Effect of CACICOL20 on corneal epithelial healing after cross-linking in patients with keratoconus

Clinical Investigation Plan

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**Study Synopsis**

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<td><strong>Background</strong></td>
<td>CACICOL20 is an ophthalmic solution based on the technology of RGTAs (ReGeneraTingAgents). It consists of large biopolymers, imitating the structure of heparansulphate. The protecting effect on different biological tissues and enhancement of wound healing has been described in several studies. Keratoconus is a relatively common disease, with incidences ranging from 1.3 to 25 per 100,000 per year across different populations. Corneal collagen cross-linking represents a treatment option for these patients, aiming to prevent progression of the disease via stabilization of corneal microstructure. Corneal epithelial removal prior to the ultraviolet A/riboflavin cross-linking procedure significantly improves the outcome of the intervention, due to ameliorated distribution of riboflavin.</td>
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<td>Randomized, double-blind, controlled, parallel group study</td>
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| **Methods** | High-resolution OCT imaging of the cornea  
Slit lamp biomicroscopy and slit lamp photography  
Visual analogue scale assessing pain |
| **Main outcome variable** | Time to closure of the epithelial wound |
| **Additional outcome variables** | Visual analogue scale (VAS) assessing pain  
Parameters derived from OCT- images |
| **Risk/benefit assessment** | There is evidence that CACICOL20 (RGTA OTR 4120) has a positive influence on wound healing in several biological tissues. Therefore, patients may benefit from adding CACICOL20 eye drops to the standard treatment regimen after corneal cross linking. All examinations used in this study are non-invasive and painless; hence the risk/benefit ratio appears acceptable. |
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1. Introduction

1.1 Background

CACICOL20 is an ophthalmic solution based on the technology of RGTAs (ReGeneraTingAgents). It consists of large biopolymers, imitating the structure of heparansulphate. The protecting effect on different biological tissues and enhancement of wound healing was described in several studies (Cejkova 2013, Aifa 2012, Barbier-Chassefière 2009). Safety and efficacy of CACICOL20 on corneal tissue was confirmed experimentally and in patients with severe corneal ulcers (Chebbi 2008, Brignole-Baudouin 2013). Furthermore CACICOL20 was toxicologically tested in mice (Charef 2010). The efficiency of CACICOL20 versus placebo is currently investigated in a randomized multicentric double-blinded study (clinicaltrials.gov, UNICOL). Mechanisms of action can be summarized as replacement of heparansulphate, preventing proteolysis of corneal matrix proteins and creating an environment allowing for recruitment of NGF (nerve growth factor) and cytokines to restore corneal integrity.

Keratoconus is a relatively common disease, with incidences ranging from 1.3 to 25 per 100,000 per year across different populations (Kennedy 1986, Rabinowitz 1998, Pearson 2000, Georgiou 2004, Nielsen 2007). Clinical signs are impairment in visual acuity, resulting from irregular astigmatism, as a consequence of corneal thinning and protrusion. Onset of the disease generally occurs in the second decade, followed by variable speed of progression. Main treatment options for patients with keratoconus are optical correction with spectacles or contact lenses, corneal collagen cross-linking, intrastromal corneal ring segments and keratoplasty (Vazirani 2013). Corneal collagen cross-linking aims to prevent progression of the disease via stabilization of corneal microstructure. It has been described that corneal cross-linking was capable of increasing the biomechanical strength of the human cornea by approximately 300% (Wollensak 2003, 2006). Corneal epithelial removal prior to the ultraviolet A/riboflavin crosslinking procedure significantly improves the outcome of the intervention, due to ameliorated distribution of riboflavin (Wollensak 2009).

The aim of the present study is to investigate the effect of CACICOL20 versus placebo on corneal epithelial closure after collagen crosslinking in patients with keratoconus. Results may lead to an improved management of patients with corneal epithelial defects by decreasing pain and enhancing epithelial wound closure.

1.2 Rationale of the study

Corneal integrity is a major issue regarding the quality of life of patients with corneal defects. Affected patients suffer from significant pain during the healing process. New therapeutic targets enhancing quality and speed of corneal healing are therefore of great interest in clinical practice.
In the present study, CACICOL20 will be administered every two days. This treatment regimen is applied in a currently ongoing large multicenter study in patients with corneal ulcers (clinicaltrials.gov, UNICOL). The results of the present study may help to find a new therapeutic scheme for patients recovering from corneal cross-linking.

