EVALUATION OF THE EFFECTS OF CHLOROQUINE PHOSPHATE EYE DROPS IN PATIENTS WITH DRY EYE SYNDROME

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Abstract

Objectives: To evaluate the efficacy and safety of 0.03% chloroquine phosphate eye drops in comparison with artificial tears for the treatment of dry eye syndrome.

Methods: Total 170 patients (121 female, 49 male) were enrolled in prospective, randomized, open label case-controlled study. Patients (mean age 53.4± 10.5 years) were randomly assigned into 2 different treatment groups. i.e. Group: 1 Carboxymethyl Cellulose (CMC), 85 patients, Dose: 0.5%, 2-4 times / day; Group: 2 Chloroquine phosphate (CHQ), 85 patients, Dose: 0.03%, twice a day. Main outcome measures included efficacy aspects viz. Lissamine Green Stain Score (LGSS), Fluorescine Stain Score (FLSS), Schirmer test, and Ocular Surface Disease Index (OSDI) - a measure of symptom frequency and impact on vision related functioning, and safety aspects such as vital signs, intraocular pressure, visual acuity, color vision and monitoring of adverse events.

Results: The most significant improvements with CHQ treatment were in LGSS from baseline (2.79±0.12) to (0.22±0.04) after treatment (p < 0.001) with the net change -2.57 (95% CI of -2.83 to -2.32). CHQ treated group also reflected significant reduction in FLSS at final visit (0.47±0.065) as compared to baseline (3.21±0.12) with a net change of -2.74 (95% CI of -3.007 to -2.451). Significant decrease in OSDI scores indicated a decrease in the effect of ocular symptoms on patients’ daily lives.

Conclusions: CHQ eye drops were found to be more effective, safe and well tolerated than artificial tears in patients with dry eye syndrome.

Keywords: Dry eye, Keratoconjunctivitis sicca, Inflammation
1. INTRODUCTION

Dry eye syndrome (DES) is “a disorder of the tear film attributable to tear deficiency or excessive evaporation that causes damage to the interpalpebral ocular surface and is associated with symptoms of discomfort.” Recent studies have revealed the inflammatory component as the main causative factor of the disorder. Cytokine and receptor mediated inflammatory cascade disintegrates the tear film layer by affecting the lacrimal gland acini and ducts and disturbs ocular surface homeostasis. Apoptosis has also been implicated in the pathogenesis of dry eyes.

Goals for treatment of patients with DES are to improve the patient’s ocular comfort and quality of life and to return the ocular surface and tear film to the normal homeostatic state. Current therapies for the management of dry eye include drugs for tear supplementation, retention, and stimulation; anti-inflammatory agents; and environmental strategies. Palliative therapies like tear substitutes are currently the most common choice of treatment but have failed to yield high success rates because they give only symptomatic improvement but do not treat underlying cause of disease. The major anti-inflammatory agents currently in use include topical corticosteroids and immunomodulatory agents. Chloroquine is a well known anti-inflammatory drug used in the treatment of rheumatoid arthritis, discoid lupus erythematosus, and amoebic hepatitis. There are studies which have shown improvement in dry eye condition when hydroxy chloroquine was used systemically for Sjogren syndrome. In addition, several studies have established the efficacy of topical chloroquine in the treatment of keratoconjunctivitis sicca in rats. These studies suggest that topical chloroquine may provide a unique opportunity to move beyond treatments that only alleviate the symptoms of dry eye disease to therapies that effectively target the inflammatory processes contributing to disease pathogenesis.

Therefore, the present study was carried out to compare the efficacy, safety and patient tolerability of topical chloroquine phosphate (0.03%) with artificial tears for the treatment of moderate-to-severe dry eye syndrome.

2. METHODS

2.1. Study Design
It was a prospective, randomized, open label, two way, split plot design study. The protocol was composed of 2 phases: a 3-week treatment phase, and a 1-week post treatment phase.

This study was conducted at Shri Krishna Hospital, Karamsad, Anand, in compliance with the institutional review board regulations (Human Research Ethics Committee) and informed consent regulations. Before initiation of study-related procedure or study medication, subject’s signed written informed consent was obtained.

