Clinical Trial Protocol CPDOXY1

Comparison between continuous and pulsed oral doxycycline treatment protocols for refractory meibomian gland dysfunction - 9 months follow-up prospective study

PROTOCOL NUMBER: CPDOXY1
VERSION NUMBER: Version 01.

STUDY CONTACTS:
Dr Paolo Fogagnolo, Principal Investigator
via di Rudini' 8, 20142, Milan, Italy
e: paolo.fogagnolo@unimi.it
p: +390281844301

SPONSOR: University of Milan
INVESTIGATOR AGREEMENT

Study title:

Comparison between continuous and pulsed oral doxycycline treatment protocols for refractory meibomian gland dysfunction - 9 months follow-up prospective study

Author approval:

This clinical study protocol has been written by the Principal Investigator.
I agree to personally conduct or supervise this study according to this protocol and to comply with its requirements, subject to ethical and safety considerations and guidelines, and to conduct the study in accordance with the Declaration of Helsinki and with the International Conference on Harmonisation guidelines on Good Clinical Practice (ICH E6), and applicable local regulatory requirements.
I agree to ensure that all associates, colleagues and employees assisting in the conduct of the study are informed about their obligations.

PI (print name) _____

Signature _____

Date _____

1. BACKGROUND

1.1 Disease

Meibomian gland dysfunction (MGD) is a chronic condition and the main cause of dry eye disease (DED) nowadays. [1-2] It affects the meibomian glands, leading to blockage of the ducts and changes in the secretion of oil. This can result in issues with the tear film, eye irritation, inflammation, and ocular surface disease. [1]

MGD can cause various eyelid symptoms, including prominent blood vessels, frothy discharge along the eyelid margin, blocked meibomian glands, and thickened or irregular eyelid margins. Patients with MGD often have rosacea or seborrheic dermatitis. MGD can lead to reduced availability of normal oil on the eyelid and tear film, resulting in increased tear film instability, bacterial growth, evaporative dry eye, and inflammation of the ocular surface. [1-2]

1.2 Current therapeutic options

The patient should understand that a complete cure is usually not possible for blepharitis. As a matter of fact, the main goal of the treatment is the control of the ocular symptoms in the majority of the cases. The main treatments for MGD are:
- Warm compresses
- Eyelid cleansing and massage to express the meibomian glands
- Antibiotics (topical and/or systemic)
- Topical anti-inflammatory agents (e.g., corticosteroids, cyclosporine)

These treatment options are often used in combination. The optimal treatment regimen often requires persistence and a trial-and-error approach.

An initial step in treating MGD patients is to recommend warm compresses and eyelid cleansing with the addition of ocular lubricants. For patients with MGD, whose chronic symptoms and signs are not adequately controlled with the previous approach, oral tetracyclines and topical antibiotics may be helpful. Doxycycline, minocycline, or tetracycline can be given daily and tapered after clinical improvement is noted. Alternatively, oral erythromycin or azithromycin can be used, especially in women of childbearing age and children. [1-2]

1.3 Devices under study

The drug in the study is oral doxycycline, which is the most commonly used oral tetracycline for treating posterior blepharitis and MGD. The rationale for using tetracyclines is based in part on small clinical trials that have reported the drugs' effectiveness in improving symptoms in patients with ocular rosacea and in improving tear break-up time in patients with rosacea and MGD. [1]

Doxycycline is a metal ion chelator and a broad-spectrum antibiotic that prevents access of acyl t-RNA to the acceptor site on the mRNA-30s ribosomal subunit complex. In addition to these established functions, other properties that have recently been ascribed to

doxycycline include differential inhibition of the activity of members of the matrix metalloproteinase (MMP) family, inhibition of MMP synthesis, inhibition of interleukin-1 synthesis, inhibition of activated B cell function, inhibition of nitric oxide (NO) synthesis by lipopolysaccharide activated macrophages, and inhibition of collagen synthesis by cultured chondrocytes. [3]

Tetracyclines also decrease lipase production in both S. epidermidis and S. aureus. However, it's worth noting that tetracyclines can cause photosensitization, gastrointestinal upset, vaginitis, and, rarely, azotemia. Tetracyclines have been implicated in cases of pseudotumor cerebri, and their metabolism may alter the effectiveness of certain medications (e.g., decrease the effectiveness of oral contraceptives and potentiate the effect of warfarin). Tetracyclines are contraindicated in pregnancy, for nursing women, and for patients with a history of hypersensitivity to tetracyclines. Furthermore, tetracyclines should not be used in children under 8 years of age since staining of teeth may occur; however, oral erythromycin may be used as an alternative. [1-2-3]

