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Content

Drops for presbyopia

Abdelkader A.[1], Kaufman H.E.[2]

[1] *Department of Ophthalmology, Faculty of medicine, Al-Azhar University, Cairo, Egypt*
[2] *Professor of Ophthalmology, Pharmacology, and Microbiology, Emeritus. LSU Medical School*
Email: mamounkader@gmail.com

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Abstract

Purpose: To test and compare in a masked fashion the efficacy of using a parasympathomimetic drug (3 % Carbachol) and an alpha agonist (0.2 % brimonidine) in both combined and separate forms to create optically beneficial miosis to pharmacologically improve vision in presbyopia.

Methods: A prospective, double-masked, randomized, placebo-controlled clinical trial. Ten naturally emmetropic and presbyopic subjects between 42 years and 58 years old with an uncorrected distance visual acuity of at least 20/20 in both eyes without additional ocular pathology are eligible for inclusion. All subjects received 3 % carbachol and 0.2 % brimonidine in both combined and separate forms, 3% carbachol alone and 0.2% brimonidine alone (control) in their non-dominant eye in a crossover manner with one week washout between tests. The subjects' pupil size and both near and distance visual acuities will be evaluated pre- and posttreatment at 1, 2, 4, and 8 hours, by a masked examiner at the same room illumination.

Results: Statistically significant improvement in near visual acuity was achieved in all subjects who received combined 3 % Carbachol and brimonidine in the same formula than separate forms or Carbachol alone or brimonidine alone ($P < 0.0001$).

Conclusions: Improving the depth of focus by making the pupil small caused statistically significant improvement in near visual acuity. The optimal formula was found to be the combined 3 % Carbachol with 0.2% Brimonidine.

Key words: Presbyopia, Carbachol, Brimonidine, depth of focus, miosis.

Introduction

Near visual acuity can be improved by increasing the depth of focus as well as by increasing the accommodation. Increased depth of focus can come from making the pupil smaller like a smaller aperture in a camera. The common traditional ways for improving vision in presbyopes was through wearing corrective lenses including pinhole spectacles or invasive procedures. Different approaches on the cornea (inlays), the crystalline lens and the sclera are being pursued to achieve surgical correction of this disability. The AccuFocus implant is an artificial pupil put under a Lasik flap in one eye to permit reading vision. It has a 1.6mm pupil in a 3.8 mm carrier. It restores reading vision through increased depth of focus. Patients do not experience dimness of vision; other eye fills in brightness. We attempt with drops to approach this effect without surgery. In this pilot study, we test the efficacy of using a parasympathomimetic drug (3 % Carbachol) and an alpha agonist (0.2 % brimonidine) in both combined and separate forms and individually to create optically beneficial miosis to pharmacologically improve vision in presbyopia. The pharmacological treatment has two purposes namely a stimulation of the parasympathetic innervation primarily and mainly by increasing depth of focus and perhaps the accommodation and its potentiation and prolongation by an alpha agonist.

Subjects and Methods

This study was begun after approval was obtained from the institutional review board and ethics Committee. Each participant gave written informed consent, and the study followed the tenets of the Declaration of Helsinki. The pharmacological stimulation protocol was developed in accordance with that used previously in the invention of Dr. Herbert Kaufman. [1]

Ten naturally emmetropic and presbyopic subjects and a mean age of 49.7 ±4.8 years (range, 42 – 58 years); 6 males and 4 females with an uncorrected distance visual acuity of at least 20/20 in both eyes without additional ocular pathology are eligible for inclusion. Participants were volunteers selected at random.

Presbyopia is considered present if an uncorrected end-point print size ≥ Jaeger (J) 5 improved by ≥ 1 optotype with the use of a lens ≥ +1.00 D. All subjects should be in good physical and ocular health and should complete a questionnaire to ascertain any contraindications for participation or predisposition to complications (eg, heart or respiratory conditions, migraines, high myopia, ocular or systemic medications, or ocular surgeries). All subjects will have a fully dilated eye examination before they are considered eligible for the study. The examination will screen for contraindications to the drugs, susceptibility to retinal detachment, ocular pathology, or peripheral retinal degeneration. Inclusion criteria were as follows: age between 43 and 56 years, emmetropia [cycloplegic spherical equivalent (SE), ±0.25 D; astigmatism, ≤0.25 D] and binocular uncorrected distance visual acuity ≥20/20. Exclusion criteria concerned patients with myopia, hyperopia and astigmatism higher than 0.25 diopter as well as those with corneal, lens and vitreous opacities, pupil irregularities, anisocoria, amblyopia, chronic general pathologies and medications that would interact unfavorably with carbachol and brimonidine. None of the patients included in the study had received any topical medication that could cause pupil mydriasis or miosis. During the study, the subjects were closely monitored and regularly asked to report on any ocular, systemic, or physiological reactions they experienced. Atropine was available in the event of adverse effects, although none was reported.

Procedures

A single dose of 3 % carbachol together with 0.2 % brimonidine in both combined and separate forms or 3 % carbachol alone or 0.2 % brimonidine alone (control) was instilled in the non-dominant eye of all subjects with one week washout between tests. In the separate form, carbachol will be installed first followed by brimonidine after 5 minutes. Initial pupil size and both near and distance visual acuities were documented before treatment and at 1, 2, 4 , and 8 hours after treatment by a masked examiner at the same room illumination. Near visual acuity was assessed at 40 cm using Jaeger (J) Eye Chart. Pupil diameter was measured using Colvard handheld Infrared pupillometer (Oasis Medical, Glendora, CA, USA). Any adverse symptoms and subject satisfaction with near and distance vision were also monitored.

Statistical analysis

Statistical analysis was performed using the Student's t-test and p value of less than 0.05 was considered statistically significant. Data were expressed as mean, range, and standard deviation (SD).

Results

In combined drops group, the mean near visual acuity (NVA) improved significantly from J-8.6 ±1.5 before treatment to J- 1.1 ±0.32 at 1 hour ($P < 0.0001$), J- 1.1 ±0.32 at 2 hours ($P < 0.0001$), J- 1.8 ±0.42 at 4 hours ($P < 0.0001$) and J- 2.3 ±0.48 at 8 hours ($P < 0.0001$) posttreatment. The mean pupil size decreased significantly from 4.3 ±0.48 mm before treatment to 1.2 ±0.26 mm at 1 hour ($P < 0.0001$), 1.25 ±0.26 mm at 2 hours ($P < 0.0001$), 1.7 ±0.25 mm at 4 hours ($P < 0.0001$) and 2.1 ±0.31 mm at 8 hours (P □ 0.0001) posttreatment.

In separate drops group, the mean near visual acuity (NVA) improved significantly from J-8.6 ±1.5 before treatment to J- 3.4 ±0.97 at 1 hour ($P < 0.0001$), J- 3.6 ±0.97 at 2 hours ($P < 0.0001$), J- 4.5 ±0.97 at 4 hours ($P < 0.0001$) and J- 5.2 ±0.79 at 8 hours ($P < 0.0001$) posttreatment. The mean pupil size decreased significantly from 4.3 ±0.48 mm before treatment to 1.95 ±0.36 mm at 1 hour ($P < 0.0001$), 2.15 ±0.24 mm at 2 hours ($P < 0.0001$), 2.52 ±0.3 mm at 4 hours ($P < 0.0001$) and 2.8 ±0.25 mm at 8 hours (P □ 0.0001) posttreatment.

In 3 % carbachol alone group, the mean near visual acuity (NVA) improved significantly from J-8.6 ±1.5 before treatment to J- 5.5 ±0.97 at 1 hour ($P < 0.0001$), J- 5.9 ±0.74 at 2 hours ($P < 0.0001$), J- 7.1 ±1.2 at 4 hours ($P = 0.023$). At 8 hours posttreatment, the improvement in mean NVA was not significant. Mean NVA was J- 7.5 ±1.08 ($P = 0.076$). The mean pupil size decreased significantly from 4.3 ±0.48 mm before treatment to 2.85 ±0.33 mm at 1 hour ($P < 0.0001$), 3.05 ±0.28 mm at 2 hours ($P < 0.0001$), 3.45 ±0.28 mm at 4 hours ($P = 0.0001$). At 8 hours posttreatment, the decrease in mean pupil size was not significant. Mean pupil size was 4 ±0.33 mm ($P = 0.12$).

In 0.2 % brimonidine alone group, no statistically significant difference in mean NVA and mean pupil size was found before treatment and at any time point after treatment (P □ 0.05).

Fig. 1 and 2 show the mean change in near visual acuity (Jaeger) and pupil size (mm) over time for treatment and control groups.

Significant improvement in mean (NVA) and mean pupil size was reported in combined 3 % Carbachol and brimonidine drops than separate forms or Carbachol alone or brimonidine alone (P □ 0.0001).

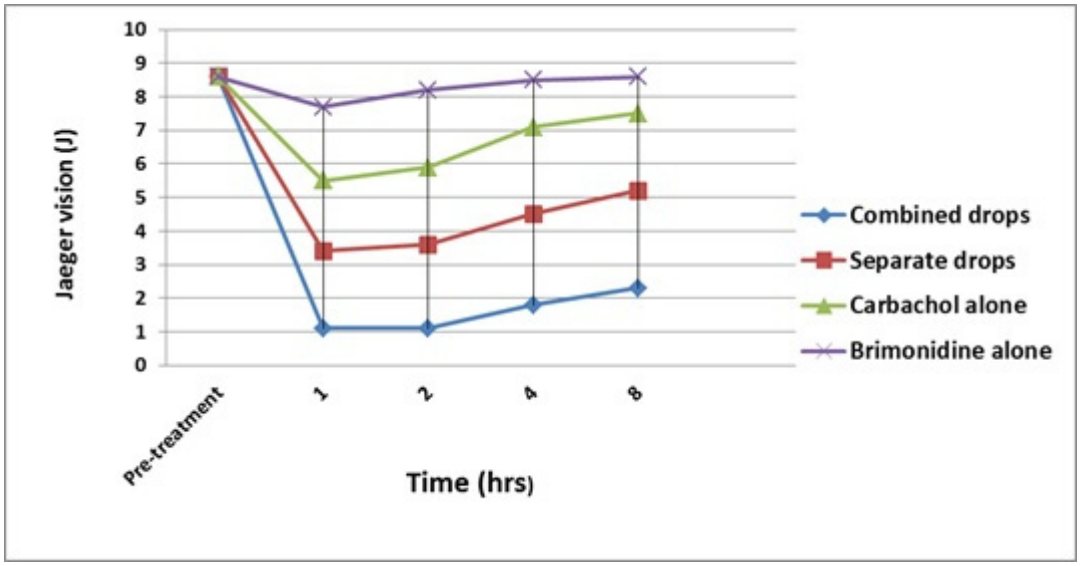

Fig. 1. Distribution of mean change in near visual acuity (Jaeger) over time for treatment and control groups

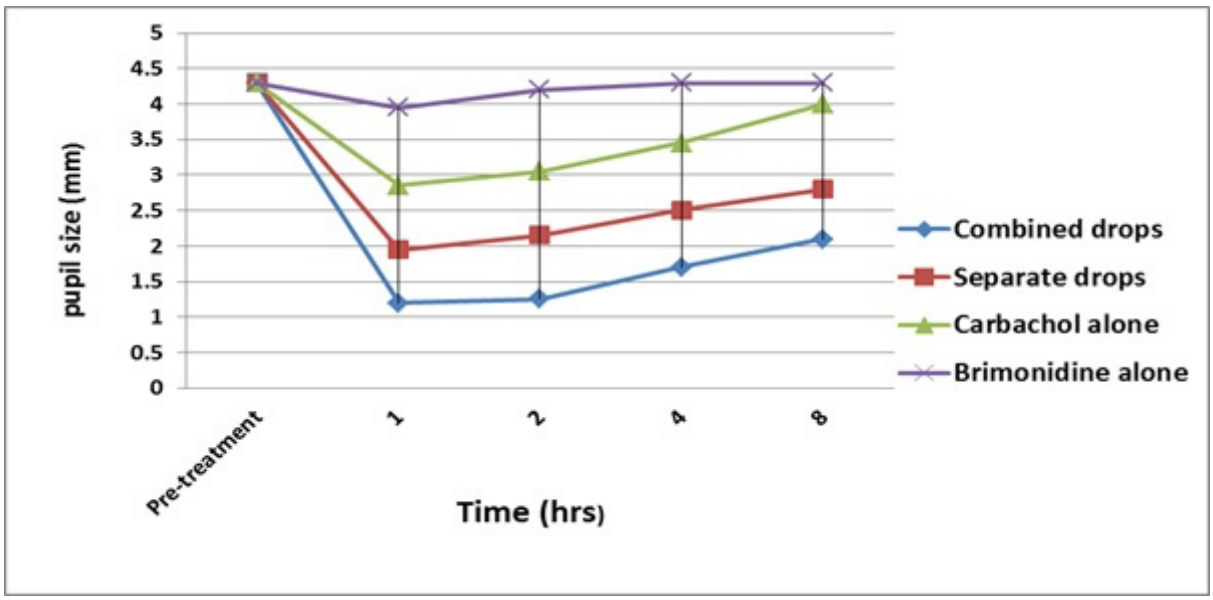

Fig. 2. Distribution of mean change in pupil size (mm) over time for treatment and control groups

Mild burning sensation was noted in some subjects in all groups but most frequently reported in carbachol alone group.

Distance visual acuity

The uncorrected distance visual acuity was 20/20 of both eyes in all subjects before treatment and remained at 20/20 at all time periods after treatment in all groups.

Discussion

Numerous research studies have been conducted to disclose the exact reasons for the development of presbyopia. [2-12] From the invention of bifocals, there have been many attempts to ameliorate presbyopia with spectacles and contact lenses. These measures include monovision, mulitifocality, and the increase in the astigmatic blur circle. All have been useful in a limited way, but none uniformly acceptable. Pinhole spectacles

have not been satisfactory since the pinhole does not move with the eye restricting the field of vision and often insufficient light reaches the retina causing dimness, even though near acuity is improved through increased depth of focus. Surgical approaches to the relief of presbyopia include primarily the use of monovision, laser ablation, intraocular lenses, and refractive lens replacement. Presbyopic refractive lenses typically are multifocal lenses, or lenses that induce pseudo accommodation, or in some cases real accommodation but require lens removal and replacement. Attempts to induce true or psuedoaccomodation by various techniques that expand the scleral ring near the limbus and cause increased tension on the zonules are also being studied. Refractive corneal inlays have been used which increase the corneal power, usually in the non- dominant eye. These appear successful, but require surgery. The most interesting surgical approach to the treatment of presbyopia has been the AccuFocus implant. [13] This is a corneal implant with a small central artificial pupil. It restores reading vision through increased depth of focus. Although there are some problems with centering the implant, and some surgical complications, it is clear that the principle of a small pupil that moves with the eye can give a good near vision and preserve distance acuity as well. Operating only on the non-dominant eye seems to avoid problems of dimness that are seen when the pupil in both eyes is made small. Patients do not experience dimness of vision; other untreated eye fills in brightness. We attempted with drops to approach this effect without surgery.

The present study used 3 % carbachol and an alpha agonist (0.2 % brimonidine) in both combined and separate forms and individually to improve vision in presbyopia through increased depth of focus. Increase depth of focus allowed many presbyopes even older ones to benefit from using the drops. Both drugs are FDA approved and have been used for years as safe and effective for glaucoma. Our technique is based on creating a pinhole effect pharmacologically increasing the depth of focus from smaller pupil. In monocular treatment, the vision in the fellow eye with the normal pupil will have some blurry near vision, but distant objects are clear and there is no diminished light perception. When the images are merged, all subjects of treatment group had clear focus at near and distance with no perception of dimness. Treating one eye only does not cause symptoms of dimness as the brain fills in brightness from the other eye. Carbachol and brimonidine can be used once daily to achieve a 8-hour effect. Brimonidine has little effect on the photopic pupil, but has been effectively used for many years to prevent excessive pupil dilatation in the dark, and thereby reduces scotopic symptoms, usually from the peripheral cornea after refractive surgery. It has not been used to ameliorate presbyopia.

Herbert Kaufman's 1 and Stephen Kaufman's [14] finding of the synergistic effect between carbachol and brimonidine has permitted one application of drops to produce miosis sufficient to improve near vision enough for most people all day long. Kaufman founded that the combination between carbachol and brimonidine was active and their effect lasted longer. Distance vision was preserved in all subjects, so that no monovision symptoms were reported. The treatment of only one eye minimizes symptoms of dimness; synergism permits use of lower doses of miotics and reduces symptoms of headache, and brimonidine eliminates any tendency of the parasympathomimetic to cause hyperemia. In this study, combined drops achieved significantly better near visual acuity than separate drops perhaps because of the penetration enhancer that is found in the combined formula. No ocular complications were detected in any treated eyes. The topical ocular miotic therapy did not precipitate retinal detachments in any of the treated eyes. This is a rare event and probably does not occur in patients free of retinal pathology [15]. There has not been a systematic study of the incidence of retinal detachment in patients treated with miotics, which were used extensively for many years for the treatment of glaucoma. Virtually all retinal detachments reported were in high myopes, and the risk in emmetropes seems minimal, though uncertain. We think the risks are very small which is why the medications were used so successful late for so long. It is therefore important that prior to starting patients on long-term miotics, a thorough examination of the retina especially its periphery must always be done. It becomes obligatory in high-risk cases of retinal detachment like high myopes, aphakics, lattice degeneration and those with history of retinal detachment in the fellow eye or in the family.

The pharmacological approach using miosis to ameliorating presbyopia is not new. Pilocarpine (a parasympathomimetic) [16] or echothiophate iodine (an acetylcholinesterase inhibitor) [17] are among the drugs studied. These have shown some efficacy, but there are no significant studies demonstrating safety and acceptability. Several studies have also evaluated the effect of brimonidine tartrate 0.2% and carbachol ophthalmic solution on pupil size and accommodation.[18-20] Additional studies are planned in the future to use the drops in presbyopia with different refractive errors as in hyperopic and myopic presbyopes and in different situations such as psaudophakic and postlasik presbyopes.

Conclusion: The monocular pharmacologic treatment of presbyopia with one drop a day of carbachol and brimonidine in the non-dominant eye, permits acceptable reading vision for many presbyopes even in older subjects. The optimal formula was found to be the combined 3 % Carbachol with 0.2% Brimonidine. Because of increased depth of focus from the smaller pupil, it does not blur distance vision or intermediate vision, as does typical monovision therapy, and the perception of normal brightness in the untreated eye eliminates symptoms of dimming from the smaller pupil of the treated eye. This active combination would also improve low non–presbyopic hyperopes and perhaps others and can be used with glasses if NVA is not enough for a special task. It is possible that, in the future, new pharmacological treatments can also be used to treat other refractive problems.

