Frontiers in Bioscience Landmark

Front. Biosci. (Landmark Ed) **2022**; 27(7): 202
https://doi.org/10.31083/j.fbl2707202

Original Research

Effects of the Topical Use of the Natural Antioxidant Alpha-Lipoic Acid on the Ocular Surface of Diabetic Patients with Dry Eye Symptoms

Anna M. Roszkowska[1,2], Rosaria Spinella[1], Giovanni W. Oliverio[1], Elisa I. Postorino[1], Giuseppe A. Signorino[1], Dario Rusciano[3,*], Pasquale Aragona[1]

[1]Ophthalmology Clinic, Department of Biomedical Sciences, University Hospital of Messina, 98125 Messina, Italy
[2]Ophthalmology Department, Andrzej Frycz Modrzewski Krakow University, 30-705 Krakow, Poland
[3]Fidia Pharmaceuticals, Research Center, 95123 Catania, Italy
*Correspondence: drusciano@fidiapharma.it (Dario Rusciano)
Academic Editor: Shikun He
Submitted: 17 February 2022 Revised: 11 March 2022 Accepted: 7 June 2022 Published: 27 June 2022

Abstract

Purpose: The purpose of this study is to investigate the effects of the treatment with eye-drops based on a combination of antioxidant and mucomimetic molecules, namely 0.1% alpha-lipoic acid (ALA) and 0.3% hydroxy-propyl-methylcellulose (HPMC) on the ocular surface of diabetic patients with dry eye symptoms. **Methods**: Seventy patients, 42 M and 28 F, aged from 50 to79 years (mean 62.1 ± 10.5), affected by type II diabetes mellitus, were enrolled and divided in two groups treated for 2 months as follows: Group 1 (35 patients), received topical ALA/HPMC three times a day, Group 2 (35 patients) received topical HPMC (0.3%) alone, three times a day. The main outcome measures were: Ocular Surface Disease Index (OSDI), tear film break-up time (TBUT), corneal fluorescein staining, Schirmer I test, corneal sensitivity. An examination of tear film morphology with confocal microscopy was carried out in a subset of patients of each group at baseline and after two months. Statistical analysis was performed with *t*-test for the parametric data and Mann-Whitney U-test or chi-squared test for the nonparametric data. **Results**: Both treatments resulted in significant improvements of BUT, OSDI and tear film morphology, although the improvements observed in group 1 showed a higher trend than what observed for group 2. Moreover, only in group 1 a significant improvement was visible for corneal staining, and no significant improvements were observed in any group for Schirmer I and sensitivity. **Conclusions**: These results confirmed the efficacy of HPMC in the treatment of diabetic dry eye and indicated that the addition of a strong self-regenerating antioxidant like ALA may give a distinctive advantage for the healing of corneal defects (as evidenced by corneal staining), beside improving HPMC efficacy on three other parameters (BUT, OSDI score, tear morphology). Therefore, the addition of a strong antioxidant like ALA can be helpful in preventing or treating ocular surface defects in diabetic patients, in which the oxidative damage is predominant.

Keywords: dry eye; ocular surface; oxidative stress; alpha-lipoic acid; hypromellose

1. Introduction

The hyperglycaemic state of diabetic patients leads to the excessive generation of free radicals (mainly reactive oxygen species: ROS) because of an increased cell mitochondrial activity [1,2]. Consequently, oxidative stress plays an unquestionable role in the aetiology of diabetic complications, such as corneal neuropathies, cataract and retinopathies with different grades of severity [2–6]. In fact, thiobarbituric acid reactive substances (TBARS), F2 isoprostanes and 8-OH-guanosine, that are considered reliable biomarkers of oxidative stress, were present in serum from diabetic patients at significantly higher levels with respect to healthy subjects [4].

Very often, diabetic complications involving the ocular surface unit result in a corneal diabetic neuropathy due to sub-basal nerve plexus alterations [7]. Therefore, several clinical studies in diabetic patients have revealed a significant involvement of the ocular surface with alterations of the corneal and conjunctival epithelium and persistent epithelial defects [8,9]. As a consequence, diabetic patients are generally considered at higher risk than non-diabetics for ocular surgery, with a prevalence of ocular surface complications [2,10]. Diabetic patients usually present decreased corneal sensitivity associated to reduced tear secretion, tear film hyperosmolarity and alterations of corneal epithelial cell junctions, finally resulting in corneal epitheliopathy and neuropathy [11–14]. Corneal involvement may manifest with punctate keratopathy, recurrent erosions, different grades of neurotrophic keratopathy and may be associated to wound healing delay and a higher risk of infection [14–17]. Diabetic patients often complain of dry eye symptoms, such as burning, stinging and foreign body sensation. Additionally, increased conjunctival squamous metaplasia with low goblet cell density and corneal sub-basal nerve alterations may also occur [11–13,17–19].

Alpha-Lipoic Acid (ALA) is a naturally occurring antioxidant also present in foods such as broccoli, spinach, yeast or meat, heart and liver [20]. Two sorts of antioxidants generally work in biological systems: those which mainly associate with a lipophilic phase (membranes or lipopro-

teins such as vitamin E and ubiquinols) and those operating in the aqueous phase such as ascorbate, glutathione and thioredoxin. ALA in its reduced form as dihydrolipoic acid (DHLA) can interact with both phases and is a bridge between them (Fig. 1A). Therefore, ALA and its reduced form DHLA, are considered powerful natural antioxidant agents with a scavenging capacity for many reactive oxygen species [21,22]. However, naturally occurring ALA in tissues (normally found at concentrations ranging between 5 and 25 nmoles/gr) is mostly found covalently attached in an amide linkage with lysine residues on enzymes and thus becomes unavailable as antioxidant (Fig. 1B). Dietary supplementation increases unbound ALA, which can now act as a potent antioxidant and counteract oxidative stress [23]. Beside its antioxidant activity, ALA may increase tissue sensitivity to insulin and lowers blood glucose levels, probably by increasing glucose uptake in muscles and fat cells through the insulin signalling cascade [24]. Lipoic acid distributes to mitochondria where it serves as a critical cofactor for mitochondrial enzymes and is considered a powerful antioxidant, since it is a direct scavenger of ROS with the ability to regenerate endogenous antioxidants such as coenzyme Q10, glutathione and vitamins E and C (Fig. 1A). The additional chelating activity of metal ions gives to ALA high and proven properties to efficiently blunt ROS levels [25].

