Comparing the Effects of THERA°PEARL Eye Mask (Bausch & Lomb Inc., New York, USA) With the Use of Blephasteam® (Spectrum Thea Pharmaceuticals LTD, Macclesfield, UK).

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Project group

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ID: 2014/1983

Date of document: 11th of October, 2017

Background

Dry Eye Disease and Meibomian Gland Dysfunction

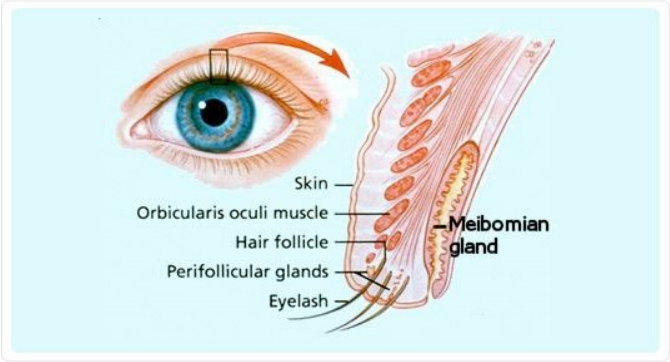
Dry eye disease (DED) affects up to 20% of the population in North America.[1] Clinically there is a plethora of irritation symptoms associated with DED, which includes ocular burning, foreign body sensation, soreness, stinging, scratchiness, photophobia and blurred vision. In 1995, the National Eye Institute workshops (in USA), defined dry eye as "a disorder of the tear film due to tear deficiency or excessive evaporation that causes damage to the interpalpebral ocular surface and is associated with symptoms of discomfort".[2] Recent studies point to the importance of the meibomian glands of the eyelids to avoid DED. Meibomian glands are located in the upper and lower eyelids and produce an oily substance that helps stop the water in the tears from evaporating, thus helping to prevent dry eyes. Meibomian gland dysfunction (MGD) is defined as "a chronic, diffuse abnormality of the meibomian glands, commonly characterized by terminal duct obstruction and/or qualitative/quantitative changes in the glandular secretion. It may result in changes of the tear film, symptoms of eye irritation, clinically apparent inflammation, and ocular surface disease."[3] The term "posterior blepharitis" is often used synonymously with MGD, however, in its early stages, MGD is not always associated with inflammation of the eyelid. The symptoms of MGD are a consequence of a reduced quantity and/or quality of meibum that is supplied to the ocular surface. The terminal ends of the ducts of the meibomian glands can become blocked with keratinized cells. Importantly, such obstruction of the duct can lead to ductal dilatation and loss of secretory cells in the acini of the gland.[4] Blocked terminal ducts reduce the quantity of meibum produced by the gland, and are also, most likely, responsible for affecting its lipid composition deleteriously. In MGD, there is a clear tendency for more branched chain fatty acids and cholesterol in the meibum, which result in a more waxy and viscous

character.

Fig 1. Meibomian glands are the oil- producing glands located in both the upper and lower eyelids. The glands normally slowly release oil into the tear film. This oil helps to stop the water in the tears from evaporating, thus helping to prevent dry eyes.

(picture: http://contactlens.org.nz)

Systematic studies of the epidemiology of MGD have been hampered by difficulties in definition and lack of standardized tools for clinical assessment. Moreover, the specialized tests required to identify MGD in an early stage are not appropriate for use in population- based epidemiological studies.[5] However, some trends are clear, i.e., Asian populations appear to have a much higher prevalence of MGD than do Caucasians.[5] A number of factors have been identified that coexist with MGD. Although causal links have not been proven, plausible mechanisms exist for connecting them with the pathophysiology of MGD, i.e., anterior blepharitis, contact lens use, Demodex mite infestation, and DED.[5] In addition, hormonal conditions, including menopause and androgen deficiency, rosacea, psoriasis, atopy, and hypertension may contribute to the illness.[5] In Tørreøyneklinikken (TØK), we have access to state-of-the-art equipment to diagnose various subgroups of DED. This gives us an opportunity to assess the relative importance of MGD in our cohort of DED patients. Our studies so far reveal that about 80 % of our patients have DED caused by MGD. The large number of patients suffering from MGD has encouraged us to compare various treatment options to optimize future treatment of the disease.