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The European perspective of the nutritional supplements in AMD prevention

García-Layana A.[1], Guirao Navarro M.C.[2]

[1] Clínica Universidad de Navarra. Pamplona (Spain)
[2] Clínica Universidad de Navarra. Pamplona (Spain)

Abstract

European legislation relating to food supplements with vitamins, minerals and other nutritional substances calls for maximum amounts of addition (the Recommended Dietary Allowances or RDA) to protect consumers safety. So, the doses of micronutrients used in the two AREDS studies are not allowed in the EU. Nevertheless, micronutrition has already become part of the day-to-day management of AMD for a considerable proportion of European ophthalmologists. Although there are obvious differences between general ophthalmologists and retinal specialists and between ophthalmologists from different nations, these differences seem relatively small in the overall context and attributable to different health care environments as well as medical custom and practice. In this context, European use of micronutrients in AMD prevention has many weaknesses, unresolved questions and difficulties extrapolating the AREDS2 results. The control group in AREDS2 received high dose supplements already shown to be effective in the prevention of AMD progression, and more than 10% added DHA/EPA supplements to their diet. These two factors might be responsible for a 'ceiling effect' within AREDS2 that could make it more difficult to demonstrate a real effect of DHA/EPA. Given the findings in some European interventional studies in this area it is necessary to urge caution in closing the chapter on omega-3, but rather suggest building on these findings with more detailed exploration of its role with different formulations and populations. Health claims regarding the role of DHA and EPA in the maintenance of vision have recently received approval from the European Food Safety Authority. There is not enough data about the effect of DHA/EPA combined with the RDA of vitamins and minerals, or the addition of another micronutrients with biological plausibility effects such as resveratrol, vitamin B or vitamin D that may also have a role in the future of AMD prevention. A European clinical trial about the micronutrition effects of these substances in AMD is essential to elucidate their individual and combined effects.

Introduction

Age related macular degeneration (AMD) is the leading cause of severe visual impairment for older people in the developed world and so, [1] not only the knowledge of its wide prevalence, but also the awareness about the increasing number of people that are expected to develop this condition [2], has led to the execution of several studies. The aim of these studies was to investigate possible effective treatments that could help us in the AMD prevention, and also in slowing AMD progression. [3,4,5,6,7,8,9,10,11,12,13,14,15,16,17] As a logical consequence, some differences in the results of the carried out studies have been found and, an opportunity to reflect on these and other realities has been given to us. [18]

Text

In the past few years, there has been a very important progress in the knowledge of AMD. [19] One of the factors that seems to be involved in the origin and evolution of the disease is oxidative stress. Due to the retina's high oxygen consumption, its high proportion of polyunsaturated fatty acids, and its exposure to visible light, retina has shown to be a tissue particularly sensitive to oxidative stress. Some "in vitro" studies have also proved that antioxidant vitamins A, C and E were protective against this photochemical damage. According to these findings, further studies have been made in order to clarify whether administering antioxidants results in a preventing or slowing down effect in AMD disease. [20]

One of the most relevant studies that were made in this regard is the Age-Related Eye Disease Study (AREDS). In this study, only the intermediate AMD patients who were randomly assigned to take antioxidants plus zinc benefited from a 25% reduction in AMD risk progression . In that same group, a 19% reduction of

the visual loss risk was seen. Therefore, the intake of zinc and antioxidants was shown to have beneficial effects on the development of advanced AMD in people with intermediate AMD. [17]

Another element that protects the retina against oxidative stress is the macular pigment, which is formed, among others, by lutein and zeaxanthin. According to this biological finding, an intake of these carotenoids showed to decrease the risk of progression to advanced AMD in observational studies. The desire of evaluating a possible additive benefit of dietary xanthopylls supplements with and without omega-3 long-chain fatty acids, led to the execution of the randomized, controlled clinical trial AREDS 2. In this study, all patients took the original AREDS formula plus either 1) lutein and zeaxanthin, 2) DHA and EPA, or 3) lutein,zeaxanthin, DHA, and EPA; however, no statistically significant overall effect on progression to advanced AMD or changes in visual acuity were observed. Despite the fact that the results from this study where not as encouraging as were expected, their interpretation should be done carefully because, due to some characteristics from the study and its population, their results may not be generalizable and the debate remains open. Furthermore, in this same study, when exploratory subgroup analyses were done, lutein plus zeaxanthin demonstrated a protective effect for progression to advanced AMD. [18]

The discovery of this nutritional supplement effect makes us reflect on the economic repercussion that their administering may have. In this regard, studies to assess cost-effectiveness and cost-utility of its administration have been performed.

Regarding cost-utility of micronutrients in AMD, the literature has shown that the cost of a good screening tool and establishing a prophylactic treatment (all estimated to cost around 22.700 £ per quality adjusted life year saved), would be less expensive than the cost of the theoretically avoidable AMD treatment. [1]

Afterwards, knowing which real benefits from administering nutritional supplements in AMD patients was an issue that was clarified in the study of David B. Rein et al. In that study, a comparison of the impact of nutritional supplementation was made by using a computerized, stochastic, agent-based model in which 20 million simulated individuals where created, and each of them received the vitamin therapy after AMD diagnosis, as well as no vitamin therapy. The result of administering the vitamin therapy yielded a cost-effectiveness ratio of 21.387 $ per quality-adjusted life-year gained, as well as lowered the percentage of visual impairment. [21]

As it would be expected, all the aforementioned has not escaped the attention of European ophthalmologist, to whom micronutrition has already become a part of the day to day AMD management. This fact was objectively determined in the study performed by Tariq Aslam et al. [22]. In this study, they evaluated through a European survey, the ophthalmologist's opinion of, and use of, micronutritional dietary supplements.

In this survey both general ophthalmologists and retinal specialists took part in it and, despite the fact that there are obvious differences between both of them and, between ophthalmologist of different nations, these discrepancies seemed relatively small in the overall context and attributable to health care environments variants as well as medical custom and practice.[22]

Despite the fact that nutritional supplements are being used in Europe, it is important to remember that, European legislation to food supplements with vitamins, minerals and other nutritional substances calls for maximum amounts of addition (the Recommended Dietary Allowances or RDA) to protect consumers safety. [23] This way, the doses of micronutrients used in the two AREDS studies; which were the ones with good outcomes for AMD, are not allowed in the European Union (EU).

Tab. 1. Difference in micronutrients dosing between the supplements used in the two AREDS studies and the ones permitted in the EU.

	AREDS formulation	Dietary reference intake (DRI)
Vitamin C	500 mg	90 mg
Vitamin E	400 IU	15 IU
Lutein Zeaxanthine	10mg 2mg	unknown
Zinc	80 mg	11 mg
Copper	2 mg	0.9 mg

For them to be allowed in Europe, their registration as a medicine would be necessary. This would involve conducting a clinical trial to prove their effectiveness. As this antioxidants and minerals substances can not be patented due to the current European legislation, the products that would be tested in that hypothetical clinical trial could be produced and sold or distributed by any producer. Therefore no pharmaceutical distributor might be interested in performing this clinical trial.

As we can see, European use of micronutrients in AMD prevention has many weaknesses and unresolved questions.

An issue that should be considered is extrapolating the AREDS 2 findings to the European population. This mayentail difficulties because of the differences that exist among the American and European dietary habits.[24,25,26,27]

In the AREDS 2 study, omega-3 supplementation seemed to have no protective effect against AMD progression. Related to this, two factors might be responsible for a ceiling effect that may have masked the omega-3 effect. In the first place, AREDS-2 control group received high dose supplements already shown to be effective in the prevention of AMD progression. Secondly, more than 10% added DHA/EPA supplements to their diet. Therefore, these two factors might have hampered demonstrating a real effect of DHA/EPA. [17]

Another important element that should be taken into account is that, a mixture of 40:20 (EPA: DHA) in triglyceride formulation – not in an ethyl-ester formula as used in the AREDS 2-, is the one that has proved to have better bioavailability and, theoretically a major impact in AMD prevention. [28]

In the study that was carried out by Eric H. Souied *et al*., only patients who achieved the highest tertile of EPA plus DHA levels in red blood cell membrane, had significantly lower incidence of choroidal neovascularization; thus, not only high doses of these elements are important, but also the bioavailability achieved by them. [29]

In addition, as AMD is not a deficiency disease, an answer to the question of whether the diet is enough to protect us against it is still unclear. The average diet fails to achieve the amount of omega-3 necessary for therapeutic effects. Results from Rees *et al*. suggested that a quantity superior to 1.35 g per day was necessary to achieve them.[30] In the publication that was made by Calder, anti-inflammatory dose-dependent actions of marine n-3 PUFAs seemed to be present at a higher dose than 2 g per day. Although a theoretical equivalent intake value was obtained, it did not seem to have a practical feasibility. [31]

One of the facts that seems to be in continuity with the previous statement is that the Omega-3 and Omega 6 ratio, which is present in food we have today, is very different from the one that was found in food in the past. Back at the dawn of time, we had fishing, hunting and collection of wild fruits. Now with fish farms, farms and crops, the ratio of Omega-3:Omega 6 fatty acids has fallen substantially. For example, if we take an egg from a rural henhouse, the Omega-3/Omega6 ratio is 1/1, while it would be 1/15 if we take it from an industrial poultry farm (citation?).

If we exclude omega-3 from the European formulation and lower the amounts of the rest of the components, there is no evidence of reaching a protective effect without the development of a European clinical trial about the micronutrition effects of these substances in AMD.

In this study, it would be interesting to check the effect of maintaining omega-3 in the European formulation, in addition to other micronutrients with biological plausibility effects (such us resveratrol, vitamin B and vitamin D), that may also have a role in the future of AMD prevention.

Conclusions

Despite the fact that Mediterranean diet doesn't always provide the appropriate amounts of all elements, a healthy diet cannot be replaced by a nutritional complex. There is evidence of a protective effect of vitamin/mineral megadoses in AMD, quantities that in Europe cannot be used as nutritional supplements allowances are based in RDA doses. Additional studies about the role of Omega-3 and other micronutrients in addition to the RDA vitamins and mineral doses are recommended. A European clinical trial about the micronutrition effects of these substances in AMD is strongly recommended, in order to best care for the many Europeans with preventable AMD progression.

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Using suprachoroidal administration as an approach to treat noninfectious uveitis – from concept through clinical data

Kissner J.[1] , Taraborelli D.[1], Noronha G.[1]*

[1] *Clearside Biomedical Inc., Alpharetta, GA (USA)*
**e-mail: glenn.noronha@clearsidebio.com*

Abstract

Clearside Biomedical Inc. is a clinical stage company developing first-in-class drugs to treat unmet blinding eye diseases where the pathologies dominantly manifest in the retina and the choroid. Clearside accesses diseased retina and choroid by accessing these posterior eye tissues through a minimally invasive suprachoroidal injection procedure. This method of administering drugs through the suprachoroidal space (SCS) then serves as the basis for drug development. In order to demonstrate proof of effect, we chose uveitis, a collection of inflammatory conditions affecting the eye, and treatment with triamcinolone acetonide, with an established scientific history for the treatment of ocular inflammatory diseases, to test out the potential for treatment through suprachoroidal drug administration.

Triamcinolone acetonide (TA) is a synthetic corticosteroid with a well-documented scientific record of anti-inflammatory action when administered locally to the eye through intra- and peri-ocular delivery methods. In order to demonstrate that suprachoroidal injection of TA could be a useful local treatment option for uveitis, a pig model of acute uveitis was run to compare local intravitreal and suprachoroidal administrations. At a 0.2 mg dose, suprachoroidal injection of TA was more effective than intravitreal injection at the same dose, and as effective as a 2 mg intravitreal dose in decreasing inflammation, demonstrating that a 10-fold lower dose administered suprachoroidally was sufficient in reducing inflammation, and matched the response seen from the 2 mg suprachoroidal and 2 mg intravitreal dose arms. Further the 0.2 mg intravitreal dose did not show reduction in inflammation in this model. In comparative pharmacokinetic studies in rabbits, ocular distribution of TA over a 90-day period following single suprachoroidal or single intravitreal administration of the same 4 mg dose in parallel groups of rabbits showed that TA was 12 times more available in the choroid and retina, with minimal amounts of drug in the anterior chamber and vitreous, compared to levels following intravitreal injection. Single- and repeat-dose toxicology studies in rabbits were conducted to evaluate the safety of suprachoroidal injection of TA. Based on the results from these toxicology studies, no nonclinical ocular tolerability or systemic toxicity findings precluded the suprachoroidal administration of TA to humans.

Both the potential for efficacy with lower dose, as well as the unique posterior localization of drug, suggested that administration of drug to the SCS could provide an effective therapy for uveitis with complications in the posterior tissues, with the potential for a safer dosing compared to other local administration routes. In a Phase 1/2 clinical study, 8 subjects with intermediate-, posterior- or pan-uveitis received a single 4 mg suprachoroidal injection of TA and were followed for 6 months post-injection. Encouraging safety and efficacy data from this clinical study continue to provide the basis for development of suprachoroidally directed therapies.

Introduction

The concept of administering drugs precisely and locally is highly valued in drug development. Historically, access of drugs to the posterior tissues of the eye has been achieved with indirect administration.

The suprachoroidal space (SCS) is approximately 30 µm thick and forms a transition zone between the choroid and the sclera (Fig. 1A). Suprachoroidal administration provides access of drug directly to posterior tissues, meeting the goal of precise, local administration. Clearside has developed a proprietary microinjector, a syringe with a microneedle, to facilitate this delivery (Fig. 1B). Following suprachoroidal administration, injected fluid spreads around the globe through the SCS and on the top of and into the choroid, distributing through the lower part of the sclera, all of the choroid and through the outer retina (Fig. 1C). Imaging and histology studies in pig and rabbit eyes confirmed this localization after suprachoroidal administration.

Fig.1. Suprachoroidal injection. (A) Location of SCS. (B) Clearside microinjector. (C) Following suprachoroidal injection, fluid spreads through the SCS.

Clearside's objective is to utilize the suprachoroidal space to develop drug treatments for posterior segment eye diseases with unmet or underserved medical need. We believe there is potential for advantages to such treatment, unique to each disease and drug or drug combination.

The precise suprachoroidal administration technique is being developed to administer drugs to the retina and the choroid. Clearside is developing drug products where a drug or drug combination will be chosen specific to each disease along with a microinjector, used to administer the drug; these items will be co-packaged. From a regulatory perspective, these products are considered as drugs.

Suprachoroidal Administration of TA to Treat Noninfectious Uveitis

This article focuses on developing triamcinolone acetonide as a local therapy for non-infectious uveitis as an example of Clearside's approach to the use of suprachoroidal administration for disease treatment. Nonclinical data in animal models provided the scientific rationale for the treatment of uveitis in humans. Administration through the suprachoroidal space provides drug in high bioavailability to the retina and choroid in animals. Improved efficacy and lowered amount of drug required were demonstrated in animal models and it is speculated that these benefits will be seen in humans.

Uveitis, the fifth most common cause of visual loss in the developed world [1], causes significant vision loss in up to 35% of affected individuals. Uveitis accounts for 10% to 15% of total blindness cases in the United States and 5% to 20% of legal blindness in other developed countries [2]. Chronic macular edema is the dominant cause of visual impairment in uveitis [1][3][4].

Uveitis is commonly treated with corticosteroids and other immunomodulatory agents administered either systemically (eg, intravenous; oral) or locally (eg, topical drops; intra-ocular or peri-ocular administration). High doses of an oral corticosteroid, usually prednisone or an equivalent, can provide ocular anti-inflammatory effects [5]; however, this oral therapy is commonly associated with systemic side effects. Topical corticosteroid eye drops may not be effective for the treatment of posterior manifestations as in the case of macular edema associated with uveitis, likely due to inadequate concentrations of drug available in the choroid and retina. Local corticosteroid therapy with intravitreal injections or the implantation of sustained delivery devices in the vitreous can be used to manage the macular edema and visual impairment associated with uveitis [6]. However, both approaches are associated with side effects due primarily to exposure of corticosteroids to the anterior segment and lens [7]-[10].

The question that we asked was whether suprachoroidal administration could provide a useful local therapy in uveitis. The value proposition of SCS therapy would be to provide robust efficacy while potentially improving the side effect profile. We believe that suprachoroidal therapy can provide a more favourable therapeutic index for uveitis patients than those achieved by other local therapies currently used to treat uveitis.

Triamcinolone acetonide (TA), a synthetic corticosteroid with anti-inflammatory action, also reduces macular edema in addition to improving the other complications associated with uveitis [11]. Clearside's therapy includes a proprietary formulation of TA, CLS-TA, which has been formulated for administration into the eye and optimized for suprachoroidal injection.

Compared to currently available treatments, CLS-TA administered via suprachoroidal injection was anticipated to have a favourable benefit to risk profile due to the unique drug distribution, which was expected to provide a higher local bioavailability of drug, robust efficacy, and a better side effect profile.

Nonclinical Development

Nonclinical studies in pigs and rabbits demonstrated the safety, pharmacokinetic exposure, and efficacy associated with suprachoroidal administration of TA. Relevant outcomes from these experiments are summarized below.

Single- and repeat-dose toxicology studies in rabbits were conducted to evaluate the safety of suprachoroidal injection of TA. A single bilateral suprachoroidal injection of TA was well tolerated, with no observed treatment-related adverse effects during the 17-week post-injection observation period. In a repeat-dose ocular toxicity study, suprachoroidal injections of TA were administered on Days 0 and 90. All animals appeared to be in good health; ophthalmic examinations and clinical observations revealed no TA- or injection-related effects over the 180-day observation period. Histopathological examination of ocular tissues indicated that repeat suprachoroidal administration of TA was well tolerated. Based on the results from these toxicology studies, no nonclinical ocular tolerability or systemic toxicity findings precluded the suprachoroidal administration of TA to humans.

Pharmacokinetic studies in rabbits demonstrated a unique distribution of drug injected suprachoroidally compared to the distribution seen following intravitreal administration. High amounts of drug were observed in the outer retina and choroid, and low amounts of drug were found in the iris-ciliary body, lens and anterior chamber when comparing ocular distribution following suprachoroidal and intravitreal injections in parallel groups of rabbits. Following suprachoroidal administration, compartmentalization of TA into the posterior segment of the eye was observed with minimal exposure to the anterior segment. The amounts of drug in the retina and sclera-choroid were 12-fold higher after suprachoroidal injection and exposures in the anterior tissues were ≤4% of those observed following intravitreal injection of TA [12] (Fig. 2A). Compared to IVT injection, concentrations in the sclera/choroid/outer retina remained substantially higher for suprachoroidal injection over at least 2 months (Fig. 2B). Systemic exposures of drug for both IVT and suprachoroidal injections were minimal.