Reports describing the effects of ALA oral supplementation in diabetic patients confirm its capability to improve insulin sensitivity, to decrease glycemia and oxidative stress, and to induce clinical improvement of neuropathy [20,25–27].

However, the systemic administration of ALA as a food supplement is expected to deliver only a small fraction to the eye, whereas if given as topical eye drops, a higher amount can be retained within the eye [28]. Therefore, given the ascertained role of oxidative stress on diabetic dry eye, the purpose of this study has been to investigate the effects of an original topical treatment with ALA-enriched artificial tears on the tear film and the ocular surface of diabetic patients with symptomatic dry eye disease.

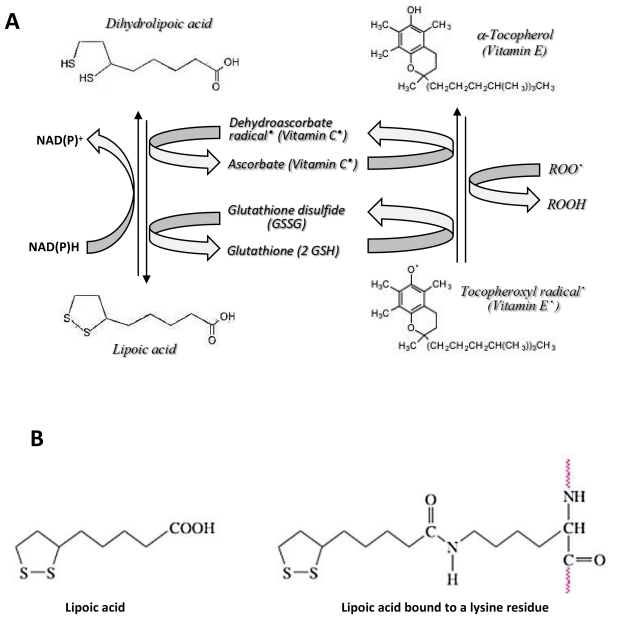

Fig. 1. Lipoic acid and its metabolic effects in living bodies. (A) After its absorption, lipoic acid is reduced enzymatically by NADH or NADPH to dihydrolipoic acid. It can regenerate glutathione and vitamin C, which in turn help the regeneration of vitamin E, thus giving a strong contribution to the antioxidant defense of cells and tissues. (B) Lipoic acid is often found near the active sites of enzymes, usually bound to the peptide by a long, flexible amide linkage with a lysine residue, and is therefore unavailable for vitamin regeneration.

2. Patients and Methods

2.1 Study Protocol

A single blind, randomized, prospective study was conducted in accordance with the tenets of the Declaration of Helsinki and was approved by the Ethical Committee of Messina with Prot.N°106/18. Seventy patients affected by type II diabetes mellitus were considered: 42 patients were males and 28 were females and their age ranged from 50 to79 years (mean 62.1 $\pm$ 10.5). All patients signed an informed consent after a full explanation of all study-related procedures. Inclusion criteria were ability and willingness to participate in the study, type II diabetes lasting for at least 10 years and an OSDI score $>$13 [29,30].

Exclusion criteria were systemic disease other than diabetes, presence of systemic treatments that could interfere with tear production other than hypoglycemic drugs or insulin, topical eye medications within the past 6 months, history of ophthalmic surgery or laser procedures, eye diseases other than dry eye and/or diabetic retinopathy, history of taking vitamin supplements. Once recruited, each patient was randomly assigned to either one of two groups: Group 1, finally included 35 patients (20 M, 15 F), who received a topical formulation containing 0.1% ALA and 0.3% HPMC (Tioretin®, Sooft Italia, Montegiorgio, Italy) three times daily for 2 months. Group 2 finally included 35 patients (22 M, 13 F), who received topical 0.3% HPMC (Next®, Sooft Italia, Montegiorgio, Italy) three times daily for 2 months. Patients were evaluated at baseline and after the 2 months treatment time. An observer, who was masked about the treatment schedule, carried out the clinical evaluations. The demographic characteristics at baseline of patients assigned to the two groups are summarized in Table 1.

2.2 Tear-Film Break Up Time

For the evaluation of tear film stability, a fluorescein tear film break-up time (TBUT) test was performed. A fluorescein strip (Haag-Streit Ag, Köniz, Switzerland) was dampened with a drop of saline, and the strip was made

Table 1. Demographic characteristics of patients assigned to the two groups.

Parameters	Group 1	Group 2	*p* value
	0.1% ALA + 0.3% HPMC	0.3% HPMC	
Age (mean ± SD)	60.25 ± 9.7	64.07 ± 11.3	*0.88**
Sex ratio (M:F)	20:15	22:13	*0.81#*

* Unpaired *T*-test.

Chi squared test.

to touch the inferior lid conjunctiva. Patients were asked to blink several times to mix fluorescein with the tear film and then to keep their eyes open without blinking; the time elapsed between the opening of the eye and the appearance of the first dry spot was measured three times per each patient. The average time was recorded as representative of the patient's TBUT and was reported in seconds. According to the classical procedure here used, TBUT values exceeding 10 seconds are usually considered as normal. Values less than 10 seconds suggest tear film instability [31,32].