2.2. Inclusion and exclusion criteria

Eligible patients were between 18 to 70 years of age. Inclusion criteria included one or more moderate dry eye–related symptoms, including itching, burning, blurred vision, foreign body sensation, dryness, photophobia, and soreness or pain i.e. a Ocular Surface Disease Index [OSDI] score between 13 and 100, best visual acuity of 6/18 or better in each eye. Both eyes were treated and included in all analysis (see statistical analysis).

Patients were excluded from study if they had any ocular disorder including ocular injury, infection, non-dry eye ocular inflammation, trauma, or surgery within the prior 6 months; any were receiving concurrent treatment that could interfere with interpretation of the study results; had any uncontrolled systemic disease or significant illness; contact lens wearer, subject that require surgical correction of dry eyes, known hypersensitivity to chloroquine, chronic alcoholics, pregnant, willing to get pregnant or nursing women or patient with Sjogren syndrome.

2.3. Study Population and Procedures

A total 340 eyes of 170 patients with bilateral dry eyes were recruited. The assignment of patients was based on simple computer-based randomization by the study statistician before the initiation of the study. An equal probability randomization procedure was used.

2.4. Sequence and duration of all study periods

All the subjects were screened and those who met all inclusion and none of exclusion criteria were recruited in this study. Patients were randomly assigned in to 2 different treatment groups i.e. Group: 1 Carboxymethyl Cellulose (CMC) (Dose: 0.5 %, 2-4 times / day), Group: 2 Chloroquine phosphate (CHQ), (Dose: 0.03%, twice a day).

All the subjects received treatment for 21 days during which they were
evaluated on visit 1 (day 0), visit 2 (day 7), visit 3 (day 14) and visit 4 (day 21). Seven days after termination of the treatment subjects were assessed on visit 5 (day 28).

2.5. Outcome Measures

After taking complete history, patients were examined in accordance with internationally accepted measurement systems i.e. Lissamine green staining score (LGSS), Fluorescein staining of cornea (FLSS), Schirmers test (without anesthesia), symptoms related ocular examination (OSDI) and safety measurements at the commencement of therapy and at every week thereafter.

2.5.1. Parameters under investigation:

2.5.1.1. Efficacy parameters

2.5.1.1.1. Objective end points: LGSS, FLSS, Schirmer’s test

Assessment of tear film and epithelial integrity was carried out by fluorescein and lissamine green dyes used to view any conjunctival and corneal epithelial abnormalities.

Lissamine green staining was performed in both eyes using impregnated lissamine green strips wetted with non preserved, balanced saline solution with results observed in the low- to moderate intensity white light of the slit lamp after 1 minute. Similarly, fluorescein was applied to the eye. Interpretation of ocular surface staining by lissamine green dye was done by van Bijsterveld grading scale \(^{15}\) and with Fluorescein dye was done by Baylor’s score \(^{1}\) to quantify the intensity of staining. Aqueous secretion was measured by schirmer test without anesthesia. \(^{16}\)

2.5.1.1.2. Subjective Endpoints: OSDI

Patient response to treatment was evaluated using the OSDI, a global assessment parameter consisting of 12 questions designed to assess the symptoms of ocular irritation consistent with dry eye disease and their impact on vision-related functioning. The questions covered three areas: ocular symptoms, environmental triggers, and vision-related function. Each question was phrased in terms of frequency and graded on a numerical scale from 0 to 4 by an OSDI score: 0= none of the time; 1= some of the time; 2= half of the time; 3= most of the time; 4= all of the time. Patient responses to all answers were then combined for a composite OSDI score ranging from 0 to 100. \(^{17}\)

2.5.1.2. Safety Parameters

Treatment safety assessments included vital signs, slit-lamp examination, best
corrected visual acuity (BCVA), macular function, intraocular pressure (IOP), color vision, dilated fundus examination, and collection of adverse events (AEs).