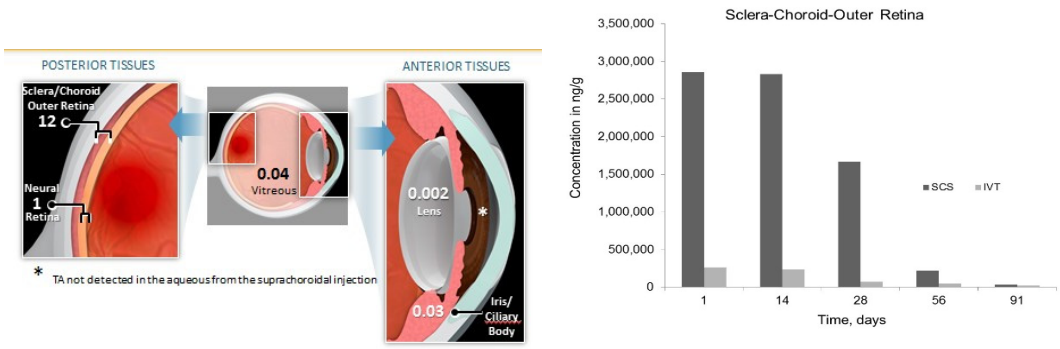

Fig.2. Exposures following suprachoroidal or IVT administration of TA. (A) Compartmentalization of TA into the posterior segment of the eye following suprachoroidal injection. Values represent SCS/IVT ratios for area under the curve over 91 days. (B) Relative to IVT injection, TA concentrations in the sclera/choroid/outer retina were much higher and prolonged.

Pharmacology studies were conducted in a pig model of acute uveitis. In this model, intraocular administration of LPS was used to induce ocular inflammation 24 hours prior to treatment with TA. One study investigated the relative efficacy of suprachoroidal and intravitreal injection in controlling inflammation and found that suprachoroidal injection of TA at one-tenth the IVT dose was sufficient to reduce inflammation [13] (Fig. 3A). The second experiment explored whether CLS-TA administered suprachoroidally could supplement or replace systemic maintenance oral corticosteroids commonly used in uveitis treatment. In this study, suprachoroidal injection of CLS-TA resulted in more rapid anti-inflammatory effect than oral prednisone (Day 1 vs Day 3), was as effective as high dose oral prednisone (Day 3), and was superior to low dose maintenance prednisone in its anti-inflammatory effect (Fig.3B). Both of these experiments showed that suprachoroidally administered TA has the potential to be useful locally for the treatment of uveitis and to provide advantages over other commonly used uveitis treatment modalities.

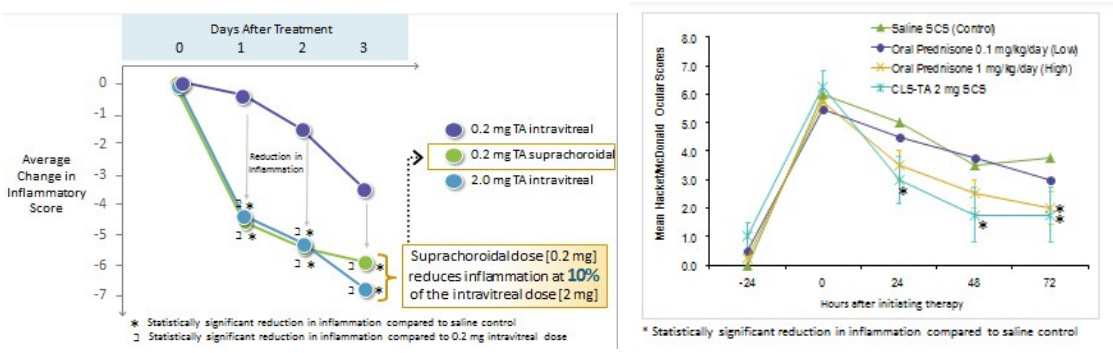

Fig.3. Pharmacology studies in a pig model of acute uveitis. The efficacy of suprachoroidal administration of TA was compared to (A) IVT injection of TA and (B) oral prednisone.

Clinical Development

A Phase 1/2 clinical study (Protocol CLS1001-101) was conducted at 3 clinical sites in the United States. The primary purpose was to evaluate the safety, tolerability, and procedure of a single suprachoroidal injection of TA in subjects with non-infectious intermediate-, posterior- or pan-uveitis. This trial is registered with ClinicalTrials.gov as # NCT01789320.

Subjects were male or non-pregnant females at least 18 years old. Each subject's study eye was required to have a best corrected visual acuity (BCVA) of +1.0 logMAR or better (20/200 Snellen equivalent) by Early Treatment of Diabetic Retinopathy Study (ETDRS) [14], as well as macular edema with a central subfield thickness (CST) of ≥ 310 microns by spectral-domain optical coherence tomography or a vitreous haze score of ≥ +1.5 based on the Standardization of Uveitis Nomenclature (SUN) Working Group criteria [15].

Key exclusion criteria for the study eye included any ocular trauma, injection of intraocular corticosteroids, steroid implant, or Ozurdex implant in the 6 months prior to study treatment; any use of Retisert™ in the 3 years prior to study treatment; history of any intraocular surgery; or presence of an anterior staphyloma. Potential subjects also were excluded if they were monocular, had ocular hypertension, had a known immunodeficiency disease for which corticosteroid therapy would be contraindicated, or had any uncontrolled systemic disease that would put them at risk due to study treatment or procedures.

In this trial, subjects were administered a single, unilateral suprachoroidal injection of 4 mg (100 μL) of TA on Day 1, then followed for 26 weeks. Study evaluations were conducted at Day 2 and Weeks 1, 2, 4, 8, 12, 16, 20 and 26.

The study enrolled 9 individuals with persistent chronic uveitis, 8 of whom received study drug and were analysed for efficacy. The subjects ranged in age from 42 to 78 years, and 2 of the 8 subjects were male. One of the 8 subjects qualified for the study based upon haze alone, with no macular edema present at enrolment.

No specific safety concerns were noted in the study. All subjects had at least one adverse event (AE). A total of 37 AEs were reported among all subjects; most AEs (95%) were mild or moderate in severity. One serious AE (SAE) occurred: severe, unrelated pulmonary emboli in a subject with a history of pulmonary emboli. No deaths were reported. Approximately half (57%) of the reported AEs were ocular events. Eye pain was the most commonly reported AE, from 4 subjects; 9 ocular AEs in 4 subjects were considered possibly related to TA.

During the study, 4 of 8 per-protocol subjects received additional, non-study therapy for uveitis; these were one at Week 8, two at Week 16, and one at Week 20. The subjects who received additional therapy were included in analyses up to and including the visit when additional therapy was administered; no imputations were performed. The remaining 4 subjects needed no additional treatment during the entire 26 weeks of the study.

Individual IOP values at baseline ranged from 10 to 19 mmHg for the 8 per-protocol subjects, and mean changes from baseline during the study did not meet the criteria for being considered as relevant by SUN criteria (Fig. 4). The maximal mean change from baseline in IOP occurred on Day 1 (+2.3 mmHg, 1 hour post-dose), and mean changes ranged from -0.1 to +1.3 mmHg during Weeks 1 to 26. No relevant increase in IOP was reported for any subject, and no subject required IOP-lowering medication.

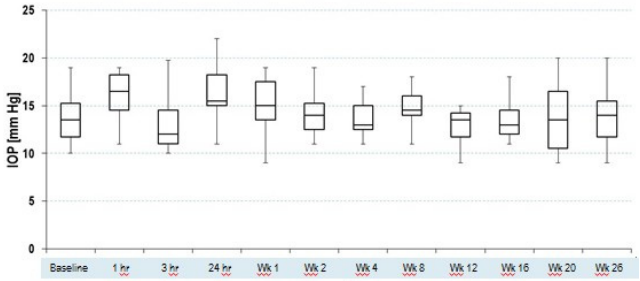

Fig. 4. IOPs at each time point for per-protocol subjects (N=8). Each box plot shows the minimum, first quartile, median, third quartile, and maximum values at the designated time point.

After a single suprachoroidal injection of TA, all 8 efficacy-evaluable subjects showed improvements in BCVA. Mean improvements ranged between 0.17 and 0.28 logMar (approximately 8 and 14 letters) (Fig. 5). Twenty-six weeks after the injection, 4 of these subjects continued to show meaningful improvements (gains of > 2 lines) in BCVA.

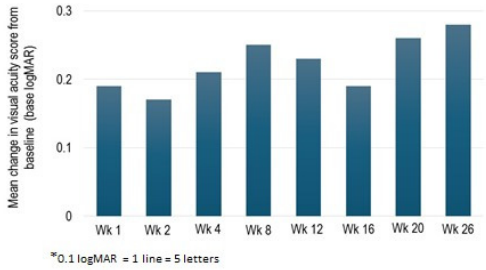

Fig. 5. Mean change from baseline in visual acuity score.

For the 7 subjects who had macular edema at baseline, mean reductions in CST ranged from 76 to 154 microns over the 26-week post-treatment observation period (Fig. 6). At Week 26, the mean reduction in CST was 107 microns. Sustained, clinically meaningful reductions in retinal thicknesses for 4 of the 7 subjects were maintained through Week 12 (Tab. 1).

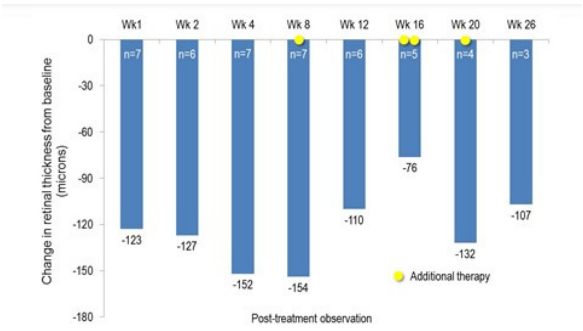

Fig. 6. Mean change from baseline in CST.

Tab. 1. Mean % reduction from baseline in CST for subjects with at least a 20% reduction.

	Wk 1	Wk 2	Wk 4	Wk 8	Wk 12	Wk 16	Wk 20	Wk 26
n*	3	3	5	4	4	2	3	2
% of subjects with 20% reduction	43	43	71	57	67	40	75	67

*Number of subjects with ≥20% reduction from baseline in CST.

Summary

To date, the Clearside uveitis program has included preclinical studies in pigs and rabbits, as well as one completed Phase 1/2 clinical study for which data are available. Preclinical toxicology, pharmacokinetics, and pharmacology studies showed that suprachoroidal administration of TA was safe and tolerable following single and repeat doses, has a unique pharmacokinetic distribution, and demonstrated robust efficacy. Phase 1/2 open label clinical data demonstrated an encouraging safety and efficacy profile for TA administered via suprachoroidal injection in subjects with non-infectious uveitis: meaningful improvements were observed in BCVA and retinal thickness, and no specific safety concerns were identified. Taken together, these data provide the rationale for continued development of TA for the treatment of uveitis through suprachoroidal administration and for SCS-directed therapies for other ocular conditions amenable to such a treatment modality. Phase 2 studies of suprachoroidal drug administration in uveitis and retinal vein occlusion (RVO) are currently ongoing.

Acknowledgments

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Nitric Oxide (NO): an emerging target for intraocular pressure (IOP) lowering

Ongini E., Impagnatiello F., Bastia E.

Nicox, Bresso (Milan), Italy

Summary

Lowering IOP can reduce the risk of progression of glaucomatous visual field loss. Current medications for lowering IOP target aqueous humor formation and/or the uveoscleral outflow pathway. With the exception of Glanatec®, a RHO kinase inhibitor approved in Japan, no therapy exists that primarily targets the conventional outflow pathway and lower IOP by enhancing aqueous humor drainage through the trabecular meshwork and Schlemm's canal. Nitric oxide (NO) is an endogenous cell-signaling molecule which is involved in multiple physiological functions in the eye, including reduction of IOP through action on conventional outflow pathway. Data from a variety of models and recent clinical studies strongly support the role of NO in lowering IOP.

Introduction

Primary open angle glaucoma (POAG) is a chronic, progressive, degeneration of the optic nerve. IOP is the only risk factor, associated with the development of POAG, whose reduction has been proven to be beneficial in patients at risk of progression[1]. Multiple data exist on the role of nitric oxide (NO) in modulating IOP and conventional outflow facility in preclinical models[2,3] and clinical studies[2,4]. In the eye, NO is produced in the major sites of outflow resistance, trabecular meshwork (TM), Schlemm's canal and collecting channels[5] by a family of three enzymes referred to as NO synthases. POAG patients show impaired NO signaling compared with control patients[6]. Accordingly, animal models characterized by alterations of the NO signaling pathway show IOP increase and aqueous humor outflow decrease[7,8]. The present study describes the IOP lowering effects of NCX 667, a novel stand-alone NO donor carrying the isomannide backbone structure, in animal models of glaucoma and ocular hypertension.

Materials and Methods

In the transient ocular hypertensive rabbit model, male New Zealand white (NZW) rabbits were injected with 0.1 ml of hypertonic saline solution (5% NaCl) into the vitreous humor of both eyes. Vehicle (phosphate buffer pH 6.0+cremophor EL 5%+DMSO 0.3%+BAC 0.2mg/ml) or NCX 667 at different doses was instilled immediately after saline injection. IOP was determined prior to (baseline) hypertonic saline and during the following 4 hours post-dosing. Similar IOP recording was used in ocular normotensive NZW rabbits prior to (baseline) and during the following 5 hours post treatment. In the ocular hypertensive non-human primate model, female cynomolgus monkeys with unilateral laser-induced elevated IOP were used. Baseline IOP was measured the day before dosing while drug-mediated IOP changes were determined by comparing vehicle and treatment groups before dosing and during the following 5 hours post treatment. In all the animal models, one topical drop of local anesthetic (proparacaine HCl 0.13%) was applied to the eye prior to each IOP measurement, which was performed with a pneumatonometer (Model 30™ Reichert, Depew, NY, USA).

Results

In ocular normotensive rabbits, NCX 667 dose-dependently lowers IOP (Figure 1). In particular, at 60 min post dosing IOP decrease is -2.7 ± 0.4, -5.0 ± 0.8 and -5.3 ± 0.8 mmHg vs. vehicle at 0.1%, 0.3% and 1%, respectively. Likewise, instillation of NCX 667 (0.1%, 0.3% and 1%) significantly lowers IOP in ocular hypertensive rabbits in a dose-dependent manner, with a maximal reduction of -9.0 ± 0.6 mmHg vs. vehicle at the highest dose tested (Figure 2). In addition, in ocular hypertensive eyes of non-human primates, ocular dosing of NCX 667 1% is effective in lowering IOP (-7.3 ± 2.3 mmHg vs. vehicle, Figure 3). NCX 667 is well tolerated following single topical dosing in rabbits and non-human primates.

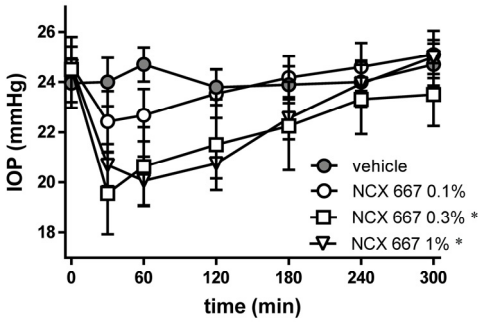

*Figure 1. NCX 667 lowers IOP in ocular normotensive NZW rabbits. IOP were measured hourly up to 5 hours post-dosing of NCX 667 (0.1%, 0.3% and 1%) or vehicle. Data are reported as mean ± SEM of n= 6. *p<0.05 vs vehicle Two way ANOVA followed by Bonferroni's multiple comparisons test.*

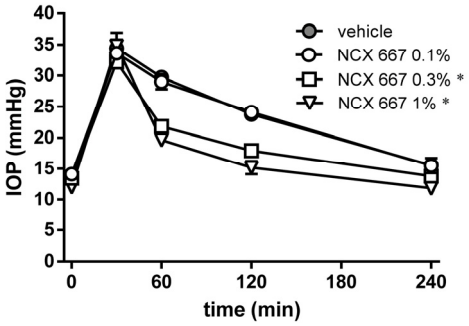

*Figure 2. NCX 667 lowers IOP in transient ocular hypertension NZW rabbits. IOP after instillation of NCX 667 (0.1%, 0.3% and 1%) or vehicle was determined in NZW rabbits injected with 0.1 mL of 5% hypertonic saline into the vitreous humor immediately before drug instillation. Data are reported as mean ± S.E.M.n=8. *p < 0.05 vs vehicle Two way ANOVA followed by Bonferroni's multiple comparisons test.*

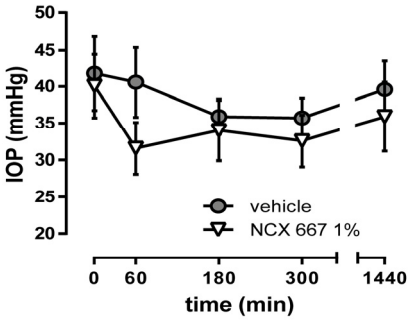

Figure 3. NCX 667 lowers IOP in ocular hypertensive non-human primates. IOP after instillation of NCX 667 (1%) or vehicle was determined in unilateral laser-induced ocular hypertensive non-human primates. Data are reported as mean ± S.E.M., n=6.

Conclusions

Data obtained confirm previous findings suggesting that targeting NO signaling pathway with exogenous NO holds promises as novel treatment strategy for glaucoma.

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Efficient and Effective Drug Delivery Systems: The Mobius™ Experience

Timm E.J.

Founder and CEO – Mobius Therapeutics™, LLC
Ed.Timm@MobiusTherapeutics.com

Mobius Therapeutics™ believes that the retasking of existing molecules is more efficient than drug discovery. New drug applications are brought to market rapidly, with less burdensome regulatory provisions. The resulting entity is enormously efficient, touching multiple subspecialties.

Glaucoma:

Primary open angle glaucoma affects approximately 3 million Americans, with only half diagnosed. The prevalence of glaucoma is far greater among those over the age of 70, African Americans and Hispanics. All therapies focus upon lowering intraocular pressure (IOP). It is treated progressively, with surgery a tertiary step. Antimetabolites are used to interrupt healing, improving efficacy. The predominant agent in use is mitomycin-c.

Mitomycin-c:

Mitomycin-c is a standard of care, but its use presents major challenges.

- It is prepared in compounding pharmacies.
- It degrades rapidly, requiring refrigeration, light-shielding, and has a shelf life of 14 days (Fig 1) [1].
- Compounded mitomycin bears a "Black Box Warning".
- It is hazardous, requiring precautions for those exposed and special handling at the time of use [2].