2.3 Corneal Fluorescein Staining

Fluorescein vital staining to evaluate the corneal epithelium, as described by Lemp, was analyzed 3 minutes after fluorescein instillation, and observed through a cobalt blue filter [33]. Five corneal areas were considered. In each area, the staining pattern was graduated with a score ranging from 0 to 3 based on the confluence of stain: 0 (absent); 1 (few isolated spots); 2 (numerous isolated spots) and 3 (confluent spots, diffuse loss of epithelium).

2.4 Schirmer I Test

A conventional Schirmer I test without anaesthesia was performed by placing the short portion of a folded Schirmer test strip (Alfa Intes, Casoria, Italy) in the lower lid margin, at the conjunction between the middle and the external third and measuring after 5 minutes the amount of wetting in mm. Less than 10 mm of wetting after 5 min without anaesthesia is considered to be abnormal and suggestive of dry eye syndrome [34].

2.5 Corneal Sensitivity Examination

Corneal sensitivity was tested by the Cochet-Bonnet esthesiometer (Luneau, SKU: 53540, France) in the central corneal area. The higher length of the filament that was felt by the cornea and resulted in eye blinking was registered. Values were expressed in cm. Values between 5.5 and 6.0 cm can be considered normal for the class of ages included in this study [35,36].

2.6 Subjective Evaluation of Ocular Discomfort (OSDI Score)

The Ocular Surface Disease Index (OSDI) is a 12-item questionnaire aimed at assessing dry eye symptoms in the recent life (7 days) of the patient. The test is made of 3 parts dealing with ocular symptoms, vision-related function, and environmental triggers. Patients value their responses on a 0 to 4 scale with 0 corresponding to "never" and 4 corresponding to "most often". A final score is calculated ranging from 0 to 100 with scores 0 to 12 falling within the normal range, 13 to 22 representing mild dry eye disease, 23 to 32 indicating moderate dry eye disease, and greater than 33 typical of severe dry eye disease [29,30].

2.7 In Vivo Confocal Microscopy

Confoscan 4 (Nidek Technologies, Padova, Italy) equipped with 20× optical system, working in no contact mode at a distance of 12 mm, was used to assess patients' tear film appearance in real time. The examined area was 460 × 690 μm with a resolution of the optical system of 1.2 μm/pixel. Examinations were performed in a subset of randomly chosen patients: 10 eyes of 10 patients from group 1 and 9 eyes of 9 patients from group 2. Two pictures per each eye were acquired before and after the 2-months' time treatment. Tear film was evaluated by considering the amount of bright speckles and debris, and the apparent density of the lipid layer [37,38].

In order to obtain a semi-quantitative assessment, fractional scores between 0 and 3 were given to the acquired images of each tear film, describing the absence (0) or the increasing presence (up to 3) of visible amounts of bright speckles and debris, and of an abnormal lipid layer; then, a cumulative average score for each group of tear films was calculated.

2.8 Statistical Analysis

Power analysis was performed to determine the number of subjects to enroll into the study taking into consideration the efficacy of 0.3% HPMC in dry eye treatment [38]. The number of subjects enrolled in the present study (n = 70: 35 controls with HPMC and 35 treated with ALA/HPMC) was based on statistical power calculation made with G Power software version 3.1 setting the power to 0.80, α to 0.05 and considering a medium effect size [38]. For each patient, only the results obtained in the worst eye were considered for statistical evaluation using the MedCalc 12.2.1.0 statistical software for Windows (MedCalc Software, Ostend, Belgium). The Student's two tailed paired *T*-test was used to compare data within each group at enrolment and after two months and the Student's two tailed *T*-test for independent data was used to compare between groups at enrolment and after two months. The Mann-Whitney U-Test was used for the analysis of nonparametric data (OSDI score, confocal microscopy score) and the chi-squared test to evaluate the sex ratio. *p* values less than 0.05 were considered statistically significant.

3. Results

Six dropouts were registered among the 70 patients enrolled: two patients from group 1 (treated), and four patients

Table 2. Signs and symptoms, average values ± SD.

Treatment		BUT (sec)	Schirmer I (mm)	Sensitivity (cm)	Corneal staining (score)	OSDI score
Group 1 (ALA/HPMC)	Baseline	3.94 ± 2.24	14.7 ± 5.47	5.61 ± 0.45	1.41 ± 0.89	24.46 ± 10.75
	2 months	5.17 ± 1.89	15.15 ± 6.13	5.50 ± 0.41	0.83 ± 0.69	18.15 ± 5.69
	p value	0.004*	0.23*	0.20*	0.02#	0.004#
Group 2 (HPMC)	Baseline	4.21 ± 1.97	15.47 ± 4.41	5.46 ± 0.42	1.40 ± 0.89	26.18 ± 9.51
	2 months	4.75 ± 1.68	16.06 ± 3.85	5.50 ± 0.45	1.29 ± 0.72	22.46 ± 7.73
	p value	0.021*	0.13*	0.75*	0.65#	0.003#

* Paired *T*-test.

\# Mann-Whitney test.

from group 2 (controls) did not show up at the two months' control. Therefore, data are based on 33 patients from group 1 and 31 patients from group 2.