The Significance of Studying Dry Dye Disease

The prevalence of DED in the Norwegian population is unknown, but based on epidemiological studies abroad, probably about 1 million Norwegians suffer from DED in one form or another. Age is an important risk factor for DED. Therefore, the prevalence of DED is expected to increase in the years to come as population ageing is unprecedented, without parallel in human history.[6] Thus, research on DED is important for a large proportion of our population. Sadly, it has been an underprioritzed field of research in the Nordic Region in sharp contrast to many other

countries. For example, at the Department of Ophthalmology, Harvard Medical School, DED is a highly prioritized field with several research groups working on improved treatment modalities. Research on DED holds the prospect to significantly improve quality of life for hundreds of thousand people in Norway. In addition, research on DED is critical to maximize the clinical outcome of some of the most severe forms of visual impairment that necessitate ocular surface stem cell treatment and/or corneal transplantations. Without an adequate tear film, these transplants will inevitably fail. Therefore, research on DED has the potential to help patients with the most severe visual impairments, either directly (in the event of keratinizing DED) or indirectly by enhancing the clinical results of other surgical procedures. In the light of DED being both a widespread disease and having huge implications for maximizing outcome of several diseases and treatments in ophthalmology, several members of our research group has taken measures to contribute to lessening DED in the Norwegian population. One example is the establishment of the Norwegian Dry Eye Clinic, which is the first specialized clinic for DED in Scandinavia.

Treatment Options for Meibomian Gland Dysfunction

There are several treatment options for MGD, but yet no consensus on the best approach. Lid scrubs using for example mild shampoo, eyelid massage and warm compresses of the eyelids, belong to the basic therapy. In addition, a large number of medications have been suggested over the past years for more severe forms of MGD. Comparative studies between possible treatment strategies are warranted to be able to conclude on the best available options.

Basic Therapy

Good eyelid hygiene, warming and massage are considered an effective mainstream therapy for MGD and blepharitis, in spite of the lack of standardization of the technique as well as the uncertainty about patient compliance. Studies comparing different methods of mainstream 4 therapy, such as eyelid warming, would allow evidence-based recommendations. The diversity of recommendations for such basic treatment is exemplified by the advice given in two leading textbooks of ophthalmology:

- “Warm wet face cloth for 5 minutes once or twice a day, and massage upper and lower lid (Moorfields Manual)”
- “Warm compresses for 15 minutes four times per day, and clean with wet cotton bud and mild (baby) shampoo (Will´s Eye Manual)”.

Recent advances

Recently, there have been two alternatives to the conventional, but somewhat unstandardized

basic treatment. Blephasteam® (Thea, France), which is eyelid-warming goggles (patented medical device) is one such option. The goggles are designed to deliver standardized conditions of moist heat therapy for 10 minutes in order to melt meibomian secretion. This dual action (moist and heat) unblocks the meibomian glands. The principal effect of this device is to improve the thickness and quality of the lipid layer of the tear film, thus enhancing tear function. THERA°PEARL Eye Mask (Bausch & Lomb Inc., New York, USA), is another mask with similar functions. It is heated in a microwave and placed on the eyelids to meld the meibomian secretion.

Additional Therapy (a Selection)

Antibiotics. A number of topical and systemic antibiotics with effect against lid-related bacteria are available. Solid evidence, however, based on randomized controlled clinical trials is lacking. This makes guidance of antimicrobial management of MGD difficult. Tetracyclines are widely used in several ocular surface diseases, including ocular rosacea, corneal angiogenesis blepharitis, recurrent erosions, and DED. These components may be helpful for blocking the vicious-circle characteristics of dry eye disease and severe MGD through 1) anti- inflammatory and anti-apoptotic properties and 2) by counteracting the free fatty acid accumulation known to be responsible for development of MGD. Despite several studies on oral tetracyclines in the treatment of MGD, additional comparative studies against other therapies are needed.[7] Interestingly, one such recent study demonstrated better effects of using a macrolide (azithromycin) compared to applying a tetracycline (doxycycline) for the treatment of MGD.[8]