2.6. Statistical analysis

Efficacy variables from subjective measurements with data collected on both eyes were analyzed by averaging the data from both the eyes. Non parametric data/ Categorical variables were analyzed by Mann-Whitney ’U’ test (comparison between two groups for Lissamine green stain score, Fluorescine stain score), Kruskall-Wallis test (comparison of ranking data at different visits within a group & for comparison between two groups), and within-group changes from baseline were evaluated with the Wilcoxon signed-rank test.

3. RESULTS

A total of 49 (28.8 %) male and 121 (71.2%) female patients were reflecting mean age of 53 ± 10.5 years (22 ~ 77). There were no statistically significant differences in age, gender, and pretreatment tear film and ocular surface parameters between the two groups (Table 1).

3.1. Outcome Measures

3.1.1. Efficacy Measures

The efficacy measures were in terms of LGSS, FLSS, Schirmer test and the global assessment scoring system - OSDI.

3.1.1.1. Efficacy Analysis of LGSS

Based on our data, group mean values, as well as the magnitude of the net change in LGSS for any particular cohort were calculated. In addition, relative changes in LGSS were calculated as the percentage change from baseline.

CHQ treated group showed significant reduction in LGSS at all the visits (P<0.05). The group mean scores was found to be (2.79±0.12) at baseline and (0.22±0.04) after treatment (P< 0.001) with the net change -2.57 (95% CI of -2.83 to -2.32). It is also to be noted that CHQ also showed significant reduction in LGSS even at visit 1 & 2 (P<0.001).

CMC treatment showed significant change in mean score only at visit 3 i.e. 1.11±0.11 (V3) from 1.98±0.13 (BL) with a net change of -0.87 (95% CI of -1.21 to -1.55) (P<0.05) (Table 2).

The above analysis indicated some differences in the treatment efficacy of two treatment groups. The next step was to make more specific comparisons to see how robust (or otherwise) any such apparent differences in efficacy
might be. Treatment with CHQ showed substantial changes in terms of significant reduction in LGSS as compared to CMC (fig 1). Analysis of comparison of both the treatments vs. baseline scores on a percentage basis was 92.21% and 44.21% respectively for CHQ and CMC treatments.

3.1.1.2. Efficacy Analysis of FLSS

CHQ treated group reflected significant reduction in FLSS from 3.21±0.13 (BL) to 0.47±0.07 (V3) (p<0.001) with the net change of -2.74 (95% CI of -3.007 to -2.451). Significant reduction was noted at the end of visit 1 (Table 2). CMC treated group indicated significant reduction in FLSS only at visit 3 from 1.52±0.01 as compared to 2.91±0.14 at baseline (P <0.05) with a net change of just -0.39 (95% CI of -1.72 to -1.06) (fig 2). Further, the percentage improvement in CHQ and CMC groups were 51% and 28% respectively.

3.1.1.3. Efficacy Analysis of Schirmer test

Baseline values for schirmer tear strip wetting scores ranged from 12.43 to 12.78 in both the treatment groups. The most consistent improvement was observed in the CHQ treated group, with mean increase in wetting length of (13.39±0.41), (14.14±0.39), (14.95±0.39) and (14.98 ±0.38) mm at week one, two, three and four respectively when compared with the baseline (12.43±0.44) values. These increases approached statistical significance at week 2 (P<0.05); week 3 (P <0.001) and week 4 (P<0.001) (table 2). The significant improvement from baseline occurred in the CMC group at treatment week 3 only (P<0.05). Further, in support of above findings, a net change of 2.52 (95% CI of 1.37 to 3.67) in mean schirmer value of CHQ treated patients was observed as compared to a net change of 1.14 (95% CI of 0.36 to 1.92) with CMC group. It is to be noted that the % improvement was found higher with CHQ treatment (20%) as compared to CMC treatment (9%).

3.1.1.4. Efficacy Analysis of OSDI

Baseline OSDI scores ranged from 54 to 61 (on a scale from 0 to 100, where 0 indicates no disability and 100 indicate complete disability) in both the treatment groups. CHQ treated group reflected highly significant reduction in OSDI at visit 3 (18.71±1.5) as compared to baseline (61.42 ± 2.11) with a net change of -42.71 (95% CI of -47.4284 to -39.8) (p<0.05) (Table 2). The mean baseline score for CMC treated group changed significantly
(p<0.05) from 54.53±1.33 (BL) to 32.29±1.16 (V3) with a net change -22.24 (95% CI of -25.69 to -18.79). Both CHQ and CMC treated groups indicated significant reduction in OSDI at every visit when compared with the baseline.