3.1 Signs and Symptoms

Table 2 illustrates the course of the different parameters in the two treatment groups. Group 1 patients, treated with the topical combination ALA/HPMC showed a significant improvement of three out of five parameters, namely BUT, corneal epithelial staining, and OSDI index. Group 2 control patients, treated with HPMC alone, showed significant improvements of the subjective parameters (OSDI) and BUT. No improvement was evident for corneal epithelial staining. Neither treatment affected tear secretion (Schirmer I) or corneal sensitivity. When comparing the results of the two groups at the end of the two months, a significant difference ($p = 0.02$) was found only for corneal epithelial staining, improving only in the ALA/HPMC group 1.

3.2 Confocal Microscopy

Fig. 2, panels A and C show confocal pictures of the tear film of two representative diabetic patients, one per each group, at enrolment. Before treatment an abnormal pattern with numerous irregular elements is clearly visible: numerous bright speckles and dark spots can be observed, likely due to the presence of dispersed lipid drops and cellular debris, sign of an abnormal tear film and the suffering of ocular surface epithelial cells. After two months of topical ALA/HPMC administration (Fig. 2B), a regular distribution of the tear film can be appreciated, with a smooth surface and no bright speckles or dark elements ($p = 0.0078$). Fig. 2D shows the tear film of the control patient after treatment for two months with HPMC alone, also showing a similar, although lesser, improvement ($p = 0.031$). The semiquantitative score assigned to the different samples are reported in Table 3. A significant improvement was observed in both groups, which were and remained statistically similar at the beginning ($p = 0.22$) and at the end ($p = 0.14$) of the treatment time.

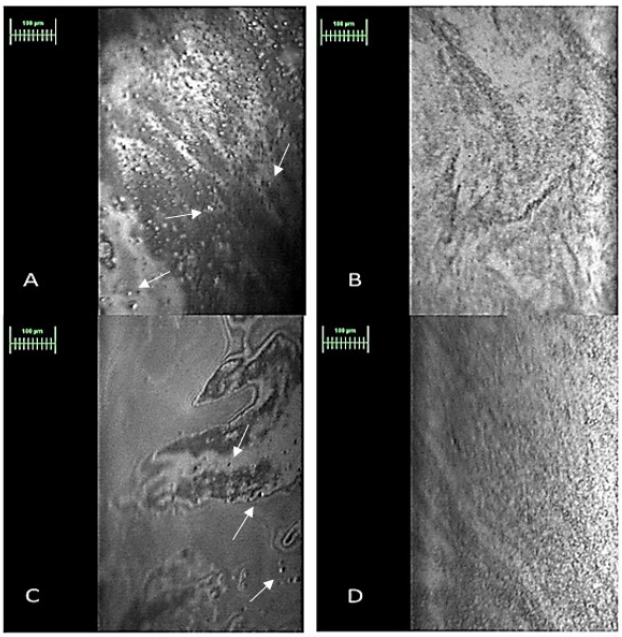

Fig. 2. Illustrative confocal images of the tear film in diabetic patients before and after treatment in both studied groups. (A) Group 1 (HPMC/ALA) before treatment: the tear film surface appears irregular with an altered lipid layer, numerous bright speckles, possibly due to exposed tips of microvilli and microplicae, and dark spots, produced by desquamating epithelial cells (arrows). (B) Group 1 after treatment: the tear film surface appears smooth with a regular distribution of the lipid layer and the absence of irregular elements. (C) Group 2 (HPMC alone) before treatment: the tear film surface shows an irregular pattern of the lipid layer with numerous presence of bright speckles (arrows). (D) Group 2 after treatment: evident improvement of the lipid layer with reduction of the irregularities can be observed. Scale bar 100 μm.

4. Discussion

A healthy ocular surface unit is necessary for proper visual acuity and diabetes is known to be a strong risk factor for its integrity. In fact, the prevalence of dry eye disease in diabetic patients is about 50–53%, and corneal epithelial lesions are present in 47–64% of affected patients who frequently report ocular discomfort and visual impairment

Table 3. Confocal analysis of tear film, the average score ± SD is reported.

Treatment	Time	Score
GROUP 1 (ALA/HPMC)	Baseline	1.81 ± 0.75
	2 months	0.94 ± 0.77
	p value	0.0078#
GROUP 2 (HPMC)	Baseline	2.22 ± 0.67
	2 months	1.44 ± 0.53
	p value	0.031#

Mann-Whitney test.

[1,8]. Goebbels *et al*. [13] presented data showing that, in comparison to healthy subjects, insulin-dependent diabetic patients showed significantly decreased reflex tearing (Schirmer test I) and significantly increased evidence of conjunctival metaplasia. Basal tear flow (Schirmer test II) and TBUT were found to be unaffected. These data are consistent with the observation that diabetic neuropathy most often involves the ophthalmic nerve, thus triggering a corneal neuropathy with loss of sensitivity [39,40], which in turn results in decreased tear secretion and altered composition of tear proteins [41,42].

Given the wide occurrence throughout the world of dry eye disease, different treatment strategies have been developed, and proposed for the treatment of eye dryness, independent of its etiology [43]. They are based on the integration of one of the three components of the tear film: mucins, water and lipids; or contain molecules with direct or indirect antinflammatory and secretagogue effects. The main objective is the stabilization of the tear film (normalization of the BUT) and the recovery of the ocular surface. Hyaluronic Acid (HA) and Hydroxy-Propyl-Methylcellulose (HPMC) are among the most used hydrating and lubricant molecules to integrate the aqueous and mucinous layers of the tear film [44]. However, it is oxidative stress the main and an early event in the pathogenic mechanism of diabetes [45], which may influence the onset and progression of late complications, especially diabetic retinopathy [46], but also including dry eye [47]. Therefore, the use of antioxidants on the ocular surface of such patients appears to be an interesting therapeutic target.