2. STUDY RATIONALE

Despite MGD's high prevalence and impact on the general population, we still lack a standardised medical treatment for moderate to severe MGD, especially for refractory cases.
Doxycycline’s main therapeutical effect on MGD inhibits the synthesis or activation of proinflammatory mediators like MMP in the corneal epithelium. Furthermore, its antimicrobial and anti-chemotactic properties are important in the lid’s microbiome regulation and are crucial to optimise the meibomian gland function (improvement of meibum fluidity and glandular secretion enhancement). However, long-term use of doxycycline can be risky due to the possibility of gastrointestinal effects. Different protocols in terms of duration and dosage have been reported for doxycycline in MGD treatment, but no clear superiority of a particular protocol has been reported. [4-5] This is why, in recent years, there has been significant interest in optimising treatment protocols with lower dosage doxycycline for better tolerability without losing the therapeutical effect. [3-4] This study compares the safety and efficacy of two daily oral doxycycline protocols for refractory meibomian gland dysfunction (MGD): LCD (low-dose continuous protocol) - 50 mg of doxycycline for three months and FPP (Full-dose pulsed protocol) - 100 mg of doxycycline for the first 15 days of the month for three months.

3. STUDY OBJECTIVES

3.1 Primary objective

To evaluate which of the protocols in the study (LCD Vs FPP) has the greatest impact on refractory MGD patient's symptoms (OSDI) on a six-month follow-up

3.2 Secondary objective

To evaluate which of the protocols in the study (LCD Vs FPP) has a greater effect on patient's signs (NIBUT; TBUT, Oxford scale) on a six-month follow-up

To evaluate which protocol is related to the lower rate of adverse events

4. STUDY DESIGN

4.1 Study Description

This spontaneous, prospective, non-randomized, parallel-group, phase IV study was conducted in compliance with the tenets of the Declaration of Helsinki.
All study participants were fully consented, and written informed consent was obtained.
This study included patients with refractory MGD. Refractory MGD is defined as symptomatic MGC (OSDI > 13 points) despite 2 months of first-line treatment with lacrimal substitutes 3 times per day and meibomian gland expression with warm compresses twice per day.
The study will consist of 3 visits: Visit 1(Baseline - day 0), Visit 2 (day 90 ± 5 - 3rd month), Visit 3 (day 180 ± 5 - 6 month). At each visit, patients will receive an OSDI questionnaire, NIBUT, type I Schirmer test and AS assessment, including TBUT, corneal staining (Oxford scale)
At baseline, eligible patients will be enrolled and treated with the assigned protocol. The investigators will assign the protocol (non-randomized) in a 1:1 ratio.
At Visit 2, NIBUT, TBUT, Oxford scale and OSDI improvement will be evaluated compared with Visit 1. Patients will be carefully asked about their compliance with the treatment. Patients who interrupted the treatment for adverse events or low compliance (<75% of the protocol adherence) will exit the study and be excluded from the analysis.
At Visit 3, the study population will be evaluated as done in Visit 2
The protocols in the study are the following:

- LCD (low-dose continuous protocol) - 50 mg of doxycycline for three months
- FPP (Full-dose pulsed protocol) - 100 mg of doxycycline for the first 15 days of the month for three months.

4.2 End of study

The enrollment period will last about 6 months, so the study will end about 6 months after the last patient enrolls.

5. STUDY ENDPOINTS

5.1 Primary endpoints

The primary endpoint of this study are:

- OSDI
- Number of patients with OSDI improvement > 7 points (clinically significant)

For these parameters, the changes occurring in the two groups at each visit vs baseline will be evaluated.

5.2 Secondary endpoints

The study's secondary endpoints are the Low- and high-tech anterior segment parameters (NIBUT, TBUT, Oxford class).

For these parameters, the changes occurring in the two groups at each visit compared to baseline will also be evaluated.

5.3 Safety endpoints

Safety assessments will consist of recording all adverse events, adverse device effects, and product deficiencies.

6. PATIENT SELECTION CRITERIA

6.1 Study participants

65 patients will be enrolled.