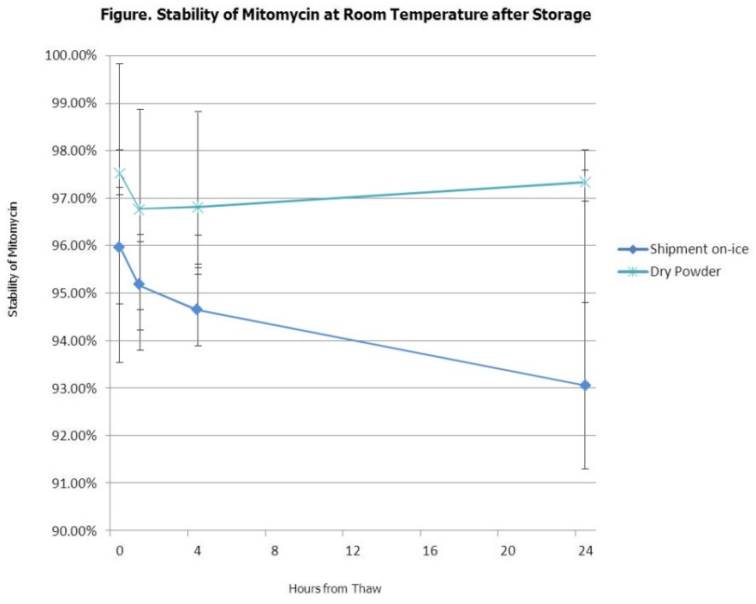

Fig 1

Mitosol® from **Mobius Therapeutics™** is an ophthalmic formulation of mitomycin, stored at room temperature with a 24 month shelf life. A single dose is prepared in surgery via closed containment. Transfer to the sterile field is compliant; disposal is accomplished via a container within the kit. Approved by FDA in 2012, ***Mitosol®*** is now the "Gold Standard" in filtering surgery. It is specified for use in forthcoming MIGS devices.

Intellectual Property and Regulatory Affairs:

The **Mobius Therapeutics™** IP portfolio consists of four issued US patents, two allowed patents, patents issued and pending in Japan, Canada, Australia, and Europe. In the USA, ***Mitosol®*** holds 3 unique Orphan Drug Designations.

Glaucoma Filtering Surgery:

The total current US market for glaucoma filtering surgery is ~88,000 procedures/year [3]. A 90% penetration equates to US revenue of $28 million. Ex-USA doubles this volume, at a lower ASP.

MIGS:

Micro-Invasive Glaucoma Surgery (MIGS) seeks to advance surgery in the glaucoma therapeutic cycle. By performing a minimally invasive procedure, IOP is relieved, extending useful vision and relieving the use of medications. First generation devices, e.g. Glaukos i-Stent, lower IOP by directing fluid to existing anatomical pathways. Second generation devices offer significantly improved efficacy by directing fluid to other sites, bypassing damaged anatomical pathways. All of these devices will require wound modulation; all have used ***Mitosol®*** in their US clinical trials. The existing volume of US patients totals more than 1.5 million per year, growing at 4% annually, creating a US market opportunity exceeding one-half billion dollars per year [4] (Fig 2).

	2013	2014	2015	2016	2017
SLT	588,000	612,500	638,021	664,605	692,297
Meds	804,000	837,500	872,396	908,746	946,610
Units	*1,392,000*	*1,450,000*	*1,510,417*	*1,573,351*	*1,638,907*

Fig 2

Ocular Surface Disease – Pterygium:

Pterygium surgery accounts for approximately 79,000 procedures/year in the USA [5]; prevalence is greater ex-USA and near the equator. The use of MMC has reduced post-surgery recurrence rates from as high as 89% to as low as 2%. A 90% penetration rate equals US revenues of M$25.5. This indication is key to strategic access to the ocular surface specialist. Patients with advanced dry eye disease are referred to and treated by this sub-specialist. Folded into a product portfolio containing products for dry eye disease, ***Mitosol®*** provides access to this market with an exclusive, patent protected, platform product. **Mobius™** anticipates US FDA approval during the first half of 2016. As the site of service (a surgery center or hospital operating theatre) is identical to that where ***Mitosol®*** is currently used, a rapid market adoption is expected.

Laser Vision Correction – Surface Ablation:

Surface ablation vision correction is 15% -20% of all refractive surgery in the USA [6]. ***Mitosol®*** has orphan drug designation for this use; patents and formulation transition unchanged. A 90% penetration rate equals revenues of M$50.4; international markets double procedures, at a lower ASP.

Pipeline: Mobius ABX

Endophthalmitis and penetrating eye injury are treated with two antibiotics: vancomycin and ceftazidime [7]. While standard of care, both remain off-label for use in ophthalmology.

While standard of care, treatment presents major challenges:

- It is prepared in compounding pharmacies.
- Use is emergent, limiting the ability to engage in inventory planning.
- The agents are highly unstable, with a shelf life of 14 days.
- Intravitreal microdosing makes “self-compounding” from a large vial dangerous.

Mobius ABX™ will utilize less burdensome provisions to gain approval of vancomycin and ceftazidime with an ophthalmic indication, accelerating the approval process and minimizing regulatory expense. **Mobius™** will utilize its existing supply chain and commercial structure to speed time to market.

Exit Strategy:

Mobius Therapeutics™ sees its exit as one of acquisition by either an existing ophthalmology manufacturer or a concern (pharma and/or device) aspirational in ophthalmology. **Mobius™** represents the opportunity to accomplish multiple strategic missions – platform products in glaucoma, ocular surface disease, refractive, and vitreoretinal ophthalmology – by way of a single acquisition.

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Effectiveness and safety of eyelid lipogel containing essential oils from Spanish Sage and Aloe Vera in chronic blepharitis caused by Demodex spp

Chudzicka-Strugała I.[1], Chudzicki W.[2], Osęka M.[3]

[1]*Department od Medical Microbiology PUMS (Poznań, Poland)*
[2]*Ophtalmology Practice in Poznan (Poznań, Poland)*
[3]*Ofta Sp. z o.o. (Warsaw, Poland)*
e-mail:ichudzicka@vp.pl

Abstract

Demodex can cause chronic blepharitis or blepharoconjunctivitis. The purpose of the study was to evaluate the effectiveness and safety of eyelid lipogel containing essential oils of Spanish Sage 0,1% or 0,2% and Aloe Vera 100% and its influence on Demodex spp. eradication time in patients with chronic blepharitis and blepharoconjuctivitis caused by Demodex sp. The eyelid lipogels eradicated Demodex in both concentrations of Spanish Sage essential oils. The Demodex eradication time was shorter in case of higher concentration of Spanish Sage essential oils. The treatment was good tolerated in patients in both concentrations.

Keywords: Demodex, blepharitis, blepharoconjunctivitis, therapy

Introduction

Demodex spp. is an external parasite. Demodex located in eyelids and Meibomam glands can cause chronic blepharitis or blepharoconjunctivitis, dry eye syndrome and chalazions. There are various therapeutic schemes against chronic blepharitis caused by Demodex but their effectiveness is questionable. Our previous in vitro study showed the effectiveness of the essential oil of Spanish Sage and Aloe Vera against Demodex sp. The eyelid lipogel containing essential oils for Spanish Sage 0,1% and Aloe Vera 100% significantly decreased subjective and objective symptoms of chronic blepharitis after 4 weeks treatment and partly eradicated Demodex sp. during 6 weeks of treatment.

Purpose

Evaluation of effectiveness and safety of eyelid lipogels with Spanish Sage essential oils 0,1% or 0,2% and Aloe Vera 100% and their influence on the eradication time of Demodex spp. in patients with chronic blepharitis and blepharoconjuctivitis caused by Demodex sp.

Material and methods

The study was performed on 90 eyes of 45 patients (27 women and 18 men, 25-75 years old) with chronic blepharitis or blepharoconjunctivitis caused by Demodex spp. The presence of Demodex infection in both eyes was confirmed by microscopic examination of 4 eyelashes from each eye before and every week from fourth week of the treatment. Eyelid lipogels containing essential oils of Spanish Sage 0,1% or 0,2% and Aloe Vera 100% were used once a day at the evening.

Results

Demodex spp. was not found in 44 eyes treated by eyelid lipogel containing essential oils of Spanish Sage 0,2% and Aloe Vera 100% after 4 weeks of treatment. Total eradication of Demodex spp. was detected in 46 eyes treated by eyelid lipogel containing essential oils of Spanish Sage 0,1% and Aloe Vera 100% after 7

weeks of treatment (+/- 2 weeks). The local tolerance of both eyelid lipogels was very good except 4 eyes of 2 patients (4,4% of all tested eyes) treated by eyelid lipogel containing essential oils of Spanish Sage 0,1% and Aloe Vera 100% and eyelid lipogel containing essential oils of Spanish Sage 0,2% and Aloe Vera 100%.

Conclusions

Eyelid lipogels containing essential oils from Spanish Sage 0,1% or 0,2% and Aloe Vera 100% are effective in eradication of Demodex spp. in patient with chronic blepharitis. Their effectiveness is dependent on concentration of essential oils from Spanish Sage. The local tolerance of eyelid lipogel is very good for both concentrations of essential oils from Spanish Sage.

The Effect of Anthocyanoside and Ginkgo Biloba Extract on Normal-Tension Glaucoma According to Presence of Diabetes

Kim J.M.[1], Choi C.Y.[1], Lee Y.W.[1], Shim S.H.[1], Choi H.[2]

[1] *Department of Ophthalmology, Kangbuk Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South Korea*
[2] *Department of Marketing, KUKJE PHARMA IND.CO.,LTD, South Korea*
e-mail:kjoonmo1@gmail.com

Abstract

Purpose: This study was performed to evaluate the effect of anthocyanoside and ginkgo biloba extract (GBE) in patients with normal tension glaucoma (NTG), according to the presence of diabetes mellitus (DM).

Methods: A chart review of patients with normal tension glaucoma was retrospectively analyzed. All patients underwent a Humphrey visual field (HVF) test and logarithm of the minimal angle of resolution best-corrected visual acuity (log MAR BCVA) was measured over a 6 months period. Changes in mean deviation (MD), pattern standard deviation (PSD) of visual field and log MAR BCVA were compared among anthocyanoside, GBE and no medication (control) groups. Patients were divided according to the presence of DM.

Results: A total of 406 NTG patients, including 151 DM patients, were included in the present study. MD was improved in the anthocyanoside and GBE groups, but not in the control group. PSD was not significantly different in all groups. BCVA was improved in the anthocyanoside group, but deteriorated in the control group. The results were similar in patients with or without DM. The generalized linear model demonstrated that systemic medication affected changes in visual indices.

Conclusions: The results from the present study suggest that anthocyanoside and GBE may be helpful for improving visual function in some patients with NTG regardless of their DM status.

Keywords: Anthocyanoside, Diabetes mellitus, Ginkgo biloba extract, Normal tension glaucoma

Introduction

Ginkgo biloba extract (GBE) and anthocyanins are considered beneficial for various vascular diseases including eye disease[1,2].

This study was performed to evaluate the effect of anthocyanoside and GBE in patients with normal tension glaucoma (NTG), according to the presence of diabetes mellitus (DM).

Methods

We performed a retrospective study with a chart review. We studied 406 NTG patients treated with anthocyanins (n=159), GBE (n=131), or no medication (n=116). The individuals was divided into two groups, depending on presence of DM.

All subjects underwent a full ophthalmic examination including Humphrey visual field, logarithm of the minimal angle of resolution best-corrected visual acuity (logMAR BCVA, intraocular pressure before and at the end of treatment.

All data were analyzed using the SPSS statistical software system version 18.0 (SPSS, Inc., Chicago, IL, USA). Paired T-tests used for differences before and after medication with visual function parameters within groups. Generalized linear model was used to association of changes of visual function with general and ocular variables. A P value of <.05 was considered statistically significant.

Results

Table 1. Characteristics of all patients.

		DM (-) (n=255)	DM (+) (n=151)	Total (n=406)
Sex	**Male**	156	109	265
	Female	99	42	141
Age (year) (mean ± SD)		51.23 (±12.91)	55.13 (±11.54)	52.68 (±12.55)
Mean follow-up (month) (mean ± SD)		23.74 (±11.33)	27.23 (±16.77)	25.04 (±13.69)
Intraocular pressure (mmHg) (mean ± SD)		13.86 (±3.10)	13.84 (±2.99)	13.85 (±3.06)
Blood pressure (mmHg) (mean ± SD)	**Diastolic**	76.70 (±8.57)	78.63 (±8.68)	77.49 (±8.65)
	Systolic	118.77 (±11.51)	123.74 (±12.78)	120.62 (±12.22)
Fasting blood sugar (mg/dL) (mean ± SD)		97.66 (±12.04)	118.14 (±28.77)	109.11 (±25.07)
Hypertension (%)		55 (21.6)	62 (41.1)	131 (31.3)

DM = diabetes mellitus, SD = standard deviation

Table 2. Patient characteristics and baseline data of treatment group.

		DM (-) (n=255)				DM (+) (n=151)			
		Control (n=80)	Anthocyanoside (n=76)	GBE (n=99)	*P*-value	Control (n=36)	Anthocyanoside (n=83)	GBE (n=32)	*P*-value
Sex	**Male**	48 (60.0%)	40 (52.6%)	68 (68.7%)	.094 †	25 (69.4%)	62 (74.7%)	22 (68.8%)	.747 †
	Female	32 (40.0%)	36 (47.4%)	31 (31.3%)		11 (30.6%)	21 (25.3%)	10 (31.3%)	
Age (year) (Mean ± SD)		50.86 (±16.48)	53.61 (±9.55)	49.71 (±11.68)	.134 *	56.11 (±11.43)	55.95 (±11.47)	51.91 (±11.62)	.205 *
Follow-up (month) (Mean ± SD)		22.34 (±9.77)	24.41 (±11.72)	24.36 (±12.17)	.409 *	24.22 (±13.54)	28.47 (±17.26)	27.41 (±18.71)	.449 *
BP (mmHg) (Mean ± SD)									
	Systolic	119.34 (±12.32)	120.72 (±10.96)	116.82 (11.05)	.073 *	123.11 (±13.50)	123.89 (±11.75)	124.06 (±14.76)	.943 *
	Diastolic	77.81 (±7.50)	77.20 (±9.44)	75.41 (±8.59)	.147 *	79.64 (±9.73)	78.66 (±8.28)	77.41 (±8.58)	.573 *
FBS (mg/dL) (Mean ± SD)		94.38 (±8.54)	101.59 (±15.83)	96.12 (±9.05)	.052 *	120.83 (±26.57)	118.08 (±29.50)	115.59 (±29.94)	.789 *
Hypertension		18 (22.5%)	22 (28.9%)	15 (15.2%)	.086 †	11 (30.6%)	41 (49.4%)	10 (31.3%)	.071 †
IOP (mmHg) (Mean ± SD)		14.16 (±3.20)	13.29 (±3.00)	14.05 (±3.07)	.157 *	14.83 (±2.85)	13.54 (±3.09)	13.50 (±2.69)	.199 *
MD (db) (Mean ± SD)		-3.953 (±4.788)	-4.868 (±5.393)	-4.861 (±5.334)	.451 *	-4.451 (±4.490)	-6.234 (±7.116)	-5.760 (±4.929)	.351 *
PSD (db) (Mean ± SD)		3.726 (±3.044)	4.611 (±3.930)	4.629 (±4.029)	.205 *	4.798 (±3.259)	5.155 (±3.750)	5.909 (±4.389)	.467 *
LogMAR BCVA (Mean ± SD)		0.077 (±0.110)	0.076 (±0.096)	0.063 (±0.087)	.535 *	0.090 (±0.148)	0.110 (±0.234)	0.100 (0.185)	.568 *

* One-way ANOVA, † Chi-square test

DM = diabetes mellitus, GBE = ginkgo biloba extract, SD = standard deviation, BP = blood pressure, FBS = fasting blood sugar, IOP = intraocular pressure, MD = mean deviation, PSD = pattern standard deviation, LogMAR BCVA = logarithm of the minimal angle of resolution best corrected visual acuity

Table 3. Best-corrected visual acuity (BCVA), visual field indices with treatment group.

DM (-) (n=255)	Control (n=80)			Anthocyanoside (n=76)			GBE (n=99)		
	Baseline (Mean ± SD)	Follow-up (Mean ± SD)	*P*-value *	Baseline (Mean ± SD)	Follow-up (Mean ± SD)	*P*-value *	Baseline (Mean ± SD)	Follow-up (Mean ± SD)	*P*-value *
MD(dB)	-3.953 (±4.788)	-4.156 (±5.567)	.654	-4.868 (±5.393)	-4.102 (±5.209)	.005	-4.861 (±5.704)	-4.013 (±5.360)	.001
PSD(dB)	3.726 (±3.044)	4.068 (±3.097)	.154	4.611 (±3.930)	4.494 (±3.943)	.438	4.629 (±4.029)	4.367 (±4.068)	.118
LogMAR BCVA	0.077 (±0.110)	0.131 (±0.238)	.022	0.076 (±0.096)	0.057 (±0.082)	.024	0.063 (±0.087)	0.054 (±0.092)	.206
DM (+) (n=151)	**Control (n=36)**			**Anthocyanoside (n=83)**			**GBE (n=32)**		
	Baseline (Mean ± SD)	Follow-up (Mean ± SD)	*P*-value *	Baseline (Mean ± SD)	Follow-up (Mean ± SD)	*P*-value *	Baseline (Mean ± SD)	Follow-up (Mean ± SD)	*P*-value *
MD(dB)	-4.451 (±4.490)	-4.261 (±4.436)	.561	-6.234 (±7.116)	-5.043 (±6.008)	.003	-5.760 (±4.929)	-4.460 (±4.914)	.025
PSD(dB)	4.798 (±3.259)	4.708 (±3.409)	.668	5.155 (±3.750)	4.818 (±3.694)	.053	5.909 (±4.389)	6.030 (±4.353)	.735
LogMAR BCVA	0.090 (±0.148)	0.130 (±0.181)	.005	0.110 (±0.234)	0.091 (±0.154)	.011	0.100 (±0.185)	0.103 (±0.233)	.809

*Paired sample *t*-test

LogMAR BCVA = logarithm of the minimal angle of resolution best corrected visual acuity, DM = diabetes mellitus, GBE = ginkgo biloba extract, MD = mean deviation, PSD = pattern standard deviation, SD = standard deviation

Table 4. Generalized linear model of general and ocular variables and changes of visual indices (after-before).

	Changes of MD		Changes of PSD		Changes of LogMAR BCVA	
	P-value	Coefficient	*P*-value	Coefficient	*P*-value	Coefficient
Gender (Female)	.818	1.118	.477	0.801	.772	1.007
Age	.002	0.948	.063	0.545	.389	0.999
Anthocyanoside	.019	2.904	.001	0.382	<.001	0.913
Ginkgo biloba extract	.037	2.958	.063	0.545	.004	0.932
Hypertension	.471	1.350	.308	0.762	.345	1.019
Diabetes	.051	2.184	.120	0.672	.121	0.971
Intraocular pressure	.228	1.076	.533	0.976	.590	1.002

MD = mean deviation, PSD = pattern standard deviation, LogMAR BCVA = logarithm of the minimal angle of resolution best corrected visual acuity

Discussion

Anthocyanins treatment appeared to be more strongly correlated with visual function improvement than GBE, especially in BCVA logMAR units. We thought, relatively small number of GBE treatment group with DM could cause such inconsistent result of HVF mean deviation, therefore this was limitation of our study.