To date, few eye drops formulations have been proposed to specifically treat the ocular surface disease (OSD) of diabetic patients. N-acetyl-carnosine has been shown to be an antioxidant prodrug, with the potential to treat several ocular pathologies in which the oxidative stress is a main agent [48]. Naltrexone (an opioid antagonist and a mild antioxidant) or Rebamipide (a scavenger of free radicals and a COX2 inhibitor) have been formulated as eye drops to treat diabetic OSD [49,50]. Most recently, Glucagon-Like Peptide-1 (GLP-1) formulated in eye drops have been used in a rat model system of diabetes-induced retinal abnormalities, showing that the enhancement of the antioxidant defense system in the diabetic retina has a neuroprotective action favouring DNA repair and neuron cells proliferation [51]. However, the great advantage of using ALA as an antioxidant comes from its very same chemical nature, in that it recycles between a reduced and an oxidized form, and both the oxidized and reduced forms of ALA exhibit antioxidant properties [52] so that this antioxidant system gets exhausted in a much longer time than other antioxidant systems. This property can be very critical in the management of diabetic dry eye, which recognizes oxidative stress due to a long-lasting hyperglycaemic state as a main etiological cause.

Systemic supplementation with ALA has already been suggested to have positive effects both in the prevention and treatment of diabetes and its systemic complications [53]. Most recently, ALA treatment has even been proposed to attenuate the symptoms of COVID-19 in diabetic patients [54]. Along this line, several other experimental studies have shown the positive effects of ALA in patients affected by dry eye and diabetic retinopathy [55]. In fact, from a mechanistic point of view, ALA can down regulate the expression of MMP-9 in the corneal epithelium and induce a better antioxidant defence on the ocular surface. Moreover, being endowed with a strong direct antioxidant effect, it can efficiently counteract oxidative stress-induced corneal surface erosion and damage to the lachrymal glands. Through the inhibition of the enzyme N-acetylglucosamine transferase, of the pro-inflammatory transcription factor NFk-B and the direct alleviation of oxidative stress, ALA may contribute to the prevention of diabetic retinopathy. Similarly, it can activate the transcription factor Nrf-2, which regulates the expression of antioxidant defence and PKA in retinal ganglion cells [55]. Indeed, Nrf-2 activation has been proposed as a strategy to prevent diabetic complications [56]. Clinical trials of ALA given as food supplement on diabetic complications such as neuropathy, nephropathy and cardiomyopathy returned favourable results, especially for pain attenuation in neuropathy, and contrast sensitivity in retinopathy [57]. More specifically, clinical trials conducted in pre-retinopathic diabetic patients showed ALA with genistein and vitamins could protect the retinal cells and decrease the inflammatory effect in diabetic patients [58]. In order to enhance the specific activity of ALA on the eye, a nanomicellar formulation of ALA as eye drops has been described, with the aim of treating diabetes-associated ocular surface disease. Such formulation showed an improved pharmacodynamics, with enhanced stability and permeability [59].

As a powerful antioxidant, ALA chelates transition metal ions, increases the levels of other antioxidants such as glutathione and vitamin E and C, therefore resulting in the scavenging of ROS and other free radicals. Based on these properties, the systemic use of ALA has been proposed for the treatment of diabetic peripheral neuropathy [20,25,26]. Since oxidative stress is clearly involved also in the pathogenesis of ocular surface alterations such as in the case of

diabetic dry eye, the topical use of strong antioxidant drops could be expected to improve tear film stability and surface integrity. In fact, in the present study, we have shown a better improvement of the ocular surface in the group of patients treated with the combination of HPMC and ALA, with respect to the group of patients taking only HPMC eye drops. It is interesting to note that although the subjective perception of improvement (as measured by the OSDI score) was similar between the two groups of patients, the average improvement in group 1 appeared better than the one measured in group 2 (Fig. 3). Moreover, the objective signs improved more in the group of patients treated with the association of HPMC and ALA (Fig. 3). We observed a 30% average improvement in BUT with HPMC/ALA (p = 0.004) vs. a 13% average improvement with HPMC alone (p = 0.021). Even more interesting was the finding of a significant 40% average reduction (p = 0.02) of the corneal epithelial staining in patients who received HPMC/ALA with respect to the control group, in which no significant variation was observed (p = 0.65). Therefore, the strong antioxidant effects of ALA added to the hygroscopic and lubricant activities of HPMC improved the efficacy of the eye drop combination. In fact, HPMC alone was indeed effective, but not on all the parameters under evaluation, and not with the same intensity as the combination with ALA. Only tear production and corneal sensitivity were not affected by either treatment, likely due to the high variability among the enrolled subjects, and to the fact that their average starting values were not in the pathologic range. On the other hand, even though the average quantity of tears was not increasing, tear quality appeared to be improving, since its stability as measured by the BUT was increasing, in parallel with corneal epithelial cells integrity as evidenced by the confocal microscopy analysis of the tear film. This technique was applied to tear film analysis by Mather in 1997 [60], however with a poor resolution of the images, likely due to the fact that they used a contact confocal procedure, which is not optimal for the observation of the tear film [61]. The technique was in fact perfected by the pioneering work of Smolek *et al.* [37] using non-contact confocal microscopy, who described the regular lipid layer distribution over a smooth surface with no bright speckles of the tear film in normal eyes. On the contrary, in dry eye the bright speckles - possibly due to exposed tips of microvilli and microplicae - could be observed with numerous dark erosions produced by desquamating epithelial cells. In a following paper, Stonecipher and Green confirmed the previous observations by evaluating the tear film in normal eyes and in moderate to severe dry eye [38]. In normal eyes the lipid layer was found to be evenly distributed over the eye surface, whereas in moderate dry eye it appeared spotted by the presence of many tear film debris; in severe dry eye, many desquamated epithelial cells with a poor tear film lipid layer with a rapid BUT could be seen. In our study, the confocal analysis of the tear film before any treatment showed severe alterations, in agreement with its instability as evidenced by the low BUT. These observed anomalies could be attributed to a thinner lipid layer, and the dark elements may likely derive from cell debris or mucous layer alterations. In our study - the first to apply non-contact confocal microscopy of the tear film in a comparative treatment analysis - we could also appreciate the same irregular pattern described by the previous authors, with numerous bright speckles corresponding to the exposed tips of microvilli as it happens in moderate and severe dry eye, and in good agreement with the more intense corneal fluorescein staining of the enrolled subjects, due to desquamating cells and deep erosions. In images taken after the two months of treatment, we could see a clear improvement of the ocular surface and the tear film, with a smooth lipid layer, reduced bright speckles, dark erosions and cellular elements in both treatment groups. However, patients in group 1, treated with the combination of ALA/HPMC displayed a 48% average improvement (p = 0.0078), better than the 35% average improvement seen in control group patients (p = 0.031) (Fig. 3), in agreement with the significant improvement of the corneal staining for patients of group 1, but not of control group 2. This confocal investigation of the tear film is a relatively new technique, with scant literature publications, so that no standard reference values (in terms of a semiquantitative analysis) are available.

