Methods for assessment of the outcome variables.

The outcome variables have been evaluated from the least to the most invasive, in the same order in which they are described in the following paragraphs. In fact, there is the possibility that tests performed before might influence the results of tests performed next.

2 *1. Assessment of ocular tolerability by Visual analogue scale (VAS)*Local ocular tolerability score: A global ocular discomfort score was determined using a 100 mm VAS on which 0 means no symptoms and 100 means the worst possible discomfort. Specific ocular symptoms that were assessed with the VAS included: foreign body sensation, burning/stinging, itching, pain, sticky feeling, blurred vision and photophobia *Assessment of frequency and severity of DED symptoms, (SANDE)*

The SANDE questionnaire is a short questionnaire to evaluate both dry eye intensity and frequency by using a 100 mm VAS. The patient symptoms of ocular dryness and/or irritation were quantified on the scale based on two questions that assess both severity and frequency of symptoms. [1]

3. Ocular Surface Disease Index (OSDI)

Symptoms of dry eye were assessed using the OSDI which was developed by the Outcomes Research Group at Allergan Inc (Irvine, Calif). The questionnaire that underlies the OSDI© is specifically designed for patients with DED and asks patients about the frequency of specific symptoms and their impact on vision-related functioning. The score reaches from 0 to 100 points. A value >12 points is defined as the cut-off for having ocular surface disease. [2,3]

4. Best corrected visual acuity

Measurement of best-corrected visual acuity was performed using ETDRS charts. This parameter was included in the safety evaluation and the assessment was performed before the subsequent exams may influence the ability of the patients to read the letters of the ETDRS chart.

5. *Tear film osmolarity*

Tear film osmolarity was measured with a commercially available instrument (TearLab®, OcuSens Inc, San Diego, USA). Tear film osmolarity was performed before any drops having been administered. The TearLab® technology uses laboratory functions on a single chip requiring less than 50 nL of tear fluid in order to measure tear osmolarity. The system uses a handheld pen on which the ophthalmologist places the laboratory chip test card. Then, the tear sample is collected minimally invasive from the lower outer tear meniscus. Special attention was paid to avoid reflex tearing. [4]

6. *Tear film break up time (TFBUT)*

After ten minutes, TFBUT was measured following the guidelines published in the Report of the International Dry Eye Work Shop (DEWS) 2007. Briefly, 5µl sodium fluorescein drops (Minims-Fluorescein Sodium 2,0%, Chauvin Pharmaceuticals Ltd. UK) were applied in the conjunctival sac of the eye. The patient was instructed to blink naturally without squeezing several times to distribute the fluorescein. Within 10 - 30 seconds after fluorescein instillation, the patient was asked to stare straight ahead without blinking, until told otherwise. The slit-lamp magnification was set at 10X, the background illumination intensity was kept constant (cobalt blue light). By means of a stopwatch, the time between last complete blink and first appearance of a dry spot was measured. TFBUT was measured twice and if the 2 readings differed by more than 2 seconds a third reading was taken. [5]

7. *Schirmer I test*

Schirmer I test (without anesthesia) was performed after 5 minutes, following the guidelines published in the Report of the International Dry Eye WorkShop. Schirmer paper strips were inserted in the unanesthetized eye over the lower lid margin, midway between the middle and outer third. The patient was then asked to close the eye. After a time of 5 minutes the wetting of the Schirmer paper was measured and reported in mm/5min. [5]

8. *Corneal sensitivity*

For the assessment of corneal sensation, the Luneau Cochet-Bonnet aesthesiometer (Western Ophthalmics Corporation©) was used. The Cochet-Bonnet aesthesiometer contains a thin, retractable, nylon monofilament that extends up to 6 cm in length. Variable pressure can be applied to the cornea by adjusting the monofilament length. The monofilament length ranges from 6 to 0.5 cm. As the monofilament length is decreased the pressure increases from 11 mm/g to 200 mm/g. The filament was retracted incrementally in 0.5 cm steps until a positive response indicating corneal sensation was given from the patient. [6]

9. Ocular surface staining

After another break of 10 minutes, lissamine green (LG) impregnated paper strips (EasyOpht, Italy) containing 1.5 mg of the dye were used to detect conjunctival and corneal epithelial defects. As grading scale of the corneal and conjunctival damage, the NEI/Industry Workshop guidelines were used. [7] In this scale, the cornea is divided into five sectors (central, superior, inferior, nasal and temporal), each of which is scored on a scale of 0–3, with a maximal score of 15. Both nasally and temporally, the conjunctiva is divided into a superior paralimbal area, an inferior paralimbal area and a peripheral area with a grading scale of 0–3 and with a maximal score of 9 for the nasal and temporal conjunctiva. LG staining was performed after Schirmer test without anesthesia in order to avoid increase of tear reflex due to ocular surface instillation of LG .

10. Intraocular pressure

After 10 minutes, intraocular pressure (IOP) was measured with a slit-lamp mounted Goldmann applanation tonometer. Before each measurement, one drop of oxybuprocainhydrochloride combined with sodium fluorescein was used for local anesthesia of the cornea.

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