Immunosuppressive medication. Most studies involving topical cyclosporine are small in sample size.[9-11] Interpretation of some of the studies is somewhat challenging due to the influence of a reduced Schirmer score or the presence of ocular rosacea. A recent, randomized double-masked study on the use of topical cyclosporine confirms its potential role in the treatment of MGD.[12] However, there are no comparative studies of a combined approach using the presumptive best choice of antibiotics and cyclosporine compared with antibiotics alone.

Ocular protection: In addition to providing the ocular surface with moist, ocular protection in the forms of drops has proved advantageous. Sodium hyaluronate and disaccharides have been included in eye drops with the aim of increasing ocular protection. Sodium hyaluronate containing solutions have been demonstrated to result in improvement in dry eye in patients compared to other lubricant solutions in several studies.[13,14] Naturally occurring disaccharides, which are a major component in Thealoz, are used with the purpose of protecting the cells of the cornea and conjunctiva as well as to give resistance to dryness. Thealoz has proved successful in clinical studies.[15,16]

Study Description

Aims: To compare the effects of THERA°PEARL Eye Mask with the use of Blephasteam®.

Study Design

All the studies will be conducted based on written consent of the patients. Moreover, they will be performed in accordance with the tenets of the Helsinki Declaration and after approval from the Regional Ethics Committee as well as. If the patients encounter any problems during the study, they will be examined, irrespective of the planned follow-ups. The patients are free to quit the study at any time for any reason.

To answer our aim, we have, in brief, chosen the following study design:

1) THERA°PEARL Eye Mask versus treatment with Blephasteam®. In both groups, 60 patients with MGD are enrolled. We aim to make the two groups comparable as to the severity of the disease, age, and gender. The patients will be examined before the treatment and after 3 months, and 6 months. Treatment for both groups will be according to the manufacturer. All the analyses mentioned in the section *analyses* are planned to be included at every examination. Inclusion criteria: Meibomian gland dysfunction and candidate for eyelid heat therapy. Diagnosis of at least mild severity will be based upon recommendation by the diagnostic subcommittee of the International Workshop on Meibomian Gland Dysfunction, as seen in Table 1.

Ocular Surface Disease Index (OSDI)	>12
Quality or expressibility score	≤20 years old: >1 >20 years old: ≥1
Non-invasive tear film break-up time (NITBUT)	<10 s in at least one eye
Schirmer-1 test	>5 mm after 5 min in at least one eye

Exclusion criteria: Glaucoma, ocular allergy, autoimmune disease, contact lens-wear during study, current punctal plugging, pregnant/lactating, candidate for topical anti-inflammatory therapy, or cicatricial MGD.

Analyses

McMonnies Dry Eye Questionnaire. The McMonnies Dry Eye Questionnaire has been considered to be the "gold standard" for examining dry eye symptoms. The validity of the theory behind the questions and the questionnaire's usefulness in clinical settings has been demonstrated in numerous research articles.[17] In addition, Ocular Surface Disease Index [OSDI] will be applied.[18]

Slit lamp examination. The cornea will be evaluated for the presence of active inflammation or structural change. The iris and anterior chamber will be assessed for sign of inflammation, whereas the eyelids will be evaluated for sign of Meibomian gland dysfunction with grading of the quality, expressibility, and volume of gland secretion, according to the Bron/Foulks scoring system.[19]