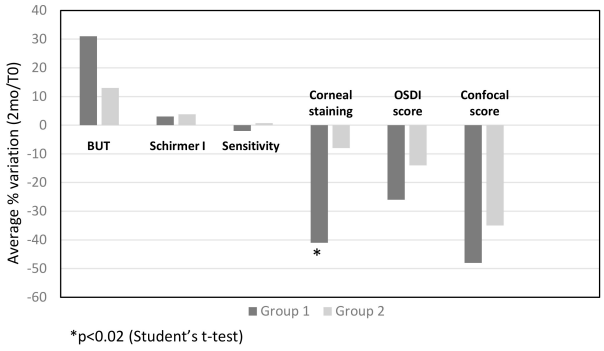

Fig. 3. The average variation of different parameters before and after two months of treatment with either ALA/HPMC or HPMC alone eye drops has been calculated as a percentage, dividing the average value obtained at the end of treatment by the initial value, as reported in Table 2.

5. Conclusions

To our knowledge, this is the first report on the topical use of a strong antioxidant such as ALA in diabetic patients with dry eye symptoms. More studies will be necessary to confirm and extend these preliminary observations, which have been done in a short time frame. Nonetheless, we believe that the additional benefits of such therapy could already be considered for diabetic patients with ocular sur-

face disorders. Furthermore, the topical use of ALA could be extended to all dry eye patients in view of the fact that oxidative stress is more than often a key element in the etiology of dry eye.

Author Contributions

Conception and design—AMR, PA. Administrative support—AMR, PA. Provision of study materials or patients—AMR, PA. Collection and assembly of data—RS, GWO, EIP, GAS. Data analysis and interpretation—AMR, RS, GWO, EIP, GAS, DR. Manuscript writing—AMR, DR, PA. Final approval of manuscript—All authors.

Ethics Approval and Consent to Participate

A single blind, randomized, prospective study was conducted in accordance with the tenets of the Declaration of Helsinki and was approved by the Ethical Committee of Messina with Prot.N°106/18.

Acknowledgment

Not applicable.

Funding

This research received no external funding.

Conflict of Interest

DR is a full time employee of Fidia Pharmaceuticals, the company selling the product Tioretin under analysis in this study. The other authors declare no conflict of interest.

References

[1] Arman MSI, Al Mahmud A, Mahmud HR, Reza AA. Free radical, oxidative stress and diabetes mellitus: A mini review. Discovery Phytomedicine. 2019; 6: 99–101.

[2] Ljubimov AV. Diabetic complications in the cornea. Vision Research. 2017; 139: 138–152.

[3] Sayin N. Ocular complications of diabetes mellitus. World Journal of Diabetes. 2015; 6: 92–108.

[4] Stephens JW, Khanolkar MP, Bain SC. The biological relevance and measurement of plasma markers of oxidative stress in diabetes and cardiovascular disease. Atherosclerosis. 2009; 202: 321–329.

[5] Kowluru RA, Chan P. Oxidative Stress and Diabetic Retinopathy. Experimental Diabetes Research. 2007; 2007: 43603.

[6] Lutty GA. Effects of Diabetes on the Eye. Investigative Opthalmology & Visual Science. 2013; 54: ORSF81–ORSF87.

[7] Roszkowska AM, Licitra C, Tumminello G, Postorino EI, Colonna MR, Aragona P. Corneal nerves in diabetes – the role of the in vivo corneal confocal microscopy of the subbasal nerve plexus in the assessment of peripheral small fibre neuropathy. Survey Ophthalmol. 2021; 66: 493–513.

[8] Aragona P, Giuffrida S, Di Stefano G, Ferreri F, Di Benedetto A, Bucolo C, *et al*. Ocular Surface Changes in Type 1 Diabetic Patients. Advances in Experimental Medicine and Biology. 2002; 506: 667–672.

[9] Lyu Y, Zeng X, Li F, Zhao S. The effect of the duration of diabetes on dry eye and corneal nerves. Contact Lens and Anterior Eye. 2019; 42: 380–385.

[10] Chung H, Tolentino FI, Cajita VN, Acosta J, Refojo MF. Reevaluation of Corneal Complications after Closed Vitrectomy. Archives of Ophthalmology. 1988; 106: 916–919.

[11] Schultz RO, Van Horn PL, Peters MA, Klewin KM, Schutten WH. Diabetic keratopathy. Transactions of the American Ophthalmological Society. 1981; 79: 180–199.