6.2 Inclusion criteria

- 18 years or older
- provided written informed consent
- OSDI score of 13 or more at the baseline visit after at least two months of first-line therapy (artificial tears and warm compresses)
- Clinical diagnosis of MGD
- Type 1 Schirmer test > 10 mm
- No previous history of allergy or sensitivity to doxycycline,
- No use of additional topical or systemic antibiotics for the prior 2 months
- No use of topical anti-inflammatory agents (ex: corticosteroids or cyclosporine) for the prior 3 months.

6.3 Exclusion criteria

- Active ocular inflammation in either eye,
- Demodex blepharitis,
- Ocular surgery within the past 3 months of baseline examination,

- Structural ocular surface and eyelid abnormalities.
- Sjögren's syndrome
- Rheumatoid arthritis
- Other systemic diseases resulting in dry eye
- Known autoimmune disease
- Doxycycline allergy or sensitivity
- History of antibiotic therapy at any time within 2 months of the commencement of study
- Use of significant calcium supplementation or other medications that could interfere with doxycycline absorption (es: Iron supplementation)

7. STUDY TREATMENT

7.1 Study supplies

Study treatment will be prescribed to the patient as for normal clinical practice

7.2 Study treatment formulation, packaging and storage

The most commonly used form of this medication in Italy is Bassado (Pfizer), which will be prescribed to every patient in this study. The active ingredient is doxycycline (as doxycycline hyclate). Each tablet contains 100 mg of doxycycline (equivalent to 115.4 mg of doxycycline hyclate). The other components are: starch, sodium lauryl sulfate, lactose, alginic acid, and magnesium stearate.

7.3 Treatment Dosage, administration, and compliance

Please note the following information:

The dosage will vary depending on the treatment plan prescribed for the patient. This may involve taking 50 mg (half a tablet) daily for three months, or 100 mg (one tablet) for 15 days each month for three months.

Patients will take the medication at home without medical supervision. However, they will be able to contact the medical staff involved in the study if they have any questions or suspect any adverse events.

7.4 Device Accountability of the Medical Device

Patients will bring the used blisters to V2 in order to perform drug accountability and calculate the compliance rate.

7.5 Concomitant and permitted and not permitted treatment

Patients will be allowed to carry on any systemic medication except:

- Multivitamins with minerals (Iron can bind to doxycycline in the gastrointestinal tract, which may prevent their absorption into the bloodstream and possibly reduce their effectiveness).
- Other oral or systemic antibiotics
- Probiotics

Patient will not be able to assume other topical medications for blepharitis or MGD treatment during the study (ex: topical antibiotics or corticosteroids) except for topical lubricating agents

8. STUDY ASSESSMENTS/VISITS

8.1 Study visits:
The study comprises three visits: Visit 1 (baseline), Visit 2 (3rd month), and Visit 3 (6th month).

8.2 Study assessments:
At each visit, the following examinations will be performed in both eyes in the following order:
- OSDI questionnaire
- NIBUT
- Schirmer test
- TBUT
- Corneal staining evaluation (Oxford scale)

All above mentioned examinations and all evaluations required by the study are part of the normal clinical practice.

9. PATIENT, STUDY, AND SITE DISCONTINUATION

9.1 End of treatment
In this prospective study, study treatments will end after three months after the last enrollment.

9.2 End of the study
Enrollment will end as soon as the target number of patients is achieved. The study will end six months after the last patient's enrollment.

10. SAFETY AND ADVERSE EVENTS

10.1 Safety evaluation

During the treatment period (from V1 to V2), patients can contact any member of the study medical staff with doubts or suspected adverse events. The investigators will determine if a drug-related adverse event (AEs) has occurred.
AEs will be coded according to the most current Medical Dictionary for Regulatory Activities (MedDRA) classification.
The investigator will record the nature, severity, treatment and outcome of the AE, and will determine their association with the investigational products or procedures involved in the clinical study.

10.2 Adverse event: Definitions

An AE is any untoward medical occurrence associated with the use of a device in humans without any judgment about relationship.
This includes events related to the investigational device or the vehicle. The investigator will determine and record the seriousness, severity, relationship, and outcome for all AEs