Tear film quantity and quality. RTVue (Optovue Inc, Fremont, CA) (http://www.optovue.com/wp-content/uploads/RTVue-Single-Sheet-Brochure_RevF.pdf) is the most comprehensive High Definition Ocular Coherence Tomography (OCT) system available today.[20] The Cornea Anterior Module unit for the RTVue system provides a rapid, non-contact, non-invasive method for producing high-quality images (5 micrometer resolution) that enable measurements of the tear meniscus. The overall tear volume can quite well be predicted by measuring the tear meniscus size[21,22] as the meniscus contains 75% to 90% of the tear volume[23]. Its size is related to the tear secretion rate[23] and the tear stability.[24] In addition, the Schirmer test, a traditional tear production measurement, will be applied with and without anesthesia. TBUT (fluoresceinn break-up time) will be used to test the quality of the tear film. TBUT will be measured after instilling 5 uL of a 2% sodium fluorescein solution. The average of three consecutive break-up times manually determined with a stopwatch will be calculated. Corneal staining will be evaluated under cobalt blue illumination 2.5 to 3.0 minutes after fluorescein instillation. The staining amplitude will be measured according to the National Eye Institute (NEI)/Industry Workshop scale.[2] Likewise, conjunctival staining will be investigated 2.5 to 3.0 minutes after 10 uL of a 1% sodium lissamine green dye is instilled. Conjunctival staining amplitude will be assessed in accordance with the NEI/Industry Workshop scale.[2]

Tear film osmolarity. TearLab TM Osmolarity system (TearLab, San Diego, CA) will be used. It requires a very small volume of tears (50nL), which will be collected at the inferior lateral meniscus.

Luminex. A multiplex Luminex® bead-based assay will be used to analyze ten cytokines (interleukin 1α [IL-1α], IL-1β, IL-1ra, IL-4, IL-6, IL-8, IL-10, IL-13, monocyte chemotactic protein-1 [MCP-1], and tumor necrosis factor-α [TNF-α]) and five metalloproteinases (MMPs) (MMP-1, MMP-2, MMP-7, MMP-9, and MMP-10). The Luminex bead-based assay is based on the principle of flow cytometry. The system allows us to simultaneously measure numerous analytes in a single microplate well, using very small sample volumes[25] while for many cytokines achieving excellent correlations to individual Enzyme-linked immunosorbent

assay (ELISA).[26] Recently, Luminex has been used to measure the cytokine content in tear

samples collected with capillary tubes[27,28] or Schirmer strips.[29] The analyses will be performed at the Department of Medical Biochemistry, Oslo University Norway. The content of cytokines and MMPs will guide us in the evaluation of the effectiveness the treatment. Moreover, it will shed light on the pathological mechanisms. As the important role of inflammation in DED has gained considerable scientific support recent years, a Luminex based approach seems pertinent in a clinical setting.

Confocal microscopy. Heidelberg Retina Tomograph with Rostock corneal module (http://www.heidelbergengineering.com/products/hrt-glaucoma-module/cornea-module/) is considered a state-of-the art diagnostic tool to evaluate the cornea at high magnification. In the present proposal, the density of presumed inflammatory cells in the central and peripheral cornea, subbasal nerve fibers, and epithelial wing cells will be investigated. Two independent investigators will evaluate the confocal pictures, and inter-observer variability will be measured to further assess the robustness of the method.

Keratograph 5M. Keratograph 5M (Oculus, Germany) technology will be used to enable meibography, which is a novel *in vivo* technique that provides photographic documentation of the meibomian gland, including the ducts and acini, under specialized illumination. In vivo confocal microscopy has the advantage of very high resolution, but at the expense of the overview. Both detailed analyses of selected glands and an overview of the general health of all the glands in the eyelids are important to be able to decide upon the best treatment strategies.

Integrative approach. The Dry Eye WorkShop (DEWS) severity scale[30] will be used as it integrates the values derived from the following analyses in one singel score: tear osmolarity, Schirmer tests, TBUT, corneal staining, meibomian score, conjunctival staining, and OSDI.

Data Management and Statistical Analysis Plan

All data will be stored in a special electronic medical record system built for management of dry eye disease. The extraction of the patient data from the system database will be carried out according to guidelines by the Regional Ethical Committee and the Norwegian Data Protection Agency. The dataset will be de-identified prior to any statistical analyses.