1.3 Risk/benefit assessment
There is evidence that CACICOL20 (RGTA OTR 4120) has a positive influence on wound healing in several biological tissues. Therefore, patients may benefit from adding CACICOL20 eye drops to the standard treatment regimen after corneal cross linking. All examinations used in this study are non-invasive and painless; hence the risk/benefit ratio appears acceptable.

2. Study objectives
To assess the effect of CACICOL20 on corneal epithelial wound closure after cross-linking in patients with keratoconus.

3. Investigational plan

3.1 Design
Randomized, double-blind, controlled, parallel group study

3.2 Selection of study population
The participants will be selected by the Department of Ophthalmology and Optometry (Medical University of Vienna). Only patients already scheduled for corneal cross linking will be included in the study.

3.2.1 Number of patients
40 patients with keratoconus

Sample size calculation
A total of 40 patients will enter this parallel-design study. The probability is 80 percent that the study will detect a treatment difference at a two-sided 0.05 significance level, if the true difference in time to

CACICOL20 CXL
corneal epithelial wound closure between treatments is 1 day or more. This is based on the assumption that the mean time to corneal epithelial wound healing is 5 days (clinical experience).

### 3.2.2 Inclusion criteria

- Men and women aged over 18 years
- Presence of keratoconus
- Scheduled for corneal cross linking
- No ophthalmic surgery in the 3 months preceding the study

### 3.2.3 Exclusion criteria

Any of the following will exclude a participant from the study:

- Participation in a clinical trial in the previous 3 weeks
- Topical use of aminoglycosid antibiotics
- Use of therapeutic or refractive contact lenses after surgery
- Known hypersensitivity to any component of the study medication or heparinoids or heparin
- Active ocular infection
- Presence of any abnormalities preventing reliable measurements as judged by the investigator
- Pregnancy, planned pregnancy or lactating

### 3.3 Medical Device

The investigator will dispense the medical device only to patients included in this study by following the procedures set out in this study protocol. The medical device will be stored in accordance with the manufacturers' instructions.

#### 3.3.1 Medical Device: dosage and route of administration

**CACICOL20 eye drops**
Thea Pharma GmbH
Route of administration: topical
Dosage: 1 drop in the study eye every two days after corneal cross linking

**Genteal HA eye drops**
Thea Pharma GmbH
Route of administration: topical
Dosage: 1 drop in the study eye every two days after corneal cross linking
Blinding:
An MD not involved in any of the study related assessments will administer the eye drops on each study day according to the randomization list.

3.3.2 Topical medication

Patients will be treated with eye drops according to the standard regimen used in the Department of Ophthalmology and Optometry after corneal cross linking.

Topical administration of 1 drop “Minims-Fluorescein Sodium 2,0%” (Chauvin Pharmaceuticals, UK) for fluorescein staining of the cornea.

3.3.3 Randomization

Randomization lists will be generated by computer software (http://randomization.com). Patients will be assigned to receive CACICOL20 or Genteal HA eye drops randomly.

3.4 Study protocol

3.4.1 Description of the study days

Study Day 1 (in the 2 weeks before corneal cross linking)

Time frame: 30 minutes
The aim, purpose and study procedures will be explained to patients that come into consideration for participation in the present study and measurements will only be started after written informed consent has been obtained.
In women of childbearing potential, a pregnancy test will be performed. Afterwards, a slit lamp examination will be performed, during which a slit lamp photograph will be taken. In addition, high-resolution OCT measurements of the cornea and the precorneal tear film of the study eye will be performed.

Study Day 2 (day of corneal cross linking):

Time frame: 15 minutes
After corneal cross linking has been performed, patients will be randomized to either receive CACICOL20 or Genteal HA eye drops in addition to the standard treatment regimen. To obtain double blinded conditions, instillation of the eye drops will be performed by a MD not involved in any of the study related procedures.
Study Days following corneal cross linking:
Time frame: 30 minutes
Patients will return to the Department of Ophthalmology every 2 days for instillation of the eye drops (CACICOL20 or Genteal HA). On every study day, a slit lamp examination and imaging of the cornea and the precorneal tear film using high-resolution OCT will be performed. In addition, a slit lamp photograph will be taken and pain will be assessed using a visual analogue scale. Patients will be assessed every two days until corneal epithelial wound closure assessed by corneal fluorescein staining is achieved.