[12] Ramos-Remus C, Suarez-Almazor M, Russell AS. Low tear production in patients with diabetes mellitus is not due to Sjögren's syndrome. Clinical and Experimental Rheumatology. 1994; 12: 375–380.

[13] Goebbels M. Tear secretion and tear film function in insulin dependent diabetics. British Journal of Ophthalmology. 2000; 84: 19–21.

[14] Inoue K, Kato S, Ohara C, Numaga J, Amano S, Oshika T. Ocular and Systemic Factors Relevant to Diabetic Keratoepitheliopathy. Cornea. 2001; 20: 798–801.

[15] Seifart U, Strempel I. The dry eye and diabetes mellitus. Ophthalmologe.1994; 91: 235–239. (In German)

[16] Shih KC, Lam KS, Tong L. A systematic review on the impact of diabetes mellitus on the ocular surface. Nutrition & Diabetes. 2017; 7: e251.

[17] Markoulli M, Flanagan J, Tummanapalli SS, Wu J, Willcox M. The impact of diabetes on corneal nerve morphology and ocular surface integrity. The Ocular Surface. 2018; 16: 45–57.

[18] He J, Bazan HEP. Mapping the Nerve Architecture of Diabetic Human Corneas. Ophthalmology. 2012; 119: 956–964.

[19] Cousen P, Cackett P, Bennett H, Swa K, Dhillon B. Tear production and corneal sensitivity in diabetes. Journal of Diabetes and its Complications. 2007; 21: 371–373.

[20] Bast A, Haenen GRMM. Lipoic acid: a multifunctional antioxidant. BioFactors. 2003; 17: 207–213.

[21] Scott BC, Aruoma OI, Evans PJ, O'neill C, Van Der Vliet A, Cross CE, *et al*. Lipoic and Dihydrolipoic Acids as Antioxidants. A Critical Evaluation. Free Radical Research. 1994; 20: 119–133.

[22] Packer L, Witt EH, Tritschler HJ. Alpha-lipoic acid as a biological antioxidant. Free Radical Biology and Medicine. 1995; 19: 227–250.

[23] Gomes MB, Negrato CA. Alpha-lipoic acid as a pleiotropic compound with potential therapeutic use in diabetes and other chronic diseases. Diabetology & Metabolic Syndrome. 2014; 6: 80.

[24] Delage B, Hagen TM Lipoic Acid. 2019. Available at: https://lpi.oregonstate.edu/mic/dietary-factors/lipoic-acid (Accessed: 9 June 2022).

[25] Smith AR, Shenvi SV, Widlansky M, Suh JH, Hagen TM. Lipoic Acid as a Potential Therapy for Chronic Diseases Associated with Oxidative Stress. Current Medicinal Chemistry. 2004; 11: 1135–1146.

[26] Ziegler D, Reljanovic M, Mehnert H, Gries F. A-Lipoic acid in the treatment of diabetic polyneuropathy in Germany: Current evidence from clinical trials. Experimental and Clinical Endocrinology & Diabetes. 1999; 107: 421–430.

[27] Singh U, Jialal I. Alpha-lipoic acid supplementation and diabetes. Nutrition Reviews. 2008; 66: 646–657.

[28] Cagini C, Leontiadis A, Ricci MA, Bartolini A, Dragoni A, Pellegrino RM. Study of alpha-lipoic acid penetration in the human aqueous after topical administration. Clinical & Experimental Ophthalmology. 2010; 38: 572–576.

[29] Maharana PK, Raghuwanashi S, Chauhan AK, Rai VG, Patterbahadur R. Comparison of the efficacy of carboxymethylcellulose 0.5%, hydroxypropyl-guar containing polyethylene glycol 300/propylene glycol, and hydroxypropyl methyl cellulose 0.3% tear substitutes in improving ocular surface disease index in cases of dry eye. Middle East African Journal of Ophthalmology. 2017; 24: 202–206.