10.3 Causality Assessment

The investigator or designee is responsible for assessing the relationship between AEs and the study drug. Additionally, the investigator or designee is responsible for providing appropriate treatment for the event and for adequately following the event until resolution. The clinical investigator or responsible designee should determine the study device relationship using the following explanations:
Certain Event or laboratory test abnormalities, with plausible time relationship to medical drug use, cannot be explained by disease or other interventions. The response to withdrawal is plausible and rechallenge satisfactory, if necessary.
Probable / Likely Event or laboratory test abnormality, with reasonable time relationship to medical device use and unlikely to be attributed to disease or other interventions. Response to withdrawal is clinically reasonable. Re-challenge not required.
Possible Event or laboratory test abnormality, with reasonable time relationship to medical device use and that could also be explained by disease or other interventions.
Information on withdrawal may be lacking or unclear.
Unlikely Event or laboratory test abnormality with a time to medical device use that makes a relationship improbable (but not impossible). Disease or other interventions provide plausible explanations.
Unrelated; A clinical event that appears before using the medical device or can be fully explained by other factors.
Conditional/Unclassified Event or laboratory test abnormality; more data for proper assessment needed or additional data under examination.
Unclassifiable; Report suggesting an adverse reaction that cannot be judged because information is insufficient or contradictory. Data cannot be supplemented or verified.

10.4 Severity Assessment

The intensity (severity) of an AE is assessed by the Investigator and graded as mild, moderate or severe, irrespective of the relationship to the device or the seriousness of the event.
Mild - Discomfort noticed, but no disruption to daily activity
Moderate - Discomfort sufficient to reduce or affect normal daily activity
Severe - Inability to work or perform normal daily activity

10.5 Outcome Assessment
The outcome assessment for an AE will be defined as follows:
• resolved
• resolved with sequelae
• ongoing
• death
• unknown

10.6 Serious Adverse Event (SAE)
An AE is considered “serious” if, it results in any of the following:
• death
• a life-threatening illness or injury, or
• a permanent impairment of a body structure or a body function, or
• required in-patient hospitalization or prolongation of existing hospitalization, or
• medical or surgical intervention to prevent life-threatening illness, injury or permanent impairment to a body structure or a body function, or
• led to fetal distress, fetal death or a congenital abnormality or birth defect.
This includes adverse AEs that might have led to an SAE if a) suitable action had not been taken or b) intervention had not been made, or c) if circumstances had been less fortunate. A planned hospitalization for a pre-existing condition, or a procedure required by the protocol, without a serious deterioration in health, is not considered to be a serious AE.

10.7 Drug Deficiency
The investigational device's inadequacy is related to its quality, safety, or performance, such as malfunction, misuse, user error, and inadequate packaging or labelling.

10.8 Adverse Device Effect
ADVERSE EVENT RELATED TO THE USE OF AN INVESTIGATIONAL MEDICAL DEVICE.
This includes any AE resulting from insufficiencies or inadequacies in the instructions for use or any malfunction of the investigational medical device. This also includes any event that is a result of a use error or intentional misuse.

UNANTICIPATED SERIOUS ADVERSE DEVICE EFFECT (USADE)
Unanticipated Serious Adverse Device Effect (USADE) is a serious adverse device effect, which by its nature, incidence, severity or outcome has not been previously identified)

10.9 Collection of Adverse Events (AE)

Any AE occurring prior to signing the ICF should be considered medical history or a preexisting condition will be collected on the Medical History eCRF.

All AEs will be collected from the signing of the ICF through the 3-year follow-up and entered into the AE section of the eCRF. Serious AEs will be collected from the signing of the ICF through the 36-month follow-up and recorded on the AE eCRF. SAEs will also be reported within 24 hours using the Serious Adverse Event Report Form.

At V2, the investigator will determine whether any AE has occurred after carefully interrogating the patient. The investigator will also determine and record the seriousness, severity, relationship, and outcome for all AEs.

10.10 Device Deficiencies

Information will be collected on investigational medical device deficiencies from the investigators. An investigation of the medical device deficiency is performed promptly to determine the root cause of the deficiency. Device deficiencies will be recorded in the eCRF, and will also be reported using the SAE Form (even if any device deficiency does not result in an AE).

10.11 Reporting of Adverse Events and/or Device Deficiency

All AEs, SAEs and device deficiencies will be reported to the regulatory authorities and IECs in accordance with the current country specific regulatory and IRB/IEC requirements.

11. STATISTICAL CONSIDERATION AND ANALYTICAL PLAN

11.1 Definition of study populations

11.1.1 All Patients Analysis Set

This analysis is not ideal for this study. It will be performed as a secondary analysis, but publication will be based on ITT analysis.

11.1.2 Intent-to-Treat (ITT) Analysis Set

Signs and symptoms will be analyzed using an intent-to-treat approach. In case of missing data, the last observation available will be carried forward. This will be the primary analysis.

