comparing the visual acuity values having been measured four weeks after the start of the therapy, it can be demonstrated that the short-term improvement in visual acuity for the control group can no longer be observed after four weeks (visual acuity control group: 0.955). The treatment group shows a stabilisation at the level observed after already 10 minutes following the application of TEARS AGAIN® (0.983). Considering the initial values, this corresponds to an average improvement in visual acuity of 3.6 % for the treatment group (factor time: $F(1.380) = 59.639$; $p > 0.001$; factor condition: $F(1.380) = 1.784$; $p > 0.05$; interaction: $F(1.380) = 12.219$; $p < 0.01$). The values for the visual acuity, measured after four weeks, do not differ significantly from those measured 6 months after the start of the therapy. Fig. 5 elucidates the changes in visual acuity for both groups during therapy.

Subjective Discomfort

Both groups did not differ significantly prior to starting the therapy regarding subjective discomfort like crusty debris on the lids, burning, sandcorn and dry feeling (Mann-Whitney-U test: $z = -0.339$; $p < 0.05$). Both groups had nearly the same number of patients with and without subjective discomfort. Table 8 shows changes in subjective discomfort during therapy as mean values (0 = no discomfort, 1 = discomfort). Fig. 6 compares the progression of discomfort of both groups at different times. Significant differences between both groups could be found already 10 minutes after the application (Mann-Whitney-U test: $z = -8.98$; $p < 0.05$). In the treatment group, the discomfort decreased in a continuous and significant manner (Wilcoxon test: $p < 0.05$). Only a little and not significant decrease was detected for the control group.

Table 8: Changes in the subjective discomfort during therapy like burning, sandcorn and dry feeling, crusty debris on the lids (a significant decrease in subjective discomfort could be observed for the treatment group after 6 months, whereas there is no significant improvement for the control group).

test condition	time	mean value	standard error
control group	before starting the therapy	0,71	0,19
	10 min. after application	0,62	0,14
	4 weeks after starting therapy	0,67	0,15
	6 months after starting therapy	0,67	0,15
treatment group	groupbefore starting the therapy	0,71	0,20
	10 min. after application	0,33	0,07
	4 weeks after starting therapy	0,36	0,14
	6 months after starting therapy	0,02	0,03

Table 9: Degree of eyelid edge parallel conjunctival folds

Slit Lamp Findings in Primary Position	
LIPCOF 0	no permanently present lid parallel conjunctival fold
LIPCOF 1	small, single-fold lid parallel conjunctival fold, being considerably smaller than a normal tear meniscus
LIPCOF 2	obvious lid parallel conjunctival fold up to the level of the normal tear meniscus, possibly multiple folds
LIPCOF 3	large lid parallel conjunctival fold exceeding significantly the height of a normal tear meniscus (in general multiple folds)
LIPCOF 4	large lid parallel conjunctival fold exceeding significantly the height of a normal tear meniscus and bulging over the inner lid edge until the outer lid edge (in general multiple folds)

Patient Satisfaction

93.2 % of patients participating in this study reported that the tolerance of TEARS AGAIN® ranged from very good to good. 23.8 % of patients felt a slight burning after the first application of TEARS AGAIN®. 361 patients (94.5 %) felt that the application as spray was more advantageous and pleasant than the previously used tear substitutes as drops. Interestingly, the amount of drugs used increased proportionally to the duration of the disease. The anamnesis of 86.4 % of patients suffering from Sicca syndrome for more than 5 years resulted in the fact that they have taken more than 5 drugs in turn (Fig. 8). When questioning the patients regarding the effectiveness of TEARS AGAIN® compared with the preparations used until that date, we could find out the following results (Fig. 7): the alternating application of various other tear substitutes led to an individual improvement of symptoms in 43% of patients, however only lasting for a short period of time. Only 12 % of the questioned patients indicated that the discomfort disappeared for a longer time period when regularly applying other tear substitutes. In the treatment group 44 % of patients showed a slight improvement or an improvement over short period of time and interestingly in 41 % of cases the symptoms disappeared for longer time. This difference was significant (Mann-Whitney-U test: $z = -9.26$; $p < 0.05$). The control group still counts for 12 % of patients an improvement of symptoms for a longer period and in 45 % of cases, no difference could be observed.