Several previous studies reported that DM is one of the risk factor of glaucoma[3,4]. Common genetic factors, premature aging process of the optic nerve caused by diabetes microscopic blood flow disorders and optic neuropathy have been proposed as the mechanisms of optic nerve damage.

Considering the mechanisms of drug, antioxidant and promotion of blood circulation, we wondered whether the diabetes influence these effects and outcome of the treatment.

Although, the larger number of study would be required, our results could be meaningful in treatment of NTG in different ways.

Conclusions

The results from the present study suggest that anthocyanoside and GBE may be helpful for improving visual function in some patients with NTG regardless of their DM status

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Ocular manifestations and choroidal thickness measured by swept-source optical coherence tomography in patients with familial hypercholesterolemia treated with oral intensive statin therapy

Manco Lavado F.[1,2], Ramoa Osorio M.R.[1,2], Díaz Soto G.[3], López Gálvez M.I.[1,2], Manzanas Leal L..[1]

[1] *Ophthalmology Department, Hospital Clínico Universitario de Valladolid, (Spain)*
[2] *IOBA (Eye Institute), University of Valladolid, (Spain)*
[3] *Endocrinology Department, Hospital Clínico Universitario de Valladolid, (Spain)*
e-mail:felixmancolavado@gmail.com

Abstract

Purpose: High levels of cholesterol have been related to macular degeneration. In animal models hypercholesterolemia damages the neurosensory retina and induces an increase in scleral and choroidal thickness. The purpose of the present study is to evaluate the anatomical findings in FH patients treated with oral intensive statin therapy using SS-OCT.

Methods: We designed a descriptive, cross-sectional and comparative study of 18 FH patients treated with statins versus 18 healthy controls. All patients underwent a complete ophthalmic examination. Primary outcomes were the presence of typical ocular manifestations of FH, and the quantitative and qualitative changes in retina and choroid of FH patients compared to healthy controls.

Results: Mean age of FH patients was 54.0 ± 11.9 years-old, 50% were male. Mean LDL-c level at diagnosis was 260.6 ± 47.6 mg/dL, but 133.7 ± 34.0 mg/dL average of twenty-years after treatment. Mean BCVA was 0.01 ± 0.04 logMAR, corneal arcus was found in 61.1%, xanthelasmas in 11.1% and one patient showed vascular narrowing in the fundus. Neither yellowish deposits nor cholesterol crystals were found. There were no qualitative changes in the retina analysed by SS-OCT. Mean subfoveal choroidal thickness (CT) was 265.17 ± 96.71 μm, nasal CT 210.90 ± 88.70 μm and temporal CT 208.20 ± 90.43 μm. In control group, the mean age was 42.3 ± 11.9 years and the mean LDL-c level was 110.6 ± 45.2 mg/dL. Choroidal and retinal thickness was within the normal range. No differences were found between both groups.

Conclusions: Intensive statin therapy prevents and reduces the ocular damage of hypercholesterolemia. The choroidal and retinal thicknesses measured by swept-source OCT in FH patients treated with oral intensive statin therapy are within the normal range.

Keywords: familial hypercholesterolemia, SS-OCT, enhanced depth imaging optical coherence tomography, choroidal thickness, retinal thickness, intensive statin therapy.

Introduction

Familial hypercholesterolemia (FH) is an autosomal dominant genetic disorder characterized by high plasma levels of low density lipoprotein-cholesterol (LDL-C), that leads to increased risk for the premature development of atherosclerosis and cardiovascular disease. The world prevalence of FH is approximately 10 million people.[1] FH is usually treated with lipid-lowering agents that lower cholesterol levels, such as statins therapy. Treatment with statins, including higher statin doses, is generally very effective, although sometimes additional therapy with other drugs is required.[2]

FH patients may have systemic and ocular manifestations. The most frequent ophthalmic manifestations are bilateral corneal arcus and xanthelasma palpebrarum. Intraocular complications such as yellowish deposits and cholesterol crystals in the retina have only rarely been observed.[3] The exact underlying mechanisms of these ocular manifestations remain unclear. Some studies have demonstrated that high levels of cholesterol have been related to macular degeneration.[4] Torres et al have published that hypercholesterolemic diet in rabbits induces an

increase in the macrophage concentration and immunoreactivity to VEGFR-1 in the choroid and sclera, resembling human AMD.[4] In addition, several studies have been performed in animal models to demonstrate that hypercholesterolemia induces: •neuronal damage due to hypoxia of the neurosensory retina,[4,5] •retinal and choroidal vascular disorders, such as tortuosity and generalized dilatation of arterioles and draining venules,[6] and •a significant increase in scleral and choroidal thickness, mainly due to a macrophage accumulation and an increase in the number of histiocytes and collagen fibres in the choroid-sclera complex.[7,8]

Cholesterol is involved in retinal cell function however, hypercholesterolemia impairs its function.[9] Retinal damage, neovascularization, and cataracts are the main complications of cholesterol overload.[9]

With all the information available, it is believed that the choroidal circulation plays an important role in the ocular manifestations of this disease.

Since the advent of newest deep-penetration optical coherence tomography (OCT) technologies, such as swept-source longer-wavelength OCT (SS-OCT) that provides a deeper range of imaging into the eye enabling high-resolution retinal and choroidal imaging, it is possible a non-invasive measurement and analysis of the retina, choroid, and in some cases, even sclera.

Therefore, it has been studied and demonstrated that the hypercholesterolemic diet induces early abnormalities in the retina, choroid and sclera in rabbits. But this issue has not been studied in humans. In this context, the present study has been designed to evaluate the ocular manifestations and the retinal and choroidal thickness assessed by SS-OCT in patients with familial hypercholesterolemia (FH patients) treated with oral intensive statin therapy.

Materials and Methods

This is a descriptive, cross-sectional and comparative study that has been conducted by the Departments of Ophthalmology and Endocrinology at the Hospital Clínico Universitario de Valladolid, Spain. The institutional review board of Hospital Clínico Universitario approved this study. The informed consent was obtained according to the principles of the Declaration of Helsinki. Primary outcomes were the presence of typical ocular manifestations of FH and the quantitative and qualitative changes in retina and choroid of FH patients compared to healthy controls. Secondary outcomes were BCVA, intraocular pressure (IOP), and lipid profile (total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglyceride).

1.1. Patients

Inclusion criteria were males and females, an age of 18 years or older, index cases of FH (diagnosis of FH based on the Simon-Broome diagnostic criteria), and patients must be receiving oral intensive statin therapy. Exclusion criteria were secondary causes of elevated blood cholesterol levels (total cholesterol and LDL-c), and / or early heart attacks or early death from heart disease or stroke, and eyes with spherical equivalent greater than 6 diopters. Subjects diagnosed with hypertension and diabetes were also excluded. Included patients were compared to age- and sex-matched healthy controls. All patients underwent a complete ophthalmic examination in both eyes including refractometry with an auto-refractometer (Auto kerato-refractometer KR-8100P; Topcon Corporation, Tokyo, Japan), distance best-correct visual acuity (BCVA) testing, slit lamp biomicroscopy, applanation tonometry, direct and indirect ophthalmoscopy through dilated pupils, SS-OCT scanning of the retina and choroid, and colour fundus photography (TRC-NW8 Non-mydriatic retinal camera; Topcon Corporation, Tokyo, Japan) obtained according to Joslin Vision Network (JVN) protocol.[10]

1.2. Deep Range Imaging OCT

Retinal and Choroidal thickness was studied with the DRI OCT-1 Atlantis SS-OCT (Topcon Corporation, Tokyo, Japan).

Automatic retinal thickness measurements were obtained through segmentation software (FastMap™, a database and analysis software). Retinal thickness (RT) was measured in three different areas: at central fovea (central foveal thickness) and 1500 μm intervals temporal and nasal to the centre of the fovea (Fig. 1).

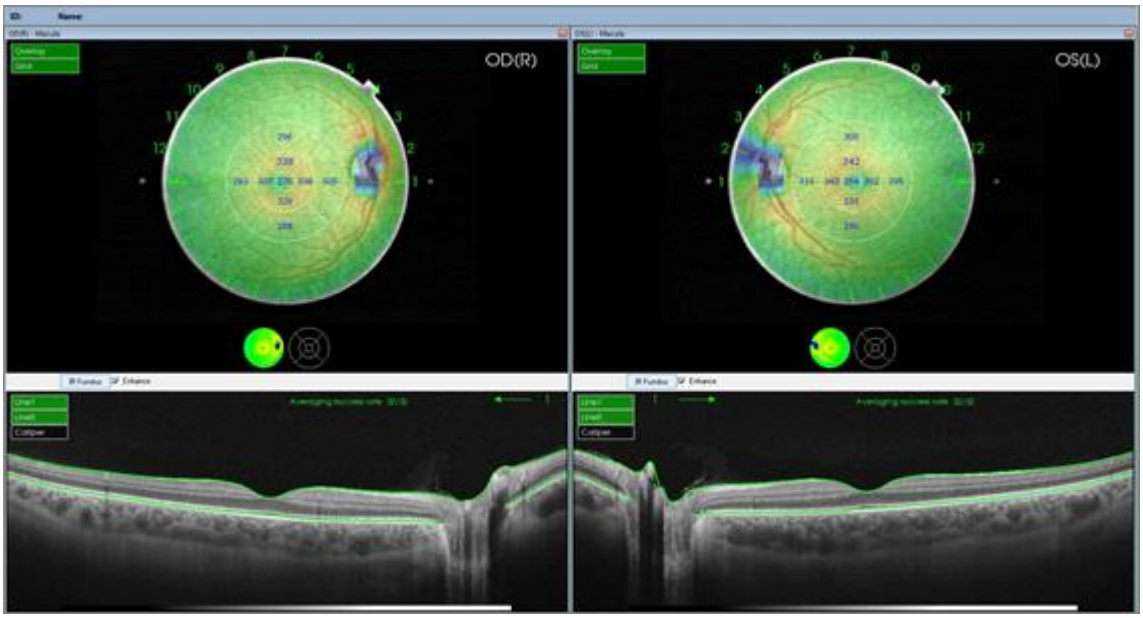

Figure 1. Automated measurements (in µm) of retinal thickness using the FastMap™ software.

Choroidal thickness was manually measured as the perpendicular distance between the external surface of the RPE and the internal surface of the sclera. It was determined under the fovea (subfoveal choroidal thickness) and 1500 µm intervals temporal and nasal to the centre of the fovea (Fig. 2).

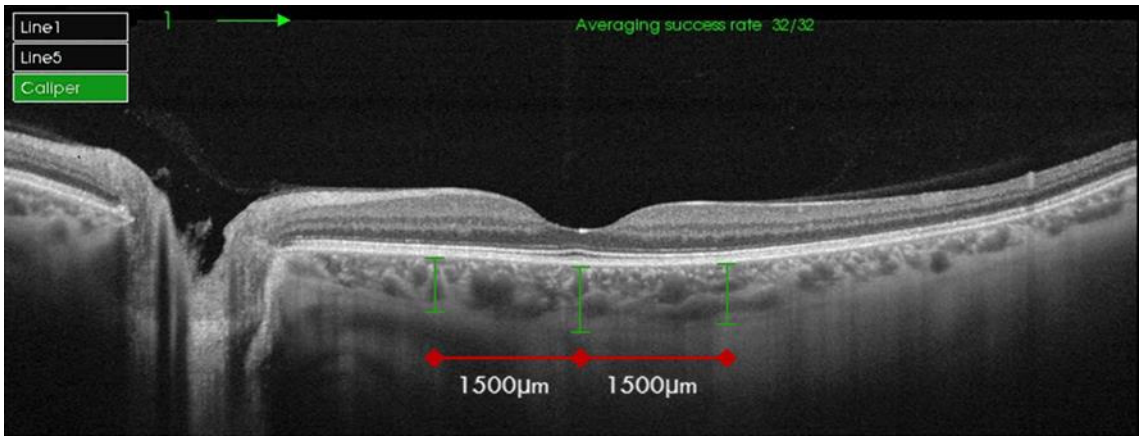

Figure 2. Manual measurements (in µm) of choroidal thickness.

All examinations were obtained in the afternoon to avoid diurnal fluctuations of choroidal thickness.[11] All images in the present study were captured by a single experienced retinal specialist.

1.3. Statistical Analysis

All the information was analysed using SPSS statistical software package for Windows (SPSS version 22.0; SPSS Inc., Chicago, IL).

Comparison between groups was performed using the Student's t-test when samples followed a normal distribution or Mann–Whitney test when parametric statistics were not possible. A p-value < 0.05 was considered statistically significant.

Results

We studied 18 cases index of FH (one person per family) treated with statins therapy and compared them with 18 age and sex matched healthy controls. All patients underwent a complete ophthalmic examination.

A total of 36 eyes of 18 index cases of FH were included. 50% were male. At diagnosis, the mean age of these patients was 34.9 ± 13.9 years-old and the mean LDL-c level was 260.6 ± 47.6 mg/dL. At the time of

the study, the mean age was 54 ± 11.9 years-old, and the evaluation of the lipid profile after statin treatment showed mean total cholesterol 208.2 ± 32.9 mg/dL, mean LDL-C 133.7 ± 34.0 mg/dL, mean HDL-C 57.4 ± 21.2 mg/dL, and mean triglyceride 102.4 ± 9 mg/dL.

The control group had very similar characteristics regarding the number of subjects examined, and the mean age and sex ratio. 36 eyes of 18 healthy controls were studied, the mean age was 42.3 ± 11.9 years-old and 50% were male. The only significant differences between both groups were the LDL-c levels, 133.7 ± 34.0 mg/dl in the FH patients group and 110.6 ± 45.2 mg/dl in the control group. The difference between the LDL-c levels in these two groups was statistically significant (p = 0.017).

Mean BCVA was 0.01 ± 0.04 logMAR and the mean IOP 14.7 ± 3.4 mmHg in the FH patients group. The complete ophthalmic examination revealed that 61.1% of FH patients had corneal arcus, 11.1% xanthelasmas, one patient (5.5%) had vascular narrowing in the fundus, and 38.8% showed no clinical feature of FH. Neither yellowish deposits nor cholesterol crystals were found in the fundus.

Mean central foveal thickness and the mean nasal and temporal retinal thickness were 250.7 ± 18.6 µm, 286.4 ± 15.9 µm and 254.7 ± 31.1 µm respectively. There were no qualitative changes in the retina analysed by SS-OCT, and no differences between FH patients group and the control group were found.

Regarding the choroidal thickness, no statistically significant differences between both groups were found (Tab. 1).

Mean CT	FH patients group	Control group	*p*-value
Subfoveal CT	265.1 ± 96.7	265.1 ± 64.9	$p = 0.99$
Nasal CT	210.9 ± 88.7	225.0 ± 71.8	$p = 0.46$
Temporal CT	208.2 ± 90.4	219.8 ± 67.3	$p = 0.54$

Table 1. Mean choroidal thickness in FH patients group versus Control group, and p-values. CT, choroidal thickness. FH, familial hypercholesterolemia.

Mean subfoveal choroidal thickness in both the FH patients group and the control group was 265.1µm. This subfoveal choroidal thickness was higher than the nasal and temporal choroidal thickness in both groups (Tab. 2).

		Nasal CT	Temporal CT
Subfoveal CT	**FH patients group**	$p < 0.0001$	$p < 0.0001$
	Control group	$p < 0.0001$	$p < 0.0001$

Table 2. P-values between subfoveal-CT versus nasal-CT, and subfoveal-CT versus temporal-CT, in both groups. CT, choroidal thickness. FH, familial hypercholesterolemia.

Discussion

Ocular manifestations of FH are little known, especially intraocular manifestations. In addition, it is unknown whether the choroid has some role in the development of these ocular manifestations. The emergence and development of an enhanced depth imaging optical coherence tomography, such as the swept-source OCT, would provide highly relevant data on that topic.

In the present study, we have shown that the most common ocular finding in FH was corneal arcus (61.1%), and the second most frequent were xanthelasmas (11.1%). The only intraocular finding noted was retinal vessel narrowing that was observed in just one patient. Some publications have reported that hypercholesterolemia (not FH) is related to retinal vascular disorders, especially arteriosclerosis and atherosclerosis.[8,12] It would be interesting to know in detail the medical history of that patient because he probably does not have a good lipid profile control. A third of patients (38.8%) showed no clinical feature of FH, this could be because the lipid profile of FH patients were within the normal range due to intensive statin therapy.

Mean retinal thickness (central foveal thickness, nasal and temporal RT) were within the normal range and are fully consistent with currently published data.[13] No qualitative changes were detected in the retina analysed by SS-OCT, and no differences between FH patients group and the control group were found. This is probably due to the efficacy of intensive statin therapy in good lipid profile control.

Regarding the choroidal thickness in FH patients, the values obtained in this study are consistent with data published in other studies that examined healthy people.[14] Our findings are virtually identical to those obtained by Margolis and Spaide.[15] They found a mean subfoveal CT of 287 ± 76 µm among the 30 subjects

(mean age, 50.4 years) in their study. We also found no statistically significant differences in choroidal thickness when comparing the FH patients group and the control group. This fact contrasts with the results of a study comparing hypercholesterolemia patients versus healthy controls, although in mentioned study, patients have not been diagnosed with FH and the study was conducted on Chinese patients.[16] Several studies have demonstrated that choroidal thickness is influenced by different factors, including age and ethnicity.[17] A sub-analysis of these current study revealed that subfoveal choroidal thickness was higher than the nasal and temporal choroidal thickness in both groups. These data are also consistent with data published so far.[14]

Mean values of lipid profile (total cholesterol, LDL-C, HDL-C, and triglyceride) in the FH patients group treated with oral intensive statin therapy since diagnosis were within the normal range, this is probably the reason we have found practically no statistically significant differences compared to the control group.

The limitations of the present study were the cross-sectional study design, the relatively small number of eyes studied, and all measurements were obtained by a single observer.

Conclusion

This study demonstrated that the intensive statin therapy prevents and reduces the ocular damage of hypercholesterolemia. The choroidal and retinal thicknesses measured by swept-source OCT in FH patients treated with oral intensive statin therapy are within the normal range. However, further studies are needed to definitely clarify the doubts regarding the ocular manifestations of familial hypercholesterolemia.