Thus, substantial change in OSDI has been achieved with the use of CHQ in terms of net reduction of score. Based upon these findings, we further tried to segregate different categories of problems i.e. ocular symptoms, vision related functions and environmental triggers in patients receiving CHQ. As shown in (fig 3), mean OSDI significantly decreased from baseline to final assessment (V3) in all 3 categories of problems indicating robust improvement with CHQ treatment. Percentage improvement in CMC and CHQ treated groups were found 40.8% and 69.5% respectively.

Both the treatments were further evaluated for the % efficacy of each group across the different category of problem. As can be seen from (fig 4), CHQ treatment showed significant improvement in ocular symptoms, vision related functions and environmental triggers as compared to CMC treatment.

### 4. DISCUSSION

Occurrence of damage to ocular surface, mainly cornea and conjunctiva are the major signs observed in DES. The extent of damage to conjunctiva can be easily evaluated by LGSS, and to the cornea with the help of FLSS. So, during the treatment of DES, improvement in both the staining score is expected. Similarly, improved secretion of lacrimal gland is desirable with the improvement in disease condition which can be measured by schirmer test.

Comparing both the therapies tested, CHQ treatment produced the most consistent improvement in objective (LGSS, FLSS and Schirmer test) as well as subjective end points (OSDI). The results of the current investigation carried out on a representative sample of mild to severe dry eye sufferers, demonstrated a superior performance for CHQ than for CMC in controlling conjunctival anomalies. The ocular parameter of interest in the present study was conjunctival staining (LGSS). The presence of such staining is indicative of ocular surface desiccation leading to symptoms; the relief of staining in that area indicates that the ocular surface has returned to a normal status.
Conjunctival staining indicates epithelial cell damage and therefore, necessarily damage to the overlying gel-like mucin layer. There is incomplete coverage of the surface by an unbroken tear film at all times between blinks leading to incomplete surface lubrication, in the exposed area. The greater efficacy of CHQ than CMC can be hypothesized to be linked to anti-inflammatory effect of CHQ as compared to symptomatic relief by lubricating effect of artificial tears.

Corneal fluorescein staining was improved significantly after CHQ treatment as early as the first week. Decreasing corneal fluorescein staining is due to the suppression of inflammation enabling normal function of ocular surface. With the deterioration of dry eye, there can be some filament and piece staining in the cornea. It has been confirmed in many clinical and elementary experiments that the inflammatory factors and the marks concerned are diminished after anti-inflammatory treatment. We also found that some patient’s vision was improved when the inflammation of ocular surface was relieved.

In the support of above, slow elongation of Schirmer value was observed after the application of CHQ and significantly good effect was observed 21 days after treatment. This can be explained by decreased inflammatory factors and improved integrity of ocular surface after the application of CHQ, so the nerves of the cornea and conjunctiva can be stimulated more effectively by blinking, the reflective secretion becoming normal, then the quality and quantity of tears might get improved, which supports the improved results of lacrimal gland secretion by Schimmers test in our experiment. Further, CHQ treated group showed significant relief from symptoms for different categories namely ocular symptoms, vision related functions and environmental triggers based on the results of % OSDI score.