All data will be checked for normality to determine the appropriate parametric or non-parametric test. To test for differences among groups, relevant Chi square test, one-way analysis of variance (ANOVA) and Kruskal–Wallis equality- of-populations rank test will be used. Where there is difference among groups, relevant Student's t test or Wilcoxon rank sum test will be performed. Chi square test will be used to evaluate the differences in the primary and secondary outcomes between groups. Logistic regression will be used to adjust for baseline differences. Statistically significant difference is based on 0.05 level of significance. All analyses will be performed with SPSS software, version 24 (IBM Corp., NY, USA).

Missing data will be handled with last observation carried forward.

Zero-hypotheses: Blephasteam® (Spectrum Thea Pharmaceuticals LTD, Macclesfield, UK) and THERA°PEARL Eye Mask (Bausch & Lomb Inc., New York, USA) differ by a clinically relevant amount. Type I error rate of 0.05 and power (type II error rate) 0.20. There are many parameters that will be measured in the study, the primary being tear film break-up time (TFBUT).

Originality and Scientific/Clinical Value

Originality: The study design is novel and may give useful information that can guide both general practitioners and ophthalmologists in their treatment of one of the largest patient groups in ophthalmology.

Presentation/Publication

The results will be presented at two international congresses, and two manuscripts will be submitted to a peer-reviewed journal of ophthalmology with high impact factor.

Project Feasibility and the Research Group

Tørreøyneklinikken (TØK) is the first private clinic in the Nordic region with DED as primary focus. Moreover, it is the first in Norway to employ osmolarity measurements, meibomography (Keratograph), LipiView (Tear Science), and in vivo confocal microscopy (Heidelberg) for setting the diagnosis as well as evaluating the effects of the treatment. All patients in TØK will be asked to enroll in research projects/systematic quality assessments. In this way, TØK will eventually be able to document the effectiveness of a variety of treatments modalities applied to different subgroups of the disease. For the present proposal, we have a broad team to carry out all the clinical examinations and analyze the clinical and the laboratory data. We also have the advantage of collaborating with Darlene A. Dartt and Neil Lagali. Dartt is a Senior Scientist and Harold F. Johnson Research Scholar at Schepens Eye Research Institute, Massachusetts Eye and Ear (SERI/MEE), Harvard Medical School and Professor at Department of Ophthalmology, Harvard Medical School. She is regarded as one of the world`s leading experts in the field. Dartt was the Director of Research at the institute for nine years and has trained five of the members of the research group (Øygunn Aass Utheim, Xiangjun Chen, Sten Ræder, Jon Roger Eidet and Tor Paaske Utheim) at SERI/MEE, Harvard Medical School. Lagali is a world-class scientist on the use of IVCM with numerous publications in this field. Tor Paaske Utheim is the recipient of 14 national and international awards and honors and is currently the youngest Adjunct Clinical Associate at SERI/MEE, Harvard Medical School. Sten Ræder is CEO of the TØK and recipient

of several awards, including three innovation prizes. Jon Roger Eidet is Director of Research at TØK and is also recipient of several awards/honors. Xiangjun Chen and Alexandar Stojanovic have numerous publications relating to the ocular surface. They have both been involved in research on dry eye disease since TØK was established in 2012.

How the Present Project Complies with the Guidelines (§ 3 b) given by ExtraStiftelsen