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Hot-melt extrusion technique (HME) to develop intravitreal inserts

Monti D.[1], Terreni E.[1], Chetoni P.[1], Burgalassi S.[1], Tampucci S.[1], Maroni A.[2], Casati F.[2], Zema L.[2], Gazzaniga A.[2]

[1]*Department of Pharmacy, University of Pisa, Pisa (Italy)*
[2]*Department of Pharmaceutical Sciences – Pharmaceutical Technology & Regulatory affairs "Maria Edvige Sangalli" Unit, University of Milan, Milan (Italy)*
daniela.monti@farm.unipi.it

Abstract

This study aimed to develop an ophthalmic insert containing fluocinolone acetonide (FA), to apply in the posterior segment of the eye. The inserts were prepared by the hot-melt extrusion technique (HME) and contained 3% FA and different polymeric materials chosen according to their suitability for extrusion and their potential use in ophthalmic field. The insert based on AMYLO-maize starch (AMYLO-FA) was selected on the basis of technological properties and *in vitro* drug release behavior. No substantial morphological changes following of AMYLO-FA in isotonic buffer solution hydration and swelling were observed up to 12 days. Drug release performance of AMYLO-FA, evaluated using Gummer-type diffusion cells to maintain the sink conditions, highlighted an anomalous non-Fickian release, and about 60% of FA content was delivered over 8 days. The release rate of FA was also determined when the receiving phase was totally replaced every 24h and 48h simulating the biological conditions of a stagnant vitreous humour. In any case, the obtained FA release was linear during the 25-day sampling period and release rates of 26.76 and 10.61 μg/day were obtained by replacing the receiving phase every 24 and 48 hours, respectively. Thus it seems that the FA release from the device was mainly influenced by the receiving environment rather than controlled from the insert.

Keywords: Hot-melt extrusion, intravitreal inserts, in vitro drug release, amylose, fluocinolone acetonide.

1. Introduction

Inflammation plays a key role in the pathological processes leading to macular edema (ME) due to retinovascular disease (i.e. diabetes and retinal vein occlusion). Prolonged-release, low dose intraocular corticosteroid delivery devices may be a promising viable long-term solution for patients diagnosed with ME. These implants are intended for long-lasting constant delivery of the drug reducing the need for repeated injections and therefore, the risk of ocular side effects associated with intravitreal injections. Both dexamethasone and fluocinolone acetonide (FA) are available as intravitreal delivery devices [1]. At the moment, three sustained-release corticosteroid implants are known: Ozurdex (Allergan Inc., Irvine, CA), which releases dexamethasone to the vitreous cavity over a six-month period, and is degraded *in vivo;* two devices that release FA, Retrisert (Bausch & Lomb, Rochester, NY), a non-biodegradable implant based on drug pellets, and Iluven (Alimera Science, Alpharetta, GA) a non-biodegradable cylindrical tube with a drug-polymer matrix core . Each has different physical characteristics and duration of release, and has been approved for different indications [2]. Ozurdex® is the only biodegradable implant.

Hence, it could be interesting to propose novel long acting intravitreal inserts for the treatment of posterior segment eye diseases. In this respect the use of hot-melt extrusion technique (HME) could be especially advantageous This technique is widely used to convert plastic raw materials into a product of uniform density by heating and forcing them through a die, generally employing a rotating screw. It is a viable technology to produce homogeneous drug incorporating polymeric matrices of different size and shape without any use of solvents. Many researchers proposed HME for the preparation of drug delivery systems (DDS), fast dissolving or prolonged release formulations to be administered by oral, transdermal, transmucosal and transungual routes [3][4][5][6].

The main goal of this study is the development of an ophthalmic insert as a new platform containing fluocinolone acetonide by exploiting HME technique to ensure the sustained-release of corticosteroids in the posterior segment of the eye [1][7]. Starches with different ratio of amylose /amylopectin were selected and technological characteristics, potential ocular tolerability and drug release performance of the inserts were preliminary evaluated.

2. Materials and Methods

2.1. Materials

Amylo-maize starch N-400 (AMYLO) was kindly provided by Roquette S.p.a. (Italy); corn starch (CS), magnesium stearate (MS), and fluocinolone Acetonide (FA) were purchased from Sigma Aldrich (Germany). All other reagents were analytical grade.

2.2. Preparation of FA inserts

Inserts were prepared by HME technique. A mixture of polymer, FA, and plasticizers was extruded at 100°C and 110 rpm through a twin-screw extruder (Haake MiniLab II, Thermo Scientific, USA). The final insert size (1.0 x 4.0 x 12.0 mm) was obtained cutting by a scalpel the cylindrical extruded product. Each insert contained 3% FA and different polymeric materials. The composition of the different formulations (AMYLO-FA, CS-FA, CS-MS-FA) is shown in Table 1. The inserts had a weight ranging between 0.033g and 0.044g.

Tab. 1. Composition of the extruded inserts.

Polymer, %w/w		Excipient, %w/w		FA, %w/w	Insert
Amylo-maize starch N-400®	97.0	-	-	3.0	AMYLO-FA
Corn starch	97.0	-	-	3.0	CS-FA
Corn starch	92.0	Magnesium Stearate	5.0	3.0	CS – MS-FA

2.3. Characterization of the inserts

2.3.1. Swelling behavior

The swelling behaviour of each type of insert was observed using a digital microscope (Dino-lite Pro, ANMO, Taiwan). Each insert was immersed in 4 mL of phosphate buffer saline (PBS; isotonic, 66.7 mM, pH 7.4) added of 20 μL of 0.02 %w/w methylene blue solution obtaining dye final concentration of 0.004 %w/w. The medium was kept at 37°C. At predetermined time intervals, an image of each insert was captured through the digital microscope. Computer analysis of the images was done using Dino Capture 2.0 Software (ANMO, Taiwan). End point of the test was loss of form, swelling, and/or the presence of fracture lines.

2.3.2. In Vitro Release Studies

In *vitro* FA release studies were performed only for AMYLO-FA using two different experimental methods. Firstly, the insert, exactly weighed (FA average content: 1.3 mg), was introduced in a metallic tiny net support and placed in the receiving chamber of a Gummer-type diffusion cell that was hermetically sealed to avoid evaporation. The Gummer-type diffusion cell was used as the most suitable container (5 mL), given that it has roughly the same volume occupied by the vitreous humour (4.6 mL) in a human adulthood eye [8]. The receiving chamber contained 5 mL of PBS that was continuously replaced with fresh fluid at a flow rate of 0.4 mL/min by a peristaltic pump (Minipuls 3; Gilson, Villers-le-Bel, France). The system was thermostated at 37°C. At predetermined time intervals, the fluid was withdrawn for analysis. Both experiments were repeated 3 fold.

At the end, the release rate of FA from AMYLO-FA inserts was also determined using another experimental method by referring to Mruthyunjaya et al. (2006) [9]. Briefly, the inserts (FA average content: 1.3 mg) was put in contact with 1 mL of PBS in glass narrow vials maintained at 37 °C. The entire solution was withdrawn for analysis at 24 or 48-hours time intervals and replaced with fresh PBS. The tests lasted 25 days. In all cases the amount of released drug was determined by HPLC. Both experimental protocols were repeated 3 fold.

2.3.3. Analytical Methods

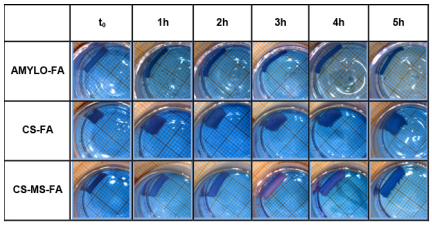
The quantitative determination of FA in receiving phase was carried out by HPLC. The apparatus consisted of Shimadzu LC-20AT system with an UV-SPD-6AV detector and the injection valve was a Rheodyne with a capacity of 20 µL. A Kinetex Phenomenex® C18 100 A (5 µm; 150 x 4.6 mm) column was employed. The mobile phase consisted of a mixture of methanol: MilliQ-water (55:45). The detection wavelength was 238 nm, the flux was 1.0 mL/min, and the retention time was 8.35 min. The amount of FA in the samples was determined by comparison with appropriate standard curve. FA stock solution was in methanol and progressive dilutions were made adding PBS in the range of 0.004 µg/mL - 5.10 µg/mL. The standard curve was linear and good (r^2= 0.993) in the detection range.

3. Results and Discussion

3.1. Swelling behaviour

Inserts containing 3% FA and AMYLO, CS or CS-MS as polymeric materials were prepared and subjected to a preliminary evaluation of the swelling behaviour for 5 hours. As shown in Fig. 1a, during the first hour of contact with PBS, used to simulate the biological fluids, inserts CS-FA and CS-MS-FA appeared hydrated, swelled, and partially eroded. Conversely, AMYLO-FA appeared hydrated but no signs of expansion or erosion were noticed. At the end of the experiment, the swelling of CS-FA and CS-MS-FA was excessive for use in *vivo*. For this reason, both of them were discarded. In the case of AMYLO-FA, the experiment was continued up to 12 days: no further substantial changes in terms of morphology occurred (Fig. 1b). The difference in hydration behaviour between AMYLO-FA and the other two inserts (CS and CS-MS) could be associated with the high amylose content (60%) of AMYLO polymer in contrast to the amylose content ranging between 15 and 30% in CS. It is known that starch contains two main polysaccharides: amylose and amylopectin in different ratios, depending on the product origin. Moreover, it is reported in literature that water uptake and solubility of starch decrease as the amylose content increases [10]. Thus the high amylose content of AMYLO-FA appears to play a key role in the interaction with the aqueous medium.

In light of the results, AMYLO-FA was selected as the best candidate for in *vitro* studies.



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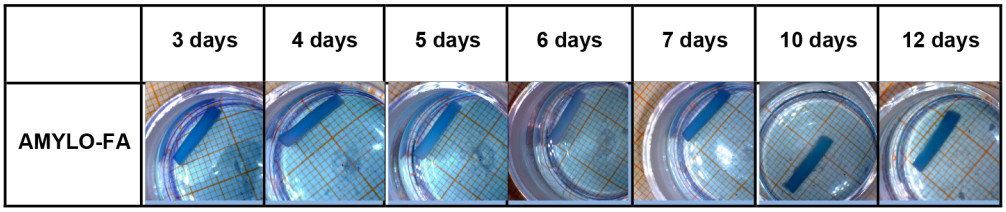

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Fig.1. Morphological changes of extruded inserts in PBS. a) until 5 hours; b) AMYLO-FA until 12 days.

3.2. In Vitro Release Studies

In *vitro* release studies of AMYLO-FA insert were carried out using Gummer-type diffusion cell method and maintaining sink conditions by continuous replacement of the receiving phase. FA release profile from AMYLO-FA insert up to 8 days is shown in Fig.2. Because of the swellable hydrophilic nature of the system, data were analyzed according to the well-known Korsmeyer and Peppas equation [11]. The obtained *n* value of 0.72 (r^2=0.9646) indicates a typical anomalous non-Fickian release characterized by an initial faster release of FA that progressively slows down tending to linearity. When aqueous fluid comes into contact with the insert, a glassy-rubbery transition occurs while drug particles at the surface start to dissolve. Soon after, drug release is therefore controlled both by glassy-rubbery transition and diffusion through the swollen barrier. AMYLO-FA insert maintained its shape without major size changes even after 12 days. This release pattern could be of interest because it would enable a rapid achievement of high drug concentrations followed by a

longer period of continuous slow release. The same OZURDEX is designed to release dexamethasone biphasically, with peak doses for initial 2 months followed by lower therapeutic doses for up to 6 months [12].

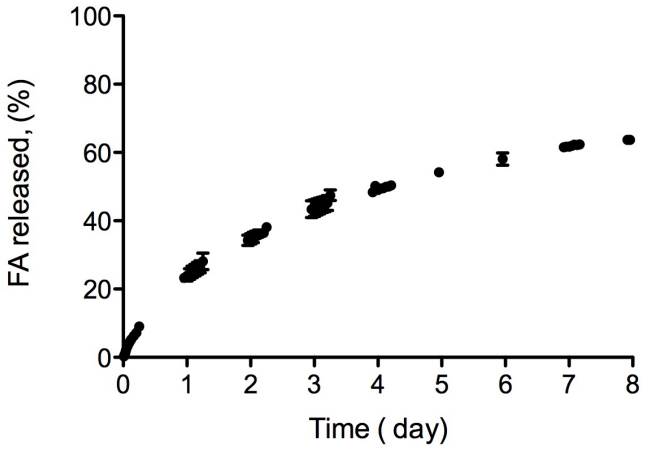

Fig.2. FA release profile from AMYLO-FA insert up to 8 days.

The release rate from AMYLO-FA inserts was also determined using another experimental method by referring to Mruthyunjaya et al. (2006)[9]. Two different experiments were performed: the first one replacing totally the receiving phase (t.r) every 24 hours, and the other one every 48 hours. In both cases a linear pattern of drug release was obtained. By way of example the experiment carried out replacing the medium every 24h is reported in Fig.3 (r^2 = 0.9917). In similar experiments, Jaffe et al. (2000)[7] and Mruthyunjaya et al. (2006)[9] have found the same linear trend**.** As summarized in Table 2, the release rates obtained are 26.76 ± 0.44 and 10.61 ± 0.51 μg/day for 24 h and 48 h t.r., respectively. At the end of the experiments (25 days), the total FA released was 674.10 ± 20.87 and 273.81 ± 10.08 μg for 24h and 48 h t.r., respectively. All data are the mean of three determinations ± standard error (SE).

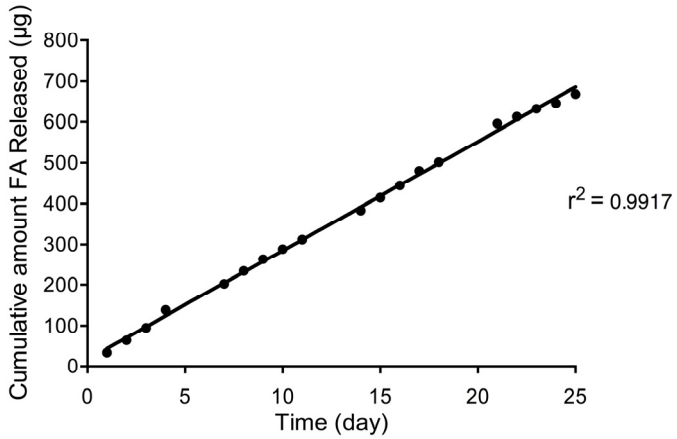

Fig.3. Cumulative FA release (μg) from AMYLO-FA a function of time. Total receiving phase replacement (t.r.) every 24h.

These results showed that both the release rate and the amount of drug released at the end of the experiment decreased by half when the sampling at longer time (48h) was carried out: so it seemed that the release of drug was mainly influenced and driven by the receiving environment rather than from the device (insert). In this experiment, the change in the receiving phase was very slow to reproduce the biological conditions of a stagnant vitreous humor. It is reported that the normal vitreous outflow is about of 0.1 μL/min [13].

Tab. 2. In vitro release parameters of FA from AMYLO-FA using t.r.

t.r.	Release Rate (μg/day)	FA Released in 25 days (μg)
24 h	26.76 ± 0.44	674.10 ± 20.87
48 h	10.61 ± 0.51	273.81 ± 10.08

4. Conclusions

AMYLO-FA intravitreal insert maintained its shape without any important morphological change following hydration and swelling over 12 days. Moreover, in the in vitro study maintaining sink conditions the insert showed an anomalous non-Fickian release. In another experiment closer to the biological condition, FA release from AMYLO-FA turned out to be mainly influenced and driven by the receiving environment rather than controlled by the device. According to the results obtained, it could be interesting to check the in *vivo* performance of AMYLO-FA. In the meanwhile, this insert has undergone gamma-ray sterilization, showing no alteration.

In light of the results, the HME technique appeared suitable for developing long acting ocular insert to apply in the posterior segment of the eye.

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Evaluation of the Safety of Repeated Subthreshold Micropulse Yellow Laser Photocoagulation in Diabetic Macular Edema Treatment

Pedanova E.K.[1], Buryakov D.A.[2], Kachalina G.F.[3], Kryl L.A.[4]

[1] *MD, PhD, (Russia)*
[2] *MD, (Russia)*
[3] *MD, PhD, (Russia)*
[4] *MD, PhD, (Russia)*
e-mail: elenamntk@mail.ru

Abstract

Background. Subthreshold micropulse yellow laser photocoagulation (SMYLP) have demonstrated its efficacy in diabetic macular edema (DME) management. High selectivity and low power parameters of SMYLP result in significantly reduced retinal damage. One of the limitations of SMYLP is transient therapeutic effect, which requires repeated laser treatment. There are no available studies aimed to assess the safety of repetitive SMYLP.

Purpose. To evaluate the safety of subthreshold micropulse laser 577-nm photocoagulation after repeated laser treatment sessions.

Material and methods. 17 patients (31 eyes) with DME who underwent three sessions of SMYLP with 1 month interval were examined at the baseline, before each treatment session and at 3-month follow-up. Retinal structure changes and functional outcomes were assessed.

Results. At 3-month follow-up mean central retinal thickness decreased from 405.34±107.22 to 378.65±111.15 ($p<0.05$), best corrected visual acuity and central retinal sensitivity improved from 0.56±0.20 to 0.62±0.30 and from 14.53±4.20 dB to 16.13±2.50 dB respectively ($p<0.05$). Autofluorescence in short-wave and infrared wavelengths didn't reveal laser induced hyperfluorescence, which was considered as lack of pigment epithelium and choriocapillaris layers damage after treatment. Optical coherence tomography didn't show any laser induced changes of neurosensory retina at any follow-up visit in each case. Microperimetry data confirmed absence of central field scotomas at final follow-up.

Conclusions. Three repeated sessions of SMYLP treatment of DME, appears to be a safe therapy technique that is not accompanied with retinal and choriocapillaris damage and functional disturbances.

Keywords: Diabetic macular edema, subthreshold micropulse yellow laser photocoagulation, autofluorescence, microperimetry, optical coherent tomography.

Introduction

Diabetic retinopathy is observed nearly in all patients with 20 years diabetes mellitus duration, and diabetic macular edema occurs in one third of cases. It is a leading cause of severe vision loss and blindness in working population [1]. Laser photocoagulation has been a “gold standard” in it’s management for many years. It reduces fluid leakage from microaneurysms, reduces the oxygen demand in retina and stimulates regeneration and migration of photoreceptors and retinal pigment epithelial (RPE) cells [2]. But due to posttreatment atrophy of pigment epithelium appear scotomas in central vision filed, decrease of central sensitivity and impairment of color vision [3]. In novel technique of Micropulse laser treatment laser energy is delivered in “trains” of microsecond pulses, where periods of laser exposure alternate with periods of relaxation. So RPE does not heat, there is no collateral temperature spread and respectively no damage of neurosensory retina [4]. It demonstrates high selectivity to RPE cells and stimulates biological cell response, what explaines mechanism of it’s action [5,6]. Subthreshold micropulse laser photocoagulation has already shown its efficacy equal to conventional laser for clinically significant macular edema [7,8,9]. But one of the most prominent limitations of micropulse is a transient, short therapeutic effect, which requires more and more sessions to be performed. To the moment there are no studies aimed to assess the safety of these repetitive micropulse sessions.