11.1.3 Safety Analysis Set

Safety analysis will evaluate the number of dropouts related to adverse events for each group

11.2 Sample size

The sample size estimate was based on a test for before-after studies using a paired t-test, defining a 1-sec change in Break-Up Time as clinically relevant and a standard deviation of 2.3 sec. Setting the error to 2.5% (one-tailed), 43 patients would be required, assuming a power of 80%. Assuming drop out, the final enrollment will be 65 patients.

11.3 Statistical analysis

11.3.1 Efficacy analysis

Treatment and analysis will be performed on the worst of the two eyes at diagnosis. Worst eye will be defined based on TBUT or, in case of identical TBUT between the two eyes, OXFORD class. In case of identical TBUT and OXFORD class, NIBUT will be considered.

11.3.1.1 Primary efficacy endpoint

Paired t-test will be used to study OSDI. Chi-square is used to study clinically significant OSDI variation

11.3.1.2 Secondary efficacy endpoints

Paired t-test will be used to study the secondary variables.

11.3.2 Compliance analysis

Patient that stopped or missed more than 25% of the assumptions (ex more than 28 cp for LCP or more than 14 cp for FPP)

11.3.3 Safety analysis

Chi-square is used for drop-out rates between groups

11.4 Independent Data Monitoring Committee and Interim Analysis

No interim analysis will be performed. No independent data monitoring will be adopted.

12. DATA MANAGEMENT

Data will be electronically collected using local software (Galileo) as part of the clinical practice using an electronic CRF. At the end of the study, data will be retrieved and extracted in Excel format.

13. MONITORING OF THE STUDY

No external monitoring will be used.

14. STUDY MANAGEMENT

14.1 Administrative Structure

Principal Investigator:
Dr. Paolo Fogagnolo

14.2 Training of study site personnel

All site personnel will provide a certificate of ICH/GCP Training

14.3 Publication of Data

We aim to publish a paper on an impacted, high-range Journal. The paper will be submitted 4 months after study conclusion.

14.4 Clinical Study Report

Dr. Paolo Fogagnolo, Università degli Studi di Milano, Italy, will write the data analysis, clinical study report, and publication draft.

14.5 Finances

This study is not sponsored, visit will be payed as for normal clinical practice according to Italian national healthcare system

14.6 Insurance

Not required for medical devices

14.7 Study timetable and end of study

	Visit 1 (day 0)	Visit 2 (day 90)	Visit 3 (day 180)
Verify inclusion and exclusion criteria	X		
Informed consent signature	X		
OSDI questionnaire	X	X	X
NIBUT	X	X	X
AS assessment of blepharitis and hyperemia	X	X	X
TBUT	X	X	X
Adherence to study treatments		X	
Prescribe study drug	X		

15. REFERENCES

[1] Amescua G, Akpek EK, Farid M et al. American Academy of Ophthalmology Preferred Practice Pattern Cornea and External Disease Panel. Blepharitis Preferred Practice Pattern®. Ophthalmology. 2019 Jan;126(1):P56-P93. doi: 10.1016/j.ophtha.2018.10.019. Epub 2018 Oct 23. Blepharitis Preferred Practice Pattern®

[2] Nelson JD, Shimazaki J, Benitez-del-Castillo JM et al. The international workshop on meibomian gland dysfunction: report of the definition and classification subcommittee. Invest Ophthalmol Vis Sci. 2011 Mar 30;52(4):1930-7. doi: 10.1167/iovs.10-6997b.

[3] Yoo SE, Lee DC, Chang MH. The effect of low-dose doxycycline therapy in chronic meibomian gland dysfunction. Korean J Ophthalmol. 2005 Dec;19(4):258-63. doi: 10.3341/kjo.2005.19.4.258.

[4] Vernhardsdottir RR, Magno MS, Hynnekleiv L et al. Antibiotic treatment for dry eye disease related to meibomian gland dysfunction and blepharitis - A review. Ocul Surf. 2022 Oct;26:211-221. doi: 10.1016/j.jtos.2022.08.010. Epub 2022 Sep 20.

[5] Ben Ephraim Noyman D, Chan CC, Mimouni M et al. Systemic antibiotic treatment for meibomian gland dysfunction. A systematic review and meta-analysis. Acta Ophthalmol. 2024 Feb;102(1):e1-e10. doi: 10.1111/aos.15681. Epub 2023 May 4