Discussion

This study shows that TEARS AGAIN® containing phospholipid liposomes is an excellent preparation to treat the Dry Eye syndrome in the majority of patients and this even for a part of patients, who could not obtain any satisfactory results using different conventional artificial tears for several years.

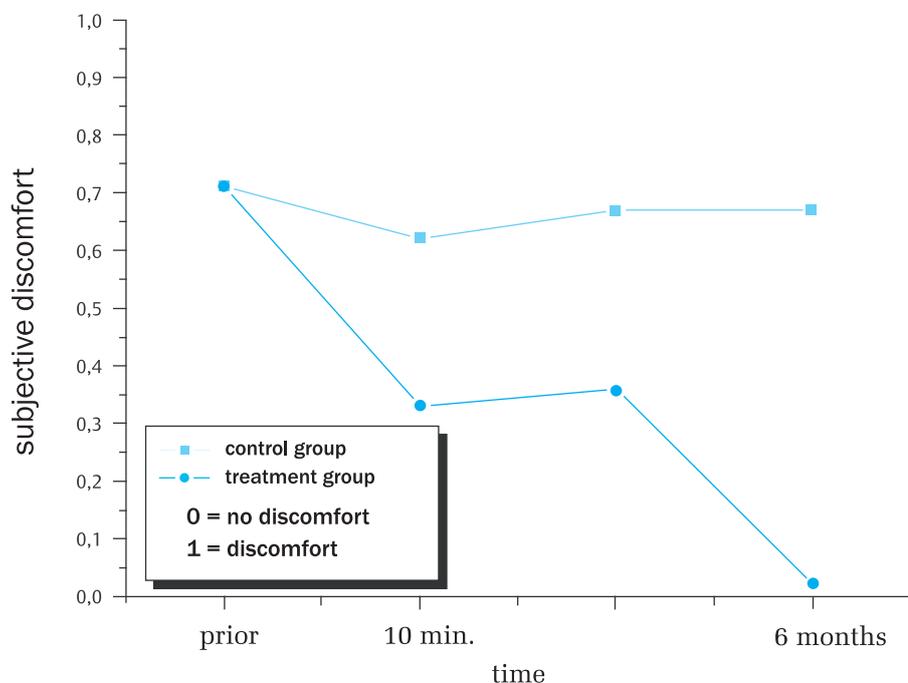


Fig. 6: Analysis of subjective discomfort as group comparison. Already 10 minutes after application, significant differences between both groups appear (Mann-Whitney-U Test: $z = -8.98$; $p \tau 0.05$). A continuous, significant decrease in discomfort could be observed for the treatment group (Wilcoxon test: $p \tau 0.05$). Only a slight, insignificant decrease in discomfort could be noted for the control group.