Purpose

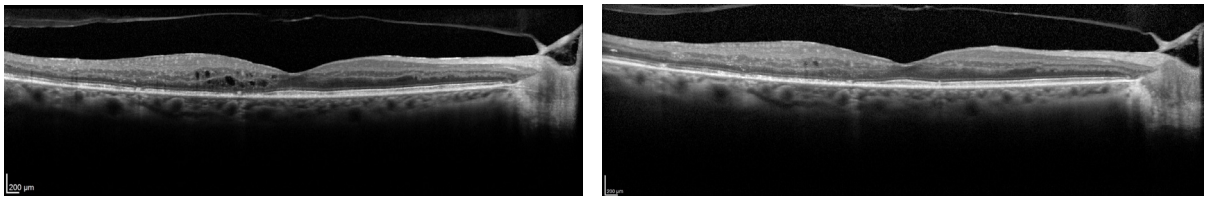
The purpose of this study is to evaluate the safety of subthreshold micropulse yellow laser photocoagulation after repeated laser treatment sessions in macular edema.

Material and methods

31 eyes with non-proliferative diabetic retinopathy and clinically significant macular edema were enrolled. They underwent three sessions of micropulse therapy with 1 month interval. They were examined at the baseline, before each session and at 3-month follow-up. For examination some standard and instrumental equipment were applied. Optical coherent tomography (OCT) and autofluorescence in both blue-wave and infra-red wavelengths were performed on Spectralis Multicolor OCT (Heidelberg, Germany). Microperimetry on MP-1 (Nidek, Italy). Laser treatment was performed on Supra 577 Y (Quantal medical, France) with yellow wavelength 577 nm and parameters listed above. Laser power was adjusted individually after testing. Micropulse duration – 0.1 ms, train duration – 100 ms, duty cycle - 5%, spot diameter 100 μm. Spots were applied on the area of edema, based on ophthalmoscopy and macular thickness map on OCT. They were applied with the distance not more than 100 microns between each of them.

Results

At 3-month follow-up mean central retinal thickness decreased from 405.34±107.22 to 378.65±111.15 ($p<0.05$), best corrected visual acuity and central retinal sensitivity improved from 0.56±0.20 to 0.62±0.30 and from 14.53±4.20 dB to 16.13±2.50 dB respectively ($p<0.05$). OCT showed reducing the amount and size of intraretinal cysts were achieved in majority of cases at final follow-up. Continuity of the inner segment – outer segment junction of the photoreceptor layer and inner limiting membrane remained stable after each of miropulse sessions in all cases, so there was not any laser induced changes of neurosensory retina (Fig. 1).

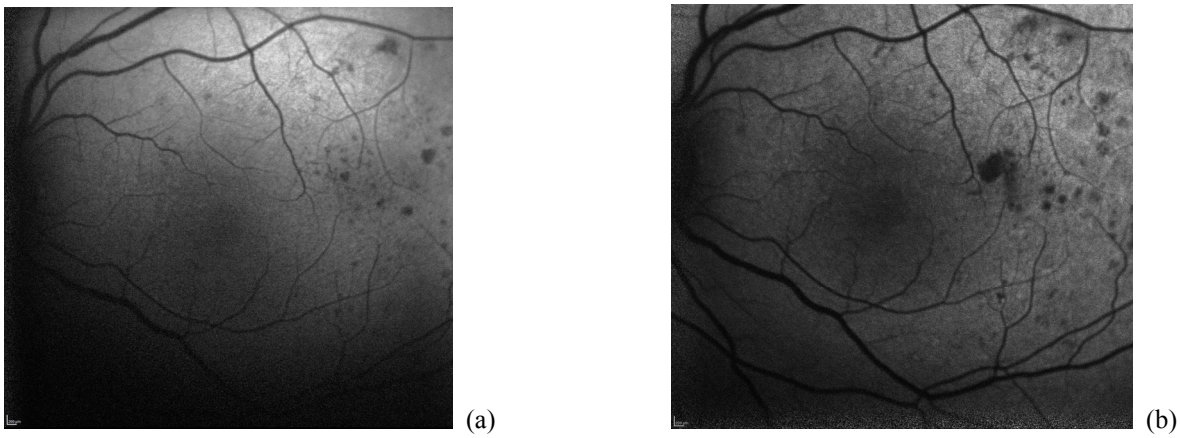


(a) (b)

Fig. 1. OCT scan before (a) and 3 months after SMYLP (b)

Reducing the amount and size of intraretinal cysts and stability of continuity of the inner segment–outer segment junction of the photoreceptor layer and inner limiting membrane after SMYLP

Autofluorescence in short-wave and infrared wavelengths didn't reveal laser induced hyperfluorescence, which was considered as lack of pigment epithelium and choriocapillaris layers damage after treatment in each case (Fig. 2).

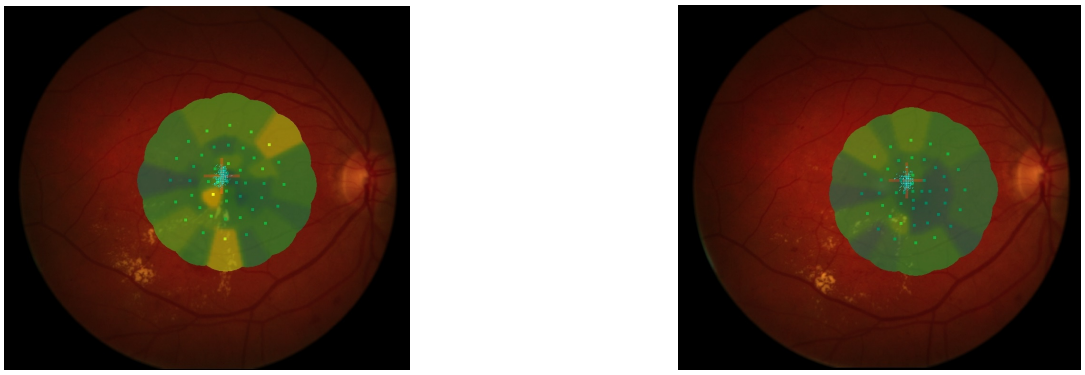


(a) (b)

Fig. 2. Short-wave autofluorescence before (a) and 3 months after SMYLP (b)

No laser induced hyperfluorescence after SMYLP

Microperimetry data confirmed absence of central field scotomas at final follow-up in all patients (Fig. 3).



(a) (b)

Fig.3. Microperimetry before (a) and 3 months after SMYLP (b)

Absence of central field scotomas after SMYLP

Conclusions

Subthreshold micropulse yellow laser photocoagulation have demonstrated its safety towards choroid-retina complex structure when low-level laser exposure parameters are applied. Three repeated sessions of SMYLP treatment of DME, appears to be a safe therapy technique that is not accompanied with retinal and choriocapillaris damage and functional disturbances.

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In Vitro and In Vivo Efficacy on Retinal Neo-Vascularization of an Innovative Broad-Range Anti-Angiogenic Synthetic Peptide (Uparant)

Rusciano D.[1], Bagnoli P.[2], Presta M.[3], Semeraro F.[4], Dal Monte M.[2], Cammalleri M.[2], Rezzola S.[3], Belleri M.[3], André H.[5], Lista L.[6], De Rosa M.[7], Pavone V.[6]

1. Sooft Italia SpA, Scientific Dept., Italy; 2. Biology Dept., University of Pisa, Italy; 3. Mol. and Translat. Medicine Dept., University of Brescia, Italy; 4. Ophthalmology Unit, University Hospital of Brescia, Italy; 5. Clinical Neurosciences Dept., Section of Ophthalmology and Vision, St. Erik Eye Hospital, Karolinska Institute, Stockholm, Sweden; 6. Department of Chemical Sciences, "Federico II" University, Naples, Italy; 7. Department of Experimental Medicine, SUN, Seconda Università di Napoli, Naples, Italy.
** Corresponding author: Sooft Italia SpA, Via Salvatore Quasimodo 136, 00144 Roma, Italy. Email: dario.rusciano@sooft.it*

Abstract

Pathologic blood neovessels growth in the macular region of the retina is responsible for several serious illnesses that can quickly lead to vision loss. Nowadays, some anti-angiogenic drugs for intravitreal use are present on the market, targeting the main angiogenic factor, VEGF. However, not all patients respond to anti-VEGF therapy (some 30% are classified as non-responders), and chronic suppression of VEGF (which appears to be also a neurotrophic factor) could exert some negative effects in the long run. We present here a new drug, a modified tetrapeptide, that works as an antagonist of the urokinase plasminogen activator cell receptor, that is able to inhibit the angiogenic response of endothelial cells to several different angiogenic and pro-inflammatory factors, since it blocks their motility and invasion independently from the trigger. We show evidence of its efficacy in widely known experimental model systems both *in vitro* and *in vivo*.

Key words: angiogenesis, retina, uparant, endothelial cells, VEGF.

Introduction

Angiogenesis – the process of new blood vessel formation – is regulated by angiogenic factors that bind to specific receptors on the surface of endothelial cells (EC), which respond by activating the mechanisms of growth, motility and invasion. Therefore, to block angiogenesis, targets can be tackled at different levels, either blocking the interaction between the angiogenic factors and their receptors, or the cell response to such stimulation, thus impairing motility and invasion of EC.

Presently, anti-angiogenic drugs mainly target the specific interaction between VEGF (the main – but not the only – angiogenic factor) and its receptors. We now present results with a new anti-angiogenic molecule that was designed to block the downstream response of EC to angiogenic stimulation blocking the motility and invasion triggered by angiogenic receptors activation. Previous evidence showed that the Ac-Arg-Glu-Arg-Phe-NH2 peptide (RERF), derived from the chemotactic sequence of the human urokinase receptor (uPAR88-92) [1], inhibits VEGF-induced angiogenesis *in vitro* and *in vivo* by preventing uPAR/Formyl-Peptide-Receptor (FPR) interaction [2]. To reduce its susceptibility to proteolytic degradation, a series of RERF analogues were generated containing C□-methyl-□-amino acids. Among them, UPARANT (Fig. 1) showed the capacity to inhibit VEGF-driven angiogenesis *in vitro* and *in vivo* more efficiently than RERF, being stable to heath sterilization and incubation in blood or plasma [3]. In addition, UPARANT competes with RERF for binding to the Formyl Peptide Receptor (FPR) and blocks VEGF-triggered signaling by preventing FPR and □v□3 integrin activities without affecting cell survival [3].

Here, we present data showing that UPARANT inhibits neovessel formation in four different *in vitro* and *ex vivo* model systems, and in two different *in vivo* murine model systems (OIR: Oxygen-Induced-Retinopathy in newborn mice, and CNV: Choroidal Neovascularization induced by laser photocoagulation in adult mice). Moreover, UPARANT is also able to prevent the sprouting of neovessels induced by the vitreous

body of diabetic retinopathic patients in the HUVEC spheroids and Embryonic Chorion Allantoidal Membrane (CAM) model systems.

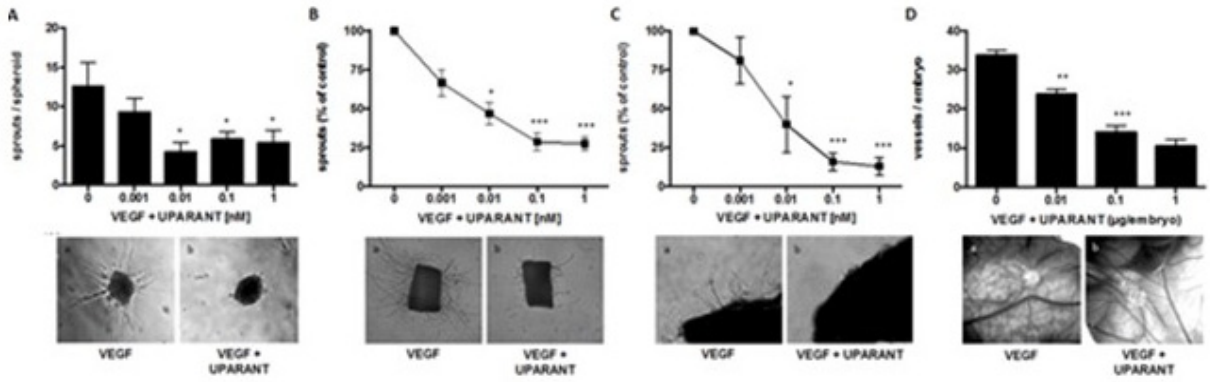

Figure 1. *A: HUVEC spheroids analyzed 24 hours after treatment with VEGF ± UPARANT (data on graph come from 50 determinations obtained in 3 different experiments). B: Murine aortic rings analyzed 5 days after treatment (data on graph come from 15 determinations obtained in 4 different experiments). C: murine retina fragments analyzed 7 days after treatment (data on graph come from 30 determinations in 4 different experiments). D: CAMs analyzed 3 days after treatment (data on graph come from 8 implants per each experimental point). Bottom insets: representative pictures of samples treated with VEGF alone (a) or together with the highest UPARANT dose tested. *p < 0.05, **p < 0.01 and ***p < 0.001 versus untreated (ANOVA).*

1.1 UPARANT inhibits angiogenesis *in vitro* and e*x vivo*.

The anti-angiogenic activity of UPARANT has been initially challenged against VEGF stimulation in a series of different simple model systems.

HUVEC (Human Vascular Endothelial Cells) spheroids (Fig. 1A); murine aortic rings (Fig. 1B); murine retina fragments (Fig. 1C) were embedded in fibrin gel. Incubation with VEGF (30 ng/ml) induced vascular sprouting, that was inhibited by increasing concentrations (from 1 pM to 1 nM) of UPARANT. Vascular sprouts invading into the fibrin gel were counted under a stereomicroscope and the quantitative analysis (mean ± SEM) reported in the graphs.

Embryonic CAMs (Fig. 1D) were implanted at day 11 with alginate beads containing 100 ng/pellet of VEGF, and increasing amounts of UPARANT (from 0.01 to 1 µg). After 3 days newly formed blood vessels converging versus the implant were counted under a stereomicroscope, and the quantitative analysis (mean ± SEM) reported in the graph.

UPARANT inhibited in a dose-dependent fashion the vascular sprouting induced by VEGF in each model system [4].

2.1 Intravitreal UPARANT inhibits retinal neovascularization in the OIR model of retinopathy

Oxygen-induced retinopathy (OIR) is a mouse model system that mimics the retinopathy of the premature (ROP), in which the shift between high O_2 tension in the incubator and normal environmental O_2 tension may result in abnormal vascularization of the still developing retina, causing serious problems of vision.

In the OIR model system, newborn mouse puppets are placed at high O2 tension at day 7 after birth (P7) for 5 days, until P12. From P12 until P17 they are returned to normal O2 tension (room air). Different doses of UPARANT-succinate in 1 µl are injected intravitreally at P12 and P15. Retinas are analyzed at P17.

Results shown in Fig. 2 indicate that treatment with UPARANT decreases the amount of neovascular tufts and at the higher dose of 6 µg (Fig. 2C) also appears to decrease the extension of the central avascular area. VEGF expression and the activation of its cognate receptor VEGFR-2 were also decreased by UPARANT administration [4].

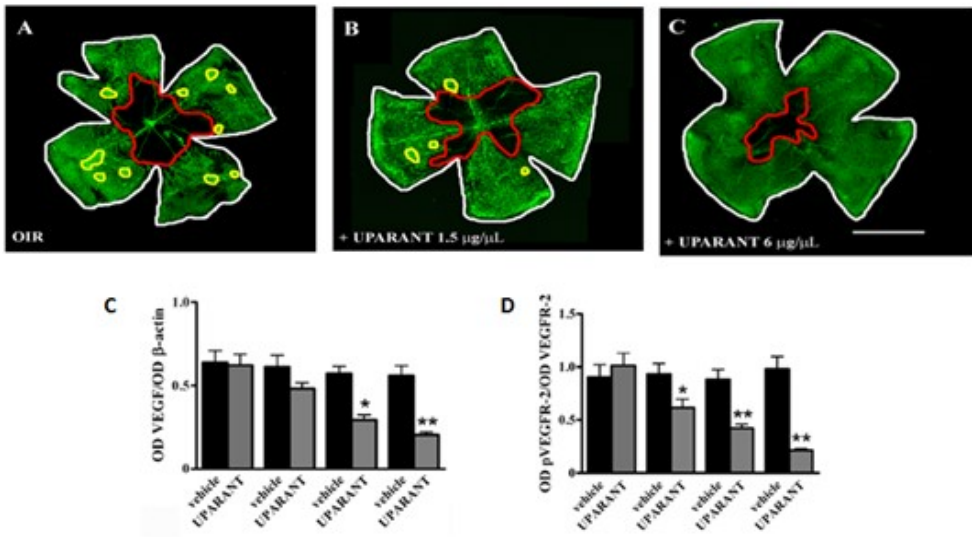

***Figure 2**: UPARANT reduces neovascular tufts. Flat-mounted retinas immunolabeled with a rat monoclonal antibody directed to the EC antigen CD31. Mice exposed to 75% ± 2% oxygen from PD7 to PD12 were intravitreally injected with vehicle (phosphate buffer) (A) or with UPARANT at 1.5 µg/µl (B), or 6 µg/µl (C) at PD12 and PD15. Retinas were explanted at PD17. Hyperoxia followed by normoxia for 5 days produced the central loss of blood vessels and the formation of neovascular tufts. Scale bar, 1.5 mm. Normalized western blot quantitative analysis of VEGF and VEGFR2 are shown in C and D. *p < 0.01 and **p < 0.001 versus vehicle-treated (ANOVA).*

2.2 UPARANT reduces BRB breakdown in the OIR model.

Newly formed blood vessels are notoriously leaky, since they lack the structural organization of mature vessels. Therefore, if a dye is injected in the blood stream, it tends to exudate from the neovessels' area, indicating in the case of the OIR model a discontinuity of the blood retinal barrier (BRB) that is formed by vascular EC. In this areas there is enhanced leakage of albumin, and the tight junction protein occludin is under-expressed. Fig. 3 shows that UPARANT treatment strongly decreases leakage from retina blood vessels, reducing the levels of tissue albumin and increasing the expression of occludin [4].

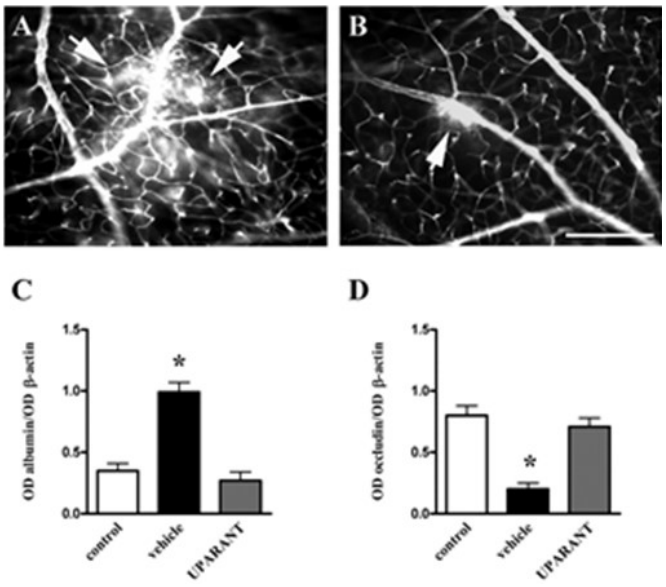

***Figure 3:** Mice were exposed to 75% ± 2% oxygen from PD7 to PD12. Mice were intravitreally injected with vehicle (phosphate buffer) or 1.5 µg/µl UPARANT at PD12 and PD15. Retinas were explanted at PD17. (A and B) Blood-retinal vascular leakage was qualitatively evaluated with the Evan's blue method in vehicle- and in UPARANT-treated retinas (A and B, respectively). Arrows: vascular leakage. Scale bar, 200 µm. (C and D) Levels of albumin (C) and occludin (D) in control, vehicle- or UPARANT-treated retinas, as evaluated by Western blot with β-actin as the loading control (*p < 0.01 vs. vehicle-treated; ANOVA). Each histogram represents the mean ± SEM of data from four independent samples.*

2.3 UPARANT restores ERG responses in the OIR mouse model of retinopathy.

Biochemical and histo-morphological changes by themselves do not necessarily imply a functional improvement, which is the goal of any therapy. Therefore, we examined the ERG response of retinas of untreated and UPARANT treated OIR animals (Fig. 4), finding that the morphological improvement correlated with a significant functional improvement [4].