1) The ability of the project to spark new actions based on the defined project aims – As the number of DED patients in the population is almost unlimited and the issues reported very diverse and complex, the project appears ideal for sparking new actions. With regard to aniridia, Norway is currently in a very favourable situation to spark new actions as both the Head of the Scientific Committee in Aniridia Europa and its president are from Norway. Our ambition is to apply for EU funding after the completion of the present project to build on our results and the expertise in the reseach group.
2) The ability of the project to solve hitherto unsolved issues in health and rehabilitation in society – The research group thinks the present projects holds great promise to solve hitherto unsolved issues for the following reasons: 1) the aims of the projects are clearly defined, 2) the available literature studied in great detail, 3) all planned methods are very well known to the project group, and 4) several scientists in the research group has an exceptional strong track record of supervision of PhD candidates and scientific achievements, please see the section *Project Feasibility and the Research Group. 5)* The project leader is currently Heading the Scientific Committee in Aniridia Europe. This should make it relatively easy to implement important results from the project on a European scale and ensure almost immediate translation of our findings.
3) The extent of the health problem in society, and whether many are or can be affected – Aniridi is a rare disease, but is regarded an excellent model for the study of stem cell deficiency of the ocular surface. This is of particular interest to many scientists in the group, in which three of the members have a PhD on the topic. The importance of the tear film in stem cell deficiency of the ocular surface can not be underestimated. Therefore, the combined study of DED and aniridia is of utmost importance in patients with aniridia.
4) The severity and complexity of the health problem – Both aniridia and DED are considered very complex health problems. Anirida can arise from X mutations and presents considerable phenotypic variations. Despite decades of intense research, mechanisms involved in DED are still poorly understood. Aniridia is a severe disease. DED, however, comes in all grades of severity. The most severe form results in ocular perforation and therefore blindness. Of note such serious consequences of DED are very rare. That said, a minimal moisture of the ocular

surface is a requisite for maintaining the integrity of the eye as the ocular surface depends on water and nutrition from the tear film to maintain their normal function.

5) Projects involving children, adolescence and elderly – By including two populations – a congenital disease and a general elderly population – in the project we hope to comply with § 3 b by ExtraStiftelsen.
6) Projects with patient contribution and administration – The project puts emphasis on patient information and involvement. These two factors are the most important measures to avoid exacerbation of DED. Unfortunately, there is little general knowledge of DED in the population. By applying for funding to carry out a PhD project on DED in a general population and in ocular disease (aniridia), we hope increase the awareness of DED and possible measures to lessen symptoms/avoid exacerbation. This is particularly important in DED as many patients enter a vicious circle without proper diagnosis and guidance. In TØK a novel software (ADEMS – A Dry Eye Management System) was developed in 2012 that increase patient contribution and awareness of DED. The further development of the program has so far been supported by the Norwegian Research Council, pharmaceutical industry and eye clinics. An improved version will be launched prior to the initiation of the present project (given it is funded). This program will allow even higher degree of patient involvement and education.
7) Projects that spark collaboration between voluntary organization and between voluntary organizations and various academic environments - The project leader is consultant at the Norwegian Association for the Blind and Partially Sighted and the Head of the Scientific Committee for Aniridia Norway. Thus, the project leader is well connected to two organizations and has an excellent collaboration with both. The present project is, therefore, very likely to boost collaboration between at least two voluntary organizations associated to ExtraStiftelsen. The project is also quite extraordinary be including no less than eleven affiliations, including both dentistry and medicine and three international partners, including Harvard Medical School. With regard to medicine, the project group includes expertise not only in ophthalmology, but also medical biochemistry, the latter is of vital importance in dry eye disease as the not only the amount, but also the content of the tear film is of a critical factor in the development and exacerbation of DED. The present project, which is based on a substantial element of previous innovation (ADEMS), is likely to increase interfacultary, interdisciplinary and international collaboration. The research group`s ADEMS is now not only a software that is in use in Norway, but also abroad.
8) Good plans for information related to the project and dissemination of the results of the projects - Several of the members of the research group has received substantial publicity based on previous scientific work, in particular the project leader with dissemination of the

scientific results in television (EuroNews, NRK Dagsrevyen, Schrødingers Katt), and newspapers (The Journal of the Norwegian Medical Association, "Everything about vision " (Norwegian journal about vision – circulation up to 700.000), Aftenposten, Ullevål-Nytt (newspaper for Oslo University Hospital, Ullevål, etc) and webpages (tidsskriftet.no, forskning.no, EuroNews.com, nrk.no, helse-sorost.no, oslo-universitetssykehus.no, medinnova.no, blindeforbundet.no, abcnyheter.no, etc). Based on this track record with regard to publicity, it is likely that the research group will succeed in ensuring broad dissemination of the results while acknowledging the source of funding.

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