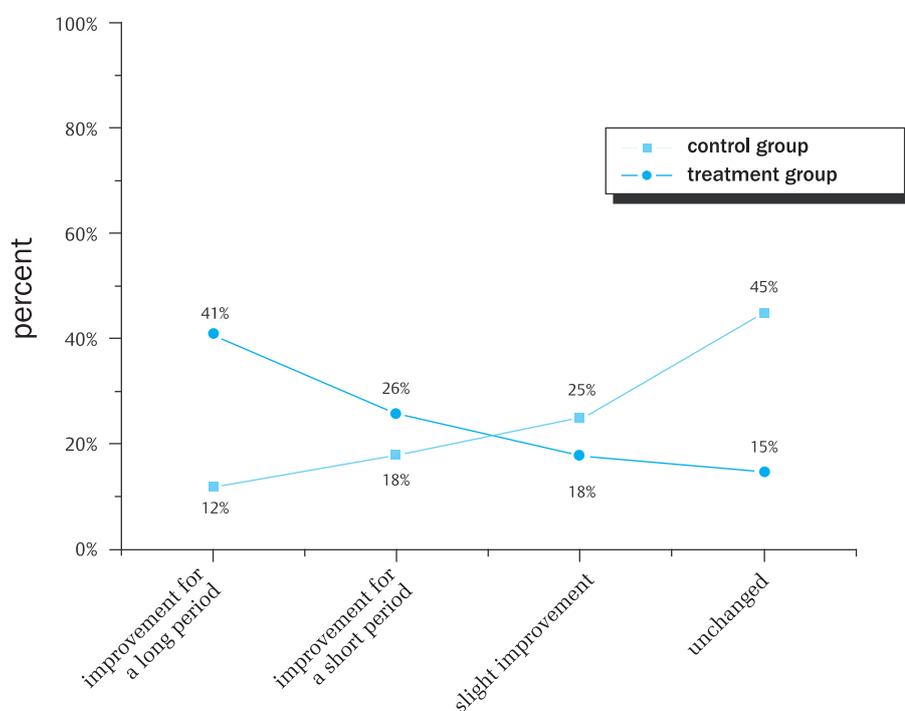


Fig. 7: Comparison of effectiveness between TEARS AGAIN® and other preparations. Altogether 85 % of cases showed an improvement of discomfort in the treatment group, whereas in the control group only 55 % showed this effect. The difference between both groups was significant.

Due to physico-chemical conditions, neutral fat (non-polar lipids) and water reject each other. For this reason, the neutral fats (non-polar lipids) can only spread on the aqueous phase of the tear film if a layer containing polar lipids between the aqueous layer and the non-polar lipids forms a stable interface. If the polar layer of the lipid phase becomes unstable, the lipid layer of the lipid phase inevitably also breaks up and can no longer protect the tear film from evaporation. Water-soluble substances are called hydrophilic; substances which can be dissolved in fats and oils are called lipophilic.

Generally water-soluble substances are not dissolvable in fats and oils and vice versa. Only few substances show both characteristics and are called amphiphilic,

meaning that they can lower the interfacial tension. The interface is understood as the phase separation layer occurring between non-miscible mediums (like e.g. between oil and water). Interface-active substances are molecules containing hydrophilic as well as lipophilic groups which accumulate at the interfaces and lower the interfacial tension (e.g. lipoproteins or lipids with polar groups). Phospholipids (Fig. 9) are amphiphilic, their non-polar fatty acid(s) interact with the lipids, the polar headgroups interact with the aqueous phase.

The behaviour of phospholipids in water is characterised by the fact that a monomolecular film of phospholipids is generated on water. Here, the phospholipid molecules align in a way that their hydrophilic heads can hydrate