The lysosomotropic effects of CHQ are widely believed to be responsible for its anti-inflammatory properties and effectiveness in the treatment of some autoimmune diseases. It is reported that CHQ decreases the production of the pro-inflammatory cytokines IFN-γ, tumour necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6) in Lipopolysacharide (LPS)- or phytohemagglutinin stimulated peripheral blood mononuclear cells, and also augmented LPS-induced expression of TNF-α, IL-1α, IL-1β and
IL-6 in monocytic and microglial cells.\textsuperscript{24} CHQ also known to exert anti-inflammatory effects via non-lysosomotropic mechanisms.\textsuperscript{25} It is shown to inhibit TNF-\(\alpha\) release in macrophages through inhibition of TNF-\(\alpha\) mRNA synthesis, thereby suggesting that it can also disrupt gene transcription\textsuperscript{25-27} but does so without interfering with posttranslational modification or release of the cytokine from macrophages.\textsuperscript{28} In human histolytic U-937 cells, CHQ has shown to decrease cell surface expression of TNF-\(\alpha\) receptors by retarding their transport to the cell surface.\textsuperscript{29} The blocking of pro-inflammatory cytokines by CHQ was shown to be protective against LPS- and\textit{Escherichia coli} DNA-induced inflammatory responses and/or sepsis in mice\textsuperscript{30}. CHQ also inhibits cytokine release into human whole blood, an effect that could be beneficial in diseases that are related to bacterial-induced inflammation.\textsuperscript{31}

CHQ inhibits metalloproteases liberated by macrophages, neutrophils and the dead or dying cells. Irrespective of some controversies regarding minute details of mechanism of action, the drug has been widely used in arthritis for decades. Topical CHQ administration may offer similar advantages in dry eyes. It is already reported that the physiological, cellular, and biochemical effects of CHQ are exerted through pleiotropic mechanisms involving both lysosomotropic-dependent and independent effects. This cornucopia of mechanisms of action has seen CHQ persist on therapeutic regimens for several diseases and conditions despite its systemic toxicity and the emergence of drug resistance in malaria parasites.\textsuperscript{32}

In any ocular insult and inflammation, there is an increased epithelial turnover that exposes immature cells to UV radiations, visible light or other environmental factors. Topical CHQ is reported as protective against UV radiations, particularly UVB and UVA induced erythema in skin.\textsuperscript{33} Besides anti-inflammatory properties, CHQ could also have photo-protective effects in conditions such as lupus erythematosus\textsuperscript{34} and could be exploited in the dry eye disease via protecting localized cell mediated inflammatory processes which contribute to the development of it.

Toxicity and adverse effects of CHQ are well documented in the literature. But they are related to high cumulative systemic dose. CHQ when given topically at 0.03\% dose twice a day for 21 days, as in present study, the total
dose reaching local ocular tissue or absorbed systemically is very minute fraction of the toxic cumulative dose. Thus in particular, the superiority of CHQ was observed in both a primary objective endpoint (lissamine green staining scores) and a primary subjective endpoint (global symptom frequency scores) in the same study. The trend observed in certain secondary objective and subjective efficacy endpoints at Day 7 and/or Day 14 demonstrated the beneficial effects of the drug at and beyond the initial (7-day) endpoint observation, providing additional reinforcement to the findings in the primary endpoints. So there is no question of any local or systemic side effect specific for CHQ.

In the present study, the most important safety findings were in terms of documenting adverse effects. The safety of CHQ is well established in our study and the benefit-to-risk evaluation is overwhelmingly positive. The importance of this study in providing the scientific evidence supporting the efficacy of CHQ in the treatment of the signs and symptoms of dry eye disease is considerable. CHQ, despite its well documented toxicity and adverse effects may have important future uses that are associated with its lysosomotropic and immunomodulatory mechanisms. Thus, the rapid treatment effect realized by administration of CHQ is highly relevant in the treatment of this disease, given its propensity to irritate subjects’ eyes, affect vision, and decrease daily quality of life.

5. CONCLUSION

It can be clearly inferred from the findings that the difference in the two groups with respect to improvement was due to the CHQ treatment suggesting independent favorable effect of it in DES. The findings of this study support the continued investigation of the use of topical CHQ as a safe and effective treatment for DES.

In conclusion, Chloroquine Phosphate eye drops can be a novel therapeutic approach for the restoration of tear formation for DES.

6. Disclosure

The authors have no proprietary or commercial interest in any materials discussed in this article.