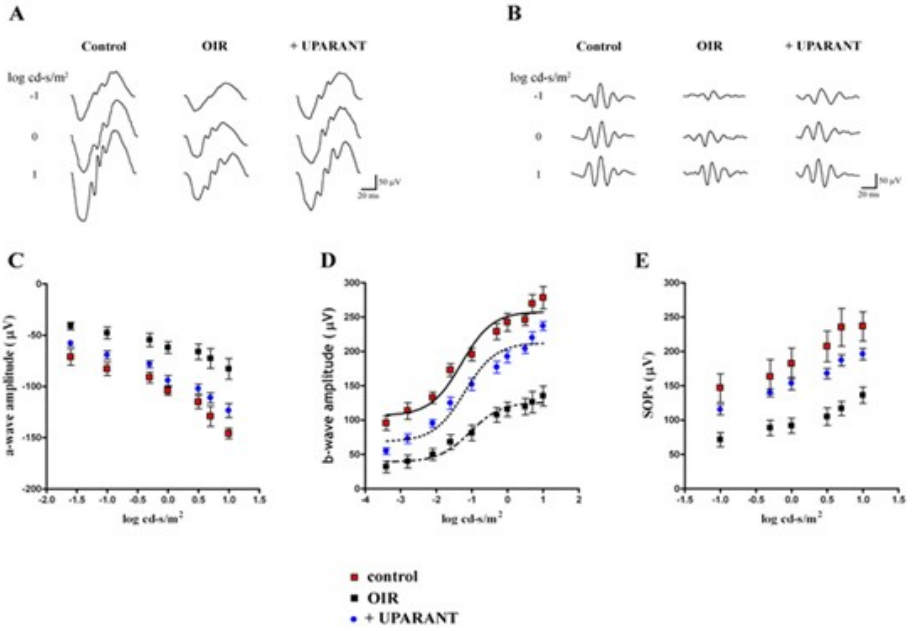

Figure 4: *(A) Representative ERG waveforms at light intensities ranging from -1 to 1 log cd-s/m2 in control, OIR and UPARANT-treated OIR mice. (B) Representative Oscillatory Potentials (OPs) extracted from ERG responses at stimulus intensities ranging from -1 to 1 log cd-s/m2 in in control, OIR and UPARANT-treated OIR mice. (C, D) a- and b-wave amplitudes (means ± SEM) in control (red squares), OIR (black squares) and UPARANT-treated OIR (blue circles) mice plotted as a function of the stimulus intensity. (E) SOPs (means ± SEM) in control (red squares), OIR (black squares) and UPARANT-treated OIR (blue circles) mice plotted as a function of the stimulus intensity.*

3.1 Intravitreal UPARANT inhibits choroidal neovascularization induced by laser photocoagulation in adult mice.

The inner retina is nourished by blood vessels originating from the central artery, that are the ones involved in OIR and diabetic retinopathy. The outer retina (containing the photoreceptor cells) is nourished by choroidal vessels, which may become deranged in case of age-related macular degeneration (AMD). A mouse model of AMD can be obtained by laser photocoagulation of some choroidal vessels, thus inducing a local ischemia and a tissue reaction that involves angiogenic and inflammatory factors. In this experimental protocol, 27 mice were laser-induced on the right eye leaving the left as a contralateral control. Animals were then randomly distributed into 3 groups: PBS (0g/L Uparant); high dose (12g/L); low dose (4g/L). At days 4, 8 and 12 both eyes were intravitreally injected according to the animals' groups (1uL/eye). On day 15 all animals were sacrificed, both eyes enucleated and microdissected from surrounding tissues, and the eyeballs fixed overnight at 4°C in 4% buffered formaldehyde solution.

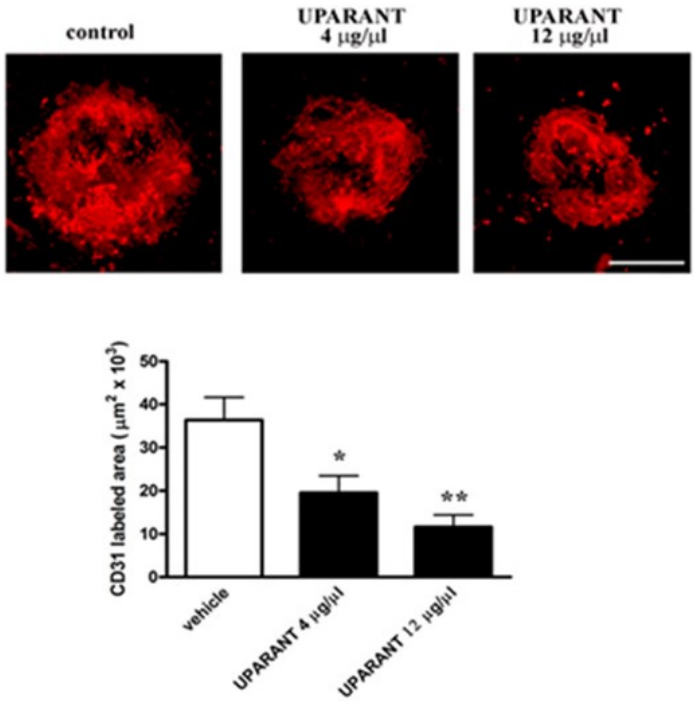

***Figure 5**: Immunofluorescence staining with CD31 of choroidal flat mounts after laser photocoagulation. Calibration bar: 150 microns. *p < 0.05 and **p < 0.01 versus vehicle-treated (ANOVA)..*

In this model system, UPARANT intravitreal treatment has shown that the neovascular invasion from choroid vessels can be efficiently counteracted by each dose, in a dose-dependent fashion (Fig. 5).

4.1 UPARANT inhibits the angiogenic activity of vitreous fluid from proliferative diabetic retinopathy patients.

A more direct evidence that UPARANT has an anti-angiogenic activity that is not limited to VEGF alone comes from a series of experiments using the vitreous humor of patients with proliferative diabetic retinopathy (PDR) as inducer of angiogenesis. It is known that in such vitreous bodies several different growth, angiogenic and inflammatory factors are present. However, if UPARANT inhibits the downstream response to any of these triggers, it should conserve its anti-angiogenic potency. Fig. 6 shows indeed that the very same concentrations of UPARANT inhibiting the VEGF response, also work in blocking the vitreous body response in two different model systems: HUVEC spheroids (Fig. 6A) and CAM (Fig. 6B) [4].

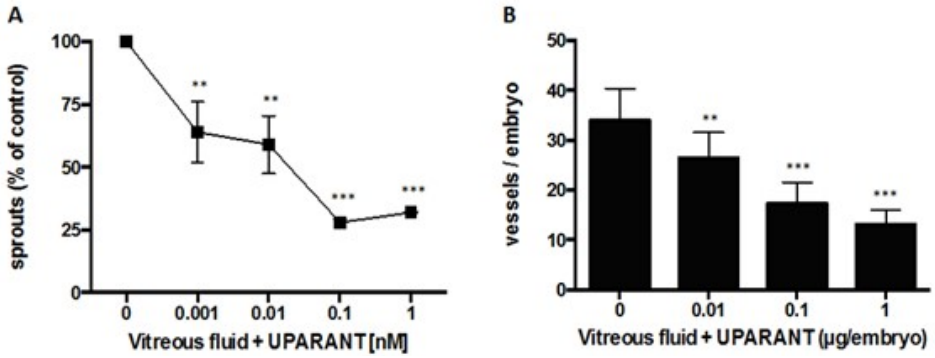

Figure 6. *A: HUVEC spheroids embedded in fibrin gel were incubated in the presence of PDR vitreous fluid (1.5 mg/ml) and increasing concentrations of UPARANT. After 24 hours vessel sprouts were counted under a stereomicroscope. Data are the mean ± SEM of 30 determinations in two independent experiments. B: CAMs were implanted at day 11 with alginate beads containing 10 mg/pellet of PDR vitreous fluid and increasing amounts of UPARANT. After 3 days, newly formed blood vessels converging versus the implant were counted under a stereomicroscope. Data are the mean ± SEM of 8 implants per each experimental point. . $^{**}p < 0.01$ and $^{***}p < 0.001$ versus untreated (ANOVA).*

Conclusions

UPARANT inhibits VEGF-mediated angiogenesis *in vitro*, *ex vivo* and *in vivo* in two different mouse model systems. Interestingly, UPARANT inhibits the angiogenic activity of vitreous fluid from PDR patients. Together, these data indicate that UPARANT may represent a promising new concept therapeutic agent for the treatment of ocular angiogenesis-dependent diseases, including ROP, AMD and PDR.

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Effects of Trehalose / Hyaluronate Solution on Tear Film Osmolarity and Ocular Surface Parameters in Glaucoma Patients on Chronic Topical Therapy with BAK-preserved Prostaglandin Eye Drops

Wasyluk J.[1], Dalz M.[1], Mędrzycka J.[1], Prost M.E.[1]

[1] *Department of Ophthalmology, Military Institute of Aviation Medicine (Warsaw, Poland)*
e-mail: jarowasyluk@wiml.waw.pl

Abstract

This paper presents the prospective open-label clinical study on influence of lubricating eye drops (artificial tears), containing combined solution of hyaluronic acid and trehalose (as active ingredients) on the ocular surface in primary open angle glaucoma patients on persistent topical therapy. All of subjects were on chronic topical antigalucoma treatment with one of two different prostaglandin analogues solutions (latanoprost or travoptost), both preserved with benzalkonium chloride (BAK). We included 22 POAG patients (44 eyes, 5 M, 17 F, mean age of 58) in this prospective, open-label clinical study. Tear film osmolarity, tear film break-up time (TBUT) and ocular hyperemia (Mc Monnies grading) were evaluated at baseline and after one month of follow up in both eyes. Significant difference in mean osmolarity was found: -4.2 ± 11.2 mOsm (p=0.002). Hyperemia measured by McMonnies grading parameters improved after one month in 26 eyes (59,09%) and significant increase of TBUT was noticed in 16 eyes (36.4%). We concluded that adding trehalose/hyaluronate solution concomitantly to the persistent glaucoma therapy with BAK-preserved prostaglandins has beneficial influence on tear film osmolarity and some ocular surface parameters in glaucoma patients and thus may have protective effect against symptoms of ocular surface disease (OSD).

Keywords: glaucoma therapy, tear film osmolarity, trehalose, hyaluronate, ocular surface disease

Introduction

1.1 Background

1.1.2 Ocular surface disease in glaucoma patients

Ocular surface disease (OSD) is the common pathological condition found among majority of glaucoma patients on long time persistent topical therapy with different types of eye drops, e.g. prostaglandins, especially on these containing benzalkonium chloride (BAK) as preservative. The disease may appear both due to pro-inflammatory pharmacological properties of PGF2α analogue itself and to BAK topical cellular toxicity as well. OSD often causes number of symptoms causing severe patient's discomfort: hyperemia, swelling, pain, foreign body sensation, dry eye, corneal and conjunctival epitheliopathy etc. and has the negative impact on visual acuity and contrast sensitivity. These may significantly and negatively influence patients everyday activities and quality of life and also compromise their adherence in long-term antiglaucoma therapy [1-6].

The major and one of most important measurable symptoms found in ocular surface disease is increased tear film osmolarity [7], which causes its destabilization and dysfunction, and in consequence increases of ocular surface disease severity. This osmolarity can be precisely assessed by specially developed diagnostic instrument – tear film osmometer, by collecting the samples of tear fluid from the lower lid margin. Fig 1.

Fig. 1. Tear film osmometer (TearLab Analyser®, TearLab Corp, USA)

1.1.3 Trehalose and hyaluronic acid

Trehalose, an alpha-linked disaccharide, can be naturally synthesized by bacteria, fungi, plants and invertebrate animals. It is implicated in anhydrobiosis, the ability of plants and animals to withstand prolonged periods of desiccation. It has high water retention capabilities and so it is commonly utilized as a component of transport solution in transplantology for example. One of the pharmacokinetic theories says that during prolonged dehydration, water may be temporary replaced by trehalose in different tissues. Its hydrating properties have also been found in dry eye syndrome caused by different ocular conditions, as topical glaucoma therapy for example [8-12], .

Hyaluronate (or hyaluronic acid), a nonsulfated glycosaminoglycan, is present in a connective tissue, body fluids (e.g. vitreous body) and epithelia and it is also constituting the plasma membranes in humans. This well-known chemical compound is widely used in dermatology, cosmetology and ocular surgery as viscoelastic (cataract and glaucoma procedures, corneal transplantation). Hyaluronate's playing important role in re-epithelization, wound healing and tissue repair, taking part in anti-inflammatory reactions, hydration and organizing the tissue matrix. It is common compound of many artificial tears eye drops, having very well established hydratating and protective effect on tear film stability in glaucoma patients [13].

Aim of the study

The purpose of this prospective open-label clinical study was to evaluate the effects of adding trehalose / hyaluronate solution (artificial tears drops ThealozDuo®, Thea) concomitantly to the topical monotherapy with BAK-preserved prostaglandins eye drops (latanoprost or travoprost) on tear film osmolarity and some ocular surface parameters in patients with primary open angle glaucoma (POAG).

Material and methods

The study protocol was accepted by local Ethical Committee (Military Institute of Aviation Medicine) and informed consent was obtained from all subjects before enrollment. 22 primary open angle glaucoma patients (44 eyes, 5 M, 17 F, mean age of 58) were included and all of them completed this clinical study.

Main inclusion criteria were: diagnosed primary open angle glaucoma on monotherapy with BAK preserved prostaglandin drops (travoprost or latanoprost) for at least one year and existence of OSD symptoms. Mean exclusion criteria: coexistence of ocular disorders other than glaucoma, any ocular surgery during last six months, artificial tears usage.

Subjects received artificial tear eye drops containing trehalose / hyaluronate solution (Thealoz Duo, Thea Laboratoires) 3 times a day as the addition to their chronic BAK-preserved prostaglandin monotherapy (latanoprost or travoprost). Tear film osmolarity was measured by TearLab Analyser®, TearLab Corp (results given in mOsm.) after taking samples of tear fluid by sterile probes from the lower conjunctival sac. Corneal tear film break-up time (TBUT) was measured (in sec.) in biomicroscope after fluorescein staining. Ocular hyperemia was assessed according to Mc Monnies grading. All above ocular surface parameters measurements were performed at baseline and after one month of follow up in both eyes at 9 am before first instillations.

Results

Tear film mean osmolarity within the studied group of glaucoma patients measured at baseline was 295.3 ± 12.3 mOsm. After one month by the end of the study, mean tear film osmolarity in this group was: 291.1 ± 9.6. Mean difference noticed was statistically significant: -4.2 ± 11.2 mOsm (p=0.002). Fig. 2.

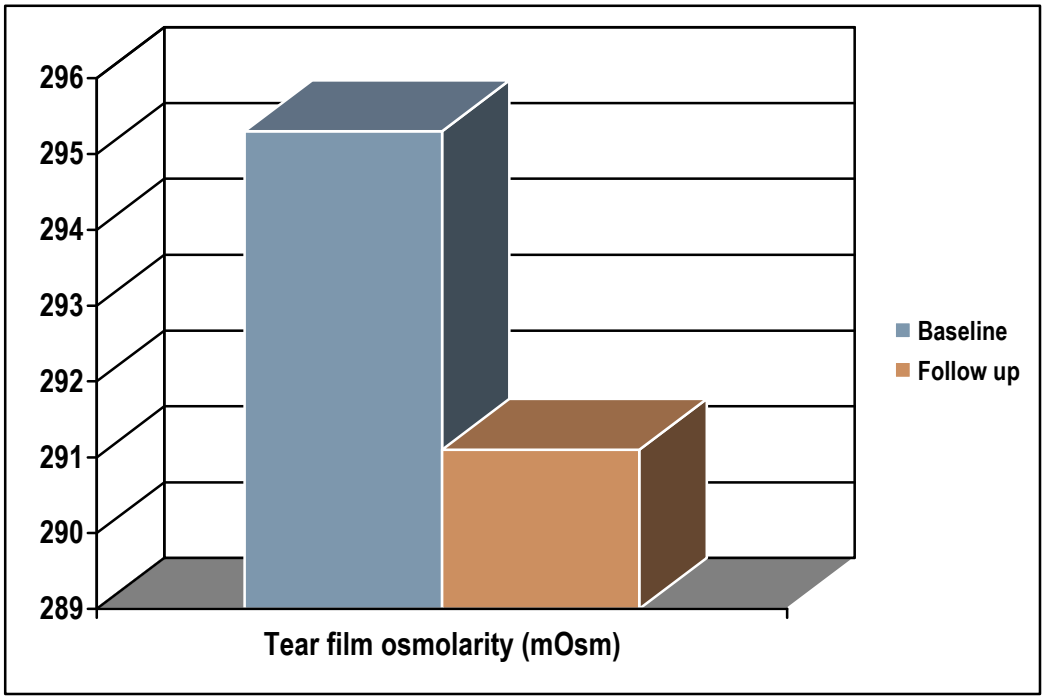

Fig. 2. Baseline and follow-up values of mean tear film osmolarity among the study group

Hyperemia, assessed in all subjects according to McMonnies grading improved after one month in 26 eyes (59,09%) and was statistically significant.

Tear break-up time (TBUT) increase at follow-up was noticed in 16 (36.4%) eyes and was statistically significant.

Conclusions

Concomitantly adding of trehalose/hyaluronate solution to the persistent glaucoma therapy with BAK-preserved prostaglandins in glaucoma patients has got statistically significant and beneficial influence on tear film osmolarity and other ocular surface parameters (tear break-up time, hyperemia grade) [14].

The study showed diminishing of ocular surface disease symptoms after on month probably due to protective and hydrating effects of treahalose and hyaluronate and increased stabilization of the tear film.

It may probably lead to better long term tolerance of topical glaucoma medications and improve patients adherence to antiglaucoma therapy and in consequence increase its efficacy [15].

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