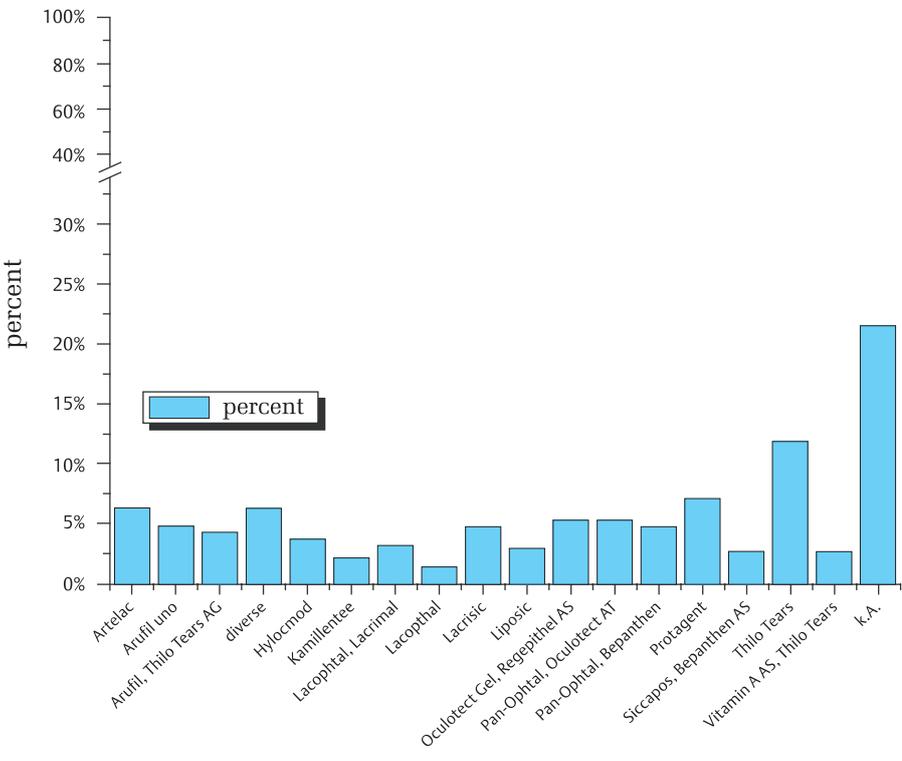


Fig. 8: Frequency of previously used tear substitutes in %

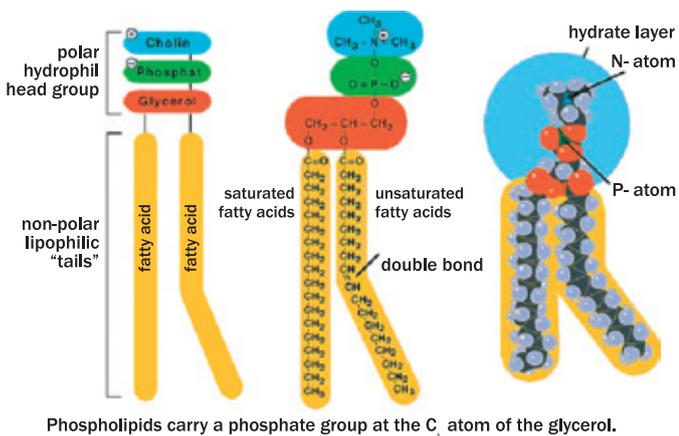


Fig. 9: phospholipid molecule

themselves. The lipophilic tails of the phospholipids jut out vertically upwards from the water surface (Fig. 10). Within the tear film, the lipophilic „tails“ of the phospholipids will be aligned to the non-polar components of the lipid layer, consequently to the non-polar fats, like above all the wax-ester, sterolester, and triglycerides. On this background, it becomes understandable that artificial tear preparations are inappropriate to successfully influence the stability of the lipid layer, even when adding triglycerides. The stability of the lipid layer depends on the structure of the polar layer, so that adding triglycerides cannot be helpful at least with reference to the required stabilisation of the lipid layer. Moreover the study demonstrated that the frequently observed inflammations of the eyelid edges (chronic blepharitis) heal surprisingly fast when using the spray containing phospholipid liposomes. Basically, an inflammation expresses that body-own protection systems are strained due to excessive free radicals. Against that, antioxidants are a natural defence line. The main component of liposomes, the phosphatidylcholine (PC) has an antioxidant effect [25]. As antioxidant in vivo, PC generates a protection [14]. Clinical and preclinical experiments demonstrated that PC protects against a multitude of chemical toxins and against undesired pharmaceutical side effects [12]. These findings meet our own results.

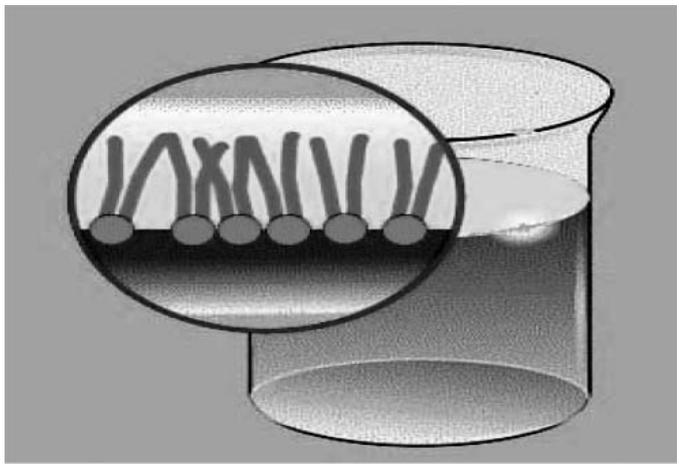


Fig. 10: Behaviour of phospholipid in water.