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Table 1. Disposition of Patients

<table>
<thead>
<tr>
<th></th>
<th>Group-1 CMC</th>
<th>Group-2 CHQ</th>
</tr>
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<tbody>
<tr>
<td>Patients enrolled</td>
<td>85 (100%)</td>
<td>85 (100%)</td>
</tr>
<tr>
<td>Patients completed</td>
<td>85 (100%)</td>
<td>82 (96.5%)</td>
</tr>
<tr>
<td>Patients discontinued</td>
<td>0 (0%)</td>
<td>3 (3.52%)</td>
</tr>
<tr>
<td>Adverse events</td>
<td>2 (0.8%)</td>
<td>2 (0.8%)</td>
</tr>
</tbody>
</table>

CMC = Carboxy Methyl Cellulose, CHQ = Chloroquine Phosphate.

Table 2: Changes of efficacy measures with different treatment groups

<table>
<thead>
<tr>
<th>Efficacy Measures</th>
<th>Baseline value (BL) (Day 0)</th>
<th>After treatment</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>V1 (Day 7)</td>
<td>V2 (Day 14)</td>
</tr>
<tr>
<td>Lissamine Green Stain Score (LGSS)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMC</td>
<td>1.98±0.13</td>
<td>1.24±0.2</td>
<td>1.11±0.11*</td>
</tr>
<tr>
<td>CHQ</td>
<td>2.79±0.12</td>
<td>0.72±0.07*</td>
<td>0.22±0.04*</td>
</tr>
<tr>
<td>Fluorescein Stain Score (FLSS)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMC</td>
<td>2.91±0.14</td>
<td>1.92±0.12</td>
<td>1.52±0.01*</td>
</tr>
<tr>
<td>CHQ</td>
<td>3.21±0.12</td>
<td>1.02±0.09*</td>
<td>0.47±0.07*</td>
</tr>
<tr>
<td>Schirmer test</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMC</td>
<td>12.78±0.3</td>
<td>13.77±0.2</td>
<td>13.92±0.2*</td>
</tr>
<tr>
<td>CHQ</td>
<td>12.43±0.4</td>
<td>14.14±0.3*</td>
<td>14.95±0.3*</td>
</tr>
<tr>
<td>Ocular Surface Disease Index (OSDI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CMC</td>
<td>54.53±1.3</td>
<td>37.92±1.2*</td>
<td>32.29±1.1*</td>
</tr>
<tr>
<td>CHQ</td>
<td>61.42±2.1</td>
<td>28.36±1.8*</td>
<td>18.71±1.5*</td>
</tr>
</tbody>
</table>

All data are presented as mean±SEM. Anova followed by Tukey’s test. Asterisk indicates significant difference from baseline (p < 0.05)
Table 3: Treatment Related Adverse Events

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>CMC (n=85)</th>
<th>CHQ (n=85)</th>
<th>Total Events (n=170)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conjunctival hyperemia</td>
<td>1 (0.4%)</td>
<td>1 (0.4%)</td>
<td>2 (1.17%)</td>
</tr>
<tr>
<td>Burning eye</td>
<td>1 (0.4%)</td>
<td>2 (0.8%)</td>
<td>3 (1.2%)</td>
</tr>
<tr>
<td>Pain in the eye</td>
<td>0</td>
<td>1 (0.4%)</td>
<td>1 (0.4%)</td>
</tr>
<tr>
<td>Visual disturbance</td>
<td>2 (2.35%)</td>
<td>0</td>
<td>2 (1.17%)</td>
</tr>
<tr>
<td>Total patients</td>
<td>2 (2.35%)</td>
<td>2 (2.35%)</td>
<td>4 (2.35%)</td>
</tr>
</tbody>
</table>

Figure 1. Change from baseline in Lissamine Green Stain Score

* indicates statistical significance (p<0.001), # indicates statistical significance (p<0.05)
Figure 2. Change from baseline in Fluorescine Stain Score

* indicates statistical significance (p<0.001), # indicates statistical significance (p<0.05)

Figure 3. Improvement in different types of problems for patients treated with CHQ

* indicates statistical significance (p<0.001)
**Figure 4.** Comparison of different treatment group for the efficacy (%) in Ocular Surface Disease Index score across the different category of problem