End of Study visit:
A final visit will be performed 7 to 14 days after corneal wound closure has been observed. Again, a slit lamp examination, slit lamp photography, OCT imaging and assessment of pain will be performed.

3.4.2 Withdrawal and replacement of patients

Patients must be withdrawn under the following circumstances:

- at their own request
- if the investigator feels it would not be in the best interests of the patient to continue
- if the patient violates the conditions laid out in the consent form/information sheet or disregards instructions by the study personnel

In all cases, the reasons why patients are withdrawn must be recorded in detail in the case report form and in the patients' medical records. Should the study be discontinued prematurely, all study materials (completed, partially completed and empty case record forms) will be retained.

Patients who do not complete the study according to the protocol will not be replaced. The data from these patients will be eligible for analysis of the conducted trial periods and for safety variables.

3.4.3 Study stopping rules

The study may be prematurely terminated if SAEs or other significant side effects related to the study procedures occurs. Should the study be discontinued prematurely, all study materials (completed, partially completed and empty case record forms) will be retained.

3.4.4 Handling of Missing and Spurious Data
Only patients for whom data are available will be included in the statistical analyses. Missing values will neither be replaced nor estimated.

### 3.4.5 Protocol deviations

The Investigator should not implement any deviation from the protocol without prior review and agreement by the Ethics Committee, except when necessary to eliminate an immediate hazard to study patients. Any deviation from the protocol will be documented in writing (note to file) by the principal investigator.

For purposes of this protocol, a major protocol deviation is defined as:
- Patient entered into the study even though he/she did not satisfy entry criteria

In case of a major protocol deviation, patients will be excluded from the study.

### 3.5 Variables and schedule of observations

#### 3.5.1 Outcome variables

Main outcome variable:
- Effect of CACICOL20 versus control on corneal epithelial closure time.

Additional outcome variables:
- Pain as assessed with visual analogue scale (VAS)
- Ultrahigh resolution OCT- images

### 4. Methods of evaluation

#### 4.1 Methods

##### 4.1.1 Slit lamp biomicroscopy and slit lamp photography

A standard slit lamp examination will be performed. For documentation purposes, a slit lamp photograph will be taken.

##### 4.1.2 High-resolution optical coherence tomography (OCT)

A spectrometer based ultrahigh resolution Spectral Domain OCT (SDOCT) system operating at 800 nm for the anterior chamber will be employed in the present study. The spectrum of the Ti:Sapphire laser light source is centered at 800 nm. With a full width at half maximum bandwidth of 170 nm, the axial...
resolution is 1.3 µm in the cornea. The transverse resolution of the employed OCT system is 21 µm at the front surface of the cornea. For measurement, patients will place their head in a modified slit lamp head rest. During the measurement period, patients will be asked to look straightforward onto an internal fixation target and to avoid blinking. Different scanning patterns, e.g. raster, circular and spiral scans, will be employed in order to visualize the corneal defect following collagen cross-linking as well as the precorneal tear film.

Post-processing of the acquired data involves, firstly, a pre-processing including contrast enhancement and registration of the OCT frame using the freeware software ImageJ (Thevenaz 1998) and, secondly, loading of the image data into software written in National Instruments LabView 2011 for automatic image analysis.

4.1.3 Visual analogue scale

A global ocular discomfort score will be determined using a 100 mm VAS on which 0 means no symptoms and 100 means the worst possible discomfort.

4.2 Adverse events

An adverse event is any event during a clinical study, including intercurrent illness or accident, which impairs the well-being of the patient; it may also take the form of an abnormal laboratory value. The term adverse event does not imply a causal relationship with the study procedures.

All patients experiencing adverse events - whether considered associated with the study procedures or not - will be monitored until symptoms subside, or until there is a satisfactory explanation for the changes observed, or until death, in which case a full pathologist's report will be supplied, if possible. All findings must be reported on an "Adverse event" page in the case record form.

All adverse events, including intercurrent illnesses, will be reported and documented as described below.

Adverse events are divided into the categories "serious" and "nonserious". This determines the procedure which must be used to report/document the adverse event (see below).

4.2.1 Definition of serious and nonserious adverse events

A serious adverse event is:
- any event that is fatal or life-threatening
- any event that is permanently disabling
- any event that requires hospitalisation
- any event that involves cancer, congenital anomaly, or occurs as a result of overdose (application of more than the stipulated dose)

Adverse events which do not fall into these categories are defined as nonserious.