[30] Schiffman RM. Reliability and Validity of the Ocular Surface Disease Index. Archives of Ophthalmology. 2000; 118: 615–621.
[31] McMonnies CW. Measurement of Symptoms Pre- and Post-treatment of Dry Eye Syndromes. Optometry and Vision Science. 2016; 93: 1431–1437.
[32] Abelson MB, Ousler GW, Nally LA, Welch D, Krenzer K. Alternative Reference Values for Tear Film Break up Time in Normal and Dry Eye Populations. Advances in Experimental Medicine and Biology. 2002; 506: 1121–1125.
[33] Lemp MA, Bron AJ, Baudouin C, Benítez del Castillo JM, Geffen D, Tauber J, *et al*. Tear Osmolarity in the Diagnosis and Management of Dry Eye Disease. American Journal of Ophthalmology. 2011; 151: 792–798.e1.
[34] Epomedicine. Schirmer's test. 2020. Available at: https://epomedicine.com/clinical-medicine/schirmers-test/ (Accessed: 9 June 2022).
[35] Aragona P, Di Pietro R. Is it safe to use topical NSAIDs for corneal sensitivity in Sjögren's syndrome patients? Expert Opinion on Drug Safety. 2007; 6: 33–43.
[36] Roszkowska AM, Colosi P, Ferreri FMB, Galasso S. Age-Related Modifications of Corneal Sensitivity. Ophthalmologica. 2004; 218: 350–355.
[37] Smolek MK, Yee RW, Khattak A, Dawson K, Winsett L. Non-contact confocal microscopy of the tear film and ocular surface of normal and dry eye patients. [abstract 4447] Association for Research in Vision & Ophthalmology (ARVO): Fort Lauderdale, Florida. 2005.
[38] Stonecipher KG, Green PT. Non-contact Confocal Microscopy of the Tear Film in Unoperated Eyes. Journal of Refractive Surgery. 2007; 23: 417–419.
[39] Iyengar MF, Soto LF, Requena D, Ruiz-Alejos AO, Huaylinos Y, Velasquez R, *et al*. Tear biomarkers and corneal sensitivity as an indicator of neuropathy in type 2 diabetes. Diabetes Research and Clinical Practice. 2020; 163: 108143.
[40] Mansoor H, Tan HC, Lin MT, Mehta JS, Liu YC. Diabetic Corneal Neuropathy. Journal of Clinical Medicine. 2020; 9: 3956.
[41] Grus FH, Sabuncuo P, Dick HB, Augustin AJ, Pfeiffer N. Changes in the tear proteins of diabetic patients. BMC Ophthalmology. 2002; 2: 4–7.
[42] Zou X, Zhang P, Xu Y, Lu L, Zou H. Quantitative Proteomics and Weighted Correlation Network Analysis of Tear Samples in Type 2 Diabetes Patients Complicated with Dry Eye. Proteomics-Clinical Applications. 2020; 14: 1900083.
[43] Alves M, Fonseca EC, Alves MF, Malki LT, Arruda GV, Reinach PS, *et al*. Dry Eye Disease Treatment: a Systematic Review of Published Trials and a Critical Appraisal of Therapeutic Strategies. The Ocular Surface. 2013; 11: 181–192.
[44] Prabhasawat P, Tesavibul N, Kasetsuwan N. Performance profile of sodium hyaluronate in patients with lipid tear deficiency: randomised, double-blind, controlled, exploratory study. British Journal of Ophthalmology. 2007; 91: 47–50.
[45] Darenskaya MA, Kolesnikova LI, Kolesnikov SI. Oxidative Stress: Pathogenetic Role in Diabetes Mellitus and its Complications and Therapeutic Approaches to Correction. Bulletin of Experimental Biology and Medicine. 2021; 171: 179–189.
[46] Kang Q, Yang C. Oxidative stress and diabetic retinopathy: Molecular mechanisms, pathogenetic role and therapeutic implications. Redox Biology. 2020; 37: 101799.
[47] Alves Mde C, Carvalheira JB, Módulo CM, Rocha EM. Tear film and ocular surface changes in diabetes mellitus. Arquivos Brasileiros de Oftalmologia. 2008; 71: 96–103.
[48] Babizhayev MA, Yermakova VN, Sakina NL, Evstigneeva RP, Rozhkova EA, Zheltukhina GA. N alpha-acetylcarnosine is a prodrug of L-carnosine in ophthalmic application as antioxidant. Clinica Chimica Acta. 1996; 254: 1–21.
[49] McLaughlin PJ, Sassani JW, Titunick MB, Zagon IS. Efficacy and safety of a novel naltrexone treatment for dry eye in type 1 diabetes. BMC Ophthalmology. 2019; 19: 35.
[50] Hayashi Y, Toshida H, Matsuzaki Y, Matsui A, Ohta T. Persistent corneal epithelial defect responding to rebamipide ophthalmic solution in a patient with diabetes. International Medical Case Reports Journal. 2016; 9: 113–116.
[51] Ramos H, Bogdanov P, Sampedro J, Huerta J, Simó R, Hernández C. Beneficial Effects of Glucagon-Like Peptide-1 (GLP-1) in Diabetes-Induced Retinal Abnormalities: Involvement of Oxidative Stress. Antioxidants. 2020; 9: 846.
[52] Gorąca A, Huk-Kolega H, Piechota A, Kleniewska P, Ciejka E, Skibska B. Lipoic acid – biological activity and therapeutic potential. Pharmacological Reports. 2011; 63: 849–858.
[53] Rochette L, Ghibu S, Muresan A, Vergely C. Alpha-lipoic acid: molecular mechanisms and therapeutic potential in diabetes. Canadian Journal of Physiology and Pharmacology. 2015; 93: 1021–1027.
[54] Cure E, Cumhur Cure M. Alpha-lipoic acid may protect patients with diabetes against COVID-19 infection. Medical Hypotheses. 2020; 143: 110185.
[55] Ajith TA. Alpha-lipoic acid: a possible pharmacological agent for treating dry eye disease and retinopathy in diabetes. Clinical and Experimental Pharmacology and Physiology. 2020; 47: 1883–1890.
[56] Tan SM, de Haan JB. Combating oxidative stress in diabetic complications with Nrf2 activators: how much is too much? Redox Report. 2014; 19: 107–117.
[57] Jeffrey S, Samraj PI, Raj BS. The Role of Alpha-lipoic Acid Supplementation in the Prevention of Diabetes Complications: a Comprehensive Review of Clinical Trials. Current Diabetes Reviews. 2021; 17: e011821190404.
[58] Nebbioso M, Federici M, Rusciano D, Evangelista M, Pescosolido N. Oxidative Stress in Preretinopathic Diabetes Subjects and Antioxidants. Diabetes Technology & Therapeutics. 2012; 14: 257–263.
[59] Alvarez-Rivera F, Fernández-Villanueva D, Concheiro A, Alvarez-Lorenzo C. Alpha-Lipoic acid in Soluplus(®) polymeric nanomicelles for ocular treatment of diabetes-associated corneal diseases. Journal of Pharmaceutical Sciences. 2016; 105: 2855–2863.
[60] Mathers WD, Lane JA, Zimmerman MB. Assessment of the Tear Film with Tandem Scanning Confocal Microscopy. Cornea. 1997; 16: 162–168.
[61] Pritchard N, Edwards K, Efron N. Non-contact laser-scanning confocal microscopy of the human cornea in vivo. Contact Lens and Anterior Eye. 2014; 37: 44–48.