During this study it became evident that already after 4 weeks the number of inflammations of the eyelid edges within the treatment group decreased significantly more than for the control group (factor time: $F_{(1,380)} = 164.373$; $p > 0.001$; interaction: $F_{(1,380)} = 53.422$; $p < 0.01$). At the end of the study, after 6 months, an overall decrease by 89.5 % of the initially diagnosed inflammations of eye-

lid edges occurred, i.e. from the initially diagnosed 59.7 % of patients suffering from blepharitis only 6.3 % remained. In particular for the serious cases of the dry eye, as for the so-called Sjögren syndrome, the discomfort are also generated by the unimpeded evaporation of the tear fluid – caused by the dysfunction of the lipid layer [26].

In the recent past it was noted that an androgen deficiency leads to dysfunctions of the meibomian glands and thus to a modified profile of the neutral and polar lipids within the lipid layer [33]. An androgen deficiency often observed during the menopause, when ageing, or when suffering from the Sjögren syndrome, essentially contributes to the dysfunction of the meibomian glands, manifesting instability of the tear film and lastly the evaporative dry eye [32].

The significant extension of the tear film break-up time and the observed improvement in visual acuity confirm the theoretical reflections that the phospholipid liposomes stabilise the lipid layer. The tear film break-up time is improved by the presence of phospholipids [22]. The lipid layer provides for a smooth surface of the tear film and is of utmost importance for its optical quality [8, 9, 27]. The also significant increase in measured tear secretion volume can be explained by the reduction in the deficiency of liquid due to evaporation based on the stabilisation of the lipid layer.

Furthermore, the observed reduction in LIPCOFs by one degree of folds in average confirms the success of the treatment using phospholipid liposomes. Of course, the therapeutic concept reaches its limits if the dysfunction of the lipid layer is not causal for the disease. When taking the statistical evaluations of Heiligenhaus et al. as a basis, altogether 22 % of patients either show an isolated dysfunction of the aqueous phase, of the mucin layer, or a combined dysfunction of both layers [4, 5] for which the use of phospholipid liposomes is not promising. Whether the stabilisation of the lipid layer alone is sufficient to produce a regeneration of the tear film when dealing with a combination of dysfunctions of the lipid layer and another phase, or whether the use of additional therapeutics is sensible here still requires clarification by carrying out further examinations. It would be conceivable that in case of dysfunction the mucin layer can regenerate itself if, due to the regulation of the evaporation rate and/or the renouncement to artificial tears containing harmful preservatives, further impairments can be avoided or minimised.

In summary, we can present as the result of this study that due to the effectiveness of phospholipid liposomes the preparation TEARS AGAIN® eye spray is an innovative and effective preparation for the dry eye treatment. All patients had been unsuccessfully treated with conventional artificial tears during years prior to the study. There were improvements for all examined parameters; however, the decrease in inflammations of eyelid edges by 89.5 % was particularly significant. In relation with the dry eye this is very meaningful, because inflamma-

tions of the eyelid edges always hint at a dysfunction of the lipid layer. When questioning the patients it occurred that the eye spray containing phospholipid liposomes led to a subjective improvement for altogether 72 %, although a slight initial burning sensation was indicated in some cases. All treated patients felt that the applications as spray were more advantageous and pleasant than the previously applied artificial tears as drops. Due to the fact that nearly 80 % of the patients with the Dry Eye syndrome also suffered from a dysfunction of the lipid phase, the phospholipid liposomes represent a revolutionary method for the therapy of the Sicca syndrome, and can therefore be considered as remedy of first choice. At this point in time, TEARS AGAIN® eye spray is compared to conventional artificial tears with reference to clinical parameters within the framework of an international clinical multi-centre study which is still running. The objective of this study will be to further research and statistically evaluate the efficiency of phospholipid liposomes.

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