4.2.2 Reporting/documentation of adverse events

Adverse events are collected by spontaneous reporting.

Serious adverse events

All serious adverse events which occur during this study, whether considered to be associated with the study medication or not, must be documented on an "Adverse event" page in the case record form.

A follow-up report including all new information obtained on the serious event must be prepared and will be collected.

All serious adverse events will be reported to the ethics committee.

Nonserious adverse events

These are to be documented on an "Adverse event" page in the case record form.

4.2.3 Assessment of severity

Regardless of the classification of an adverse event as serious or nonserious (see above), its severity must be assessed as mild, moderate or severe, according to medical criteria alone:

mild = does not interfere with routine activities, acceptable

moderate = interferes with routine activities

severe = impossible to perform routine activities, considered as unacceptable by the physician, requires treatment, requires discontinuation of study, or has residual effect.
It should be noted that a severe adverse event need not be serious in nature and that a serious adverse event need not, by definition, be severe. Regardless of severity, all serious adverse events must be reported on as above. In addition the causal relationship between the adverse event and the study procedures will be assessed by the investigator. (Definite, Probable, Possible, Unlikely, Unrelated)

4.3 Data handling procedures

A case record form will be completed for each patient. The entries will be checked by trained personnel and any errors or inconsistencies will be checked immediately. Analysis will be done intent to treat (ITT). The ITT analysis dataset contains data from all patients who entered the study.

4.4 Biometric methods

4.4.1 Biometric methods - outcome variables

Differences between CACICOL20 and control in time to closure will be assessed using Student’s t-test. To detect possible differences between the medical test device and control in tear film thickness, size in corneal lesion and VAS score after corneal cross-linking a two-way analysis of variance (ANOVA) with repeated measures will be calculated. Descriptive results (frequencies and percentages or mean and standard deviation) will be given for all co-variables.

4.4.2 Biometric methods - Adverse events/Safety investigations

All adverse events will be properly listed and summarized by an appropriate method.

5. Ethical and legal aspects

The study will be carried out in accordance with the ICH-GCP guideline. The study will be performed in accordance with the guidelines of the Declaration of Helsinki (1964), including current revisions.

5.1 Informed consent of patients

Before being admitted to the clinical study, the patient must have consented to participate after the nature, scope and possible consequences of the clinical study have been explained in a form understandable to him.

The patient must give consent in writing. The patient's consent will be confirmed by the signature of the investigator.
If any new information arises the patients will be informed by the investigator.

5.2 Acknowledgment/approval of the study

Before the start of the study, the study protocol will be submitted to the Ethics Committee of the Medical University of Vienna.

5.3 Insurance

All patients participating in this clinical study will be insured through the Department of Clinical Pharmacology.

5.4 Confidentiality

All patients’ names will be kept confidential in the investigator's files. Patients will be identified throughout documentation and evaluation by the number allotted to them during the study. The patients will be told that all study findings will be stored and handled in strictest confidence.

5.5 Data quality assurance

5.5.1 Monitoring

5.5.2 Auditing and inspections

The investigator will permit study-related monitoring, audits, review of the Ethics Committee, and regulatory inspection(s) to evaluate study conduct and compliance with the protocol, standard operating procedures, other written instructions/agreements, ICH GCP, and applicable regulatory guidelines/requirements. For this purpose, the investigator will provide direct access to source data documents.

6. Documentation and use of study findings

6.1 Documentation of study findings

All findings collected during the study will be entered on the case record forms provided by the Department of Clinical Pharmacology.
All entries in the case report forms will be made legibly in black ink. If corrections are made to entries in the case record form, the words or figures will be ringed and a single stroke drawn through them. The correct value will be entered beside the old entry and date and the correction will be initialed. Incorrect entries must not be covered with correcting fluid, or obliterated, or made illegible in any way.

Case report forms will be completed immediately after the final examination.

6.2 Use of study findings

The findings of this study will be published by the investigators in a scientific journal and presented at scientific meetings. The manuscript will be circulated to all co-investigators before submission.

7. Protocol amendments

If any modifications (such as selection of additional variables) become necessary or desirable, these will be documented in writing; major changes require the approval of all investigators and the ethics committee.
8. References


CACICOL20 CXL