A New Long Acting Liposomal Topical Antifungal Formula: Human Clinical Study

MOHAMED S. ABD EL-RHAMAN, M.D. 1; WAEL SOLIMAN, M.D. 1; FAWZIA HABIB, Ph.D. 2 and DINA FATHALLA, Ph.D. 2

The Departments of Ophthalmology, Faculty of Medicine 1 and Pharmaceutics, Faculty of Pharmacy 2, Assiut University.

Abstract

Purpose: To study the clinical effect of topical controlled release ophthalmic fluconazole liposomal formulation on patients with candida keratitis.

Methods: This prospective uncontrolled study included 11 eyes of 11 patients with Candida Albicans keratitis (proved by cultures).

All were treated with topical liposomal fluconazole (2mg/ml) three times daily. The response to the treatment was divided into 3 categories: Complete improvement: Complete healing with scar formation at the end of one month, partial improvement: Decrease in the ulcer size at the end of one month and No improvement: Includes extension of ulcer size and or perforation which necessitates other approaches of management. The patients were examined daily over a 30-day period and results were recorded.

Results: Eleven eyes with candida albicans as proved with laboratory cultures were included in this study (7 males and 4 females). Three of the included patients in this study had diabetes mellitus and 2 patients had rheumatoid arthritis. Mean corneal ulcer diameter (mean of both horizontal and vertical diameters) was 5.5mm (ranges from 3.5 to 6.5mm). Mean duration of the ulcers at presentation was 7.6 days (ranges fro 3 to 14 days). Eight patients improved after 1 month while 1 patient had partial improvement and 2 patients did not improve and underwent amniotic membrane transplantation. One of the non improved patients progressed to perforation and keratoplasty was done. Mean best corrected visual acuity was 1/60 which was not improved at the end the study.

Conclusion: Therapy with topical liposomal fluconazole (2mg/ml) carries high success rate and fast effect in treating patients with candida albicans keratitis.

Key Words: Candida keratitis – Fluconazole liposomal formulation.

Introduction

The treatment of fungal keratitis is often disappointing because of limited tissue penetration, narrow antimicrobial spectrum, and toxicity of the currently available antifungal agents [1].

Fungal infections of the cornea, especially Candida, are considered a major problem in ophthalmology because of diagnostic and therapeutic difficulties. In a study carried out recently Candida was the most common cause of fungal endophthalmitis [2]. Fungal keratitis can impair vision or lead to total blindness. So various specific antifungal agents have been used [3]. Fungal keratitis is not rare in Egypt especially in the countryside regions, and it represents infection of the cornea that can potentially cause blindness [4]. The high incidence of fungal keratitis is due to the agricultural environment and suitable high temperature which help the abundance of fungi in the surrounding environment. Also occupational trauma with plant origin or hard object during farming, the major economic activity of the population, misdiagnosis and delayed presentation aggravate the problem. Early diagnosis and adequate dosing is essential to prevent recourse to therapeutic penetrating keratoplasty (TPK) [5].

Liposomes are artificial closed vesicles consisting of lipid bilayers (or lamellae) enclosing an equal number of aqueous compartments [6]. Vesicular drug delivery systems used in ophthalmic carrier, such as liposomes and niosomes, help in providing prolonged and controlled action at the corneal surface and preventing the metabolism of the drug by enzymes present at the tear/corneal...
The discovery of liposomes has opened new opportunities for novel applications in ocular drug delivery, their efficacy as vectors and their ability to increase ocular absorption have ensured their success. Liposomes offer advantages over most ophthalmic delivery systems in being completely biodegradable and relatively non-toxic. Fluconazole is a bis-triazole compound that exhibits a broad spectrum antifungal activity belonging to the group of triazoles. It is effective against many fungal species including Candida [8]. It acts as a fungistatic agent. The high penetration into the aqueous humour and low toxicity of fluconazole, make it a good candidate for consideration as a topical ocular antifungal agent [9]. Topical Fluconazole controlled release loaded liposomes had been tried by our group in rabbits and achieved good results [10]. This study was done as a continuation of our previously published work to show the clinical effect of the same formula that used before in rabbits in patients with candida keratitis.

Material and Methods

Preparation of fluconazole-loaded liposomes eye drops. All the steps were performed under aseptic conditions. Fluconazole liposomes were prepared using the reverse-phase evaporation technique [11]. Fluconazole eye drops were prepared by diluting the optimized liposomes preparations with Sørensen’s modified phosphate buffer pH 7 containing 0.01 % benzalkonium chloride as preservatives, so that the eye drops contained the equivalent amounts of 0.2% of fluconazole-loaded liposomes. We suspected candida keratitis in 20 patients, For all of them we stopped any previous medications for 48h then cultures for bacteria and fungi were done. After getting samples for culture, we immediately started both gatifloxacin 0.3% eye drops every hour for the first 24 hours and 4 times daily after that and with fluconazole loaded liposomes 4 times/day, cyclopentolate 1 % 3 times/day and bandage until the results of the cultures confirmed the Candida albican diagnosis. We had only 11 patients proved to have candida keratitis with laboratory cultures. So 11 patients (7 males and 4 females) with proved candida keratitis were included in this study Fig. (1).

Six of our patients gave a history of trauma by objects of plant origin during work in the countryside region.

They all underwent laboratory work up to confirm diagnosis. Three of the included patient in this study had diabetes mellitus for more than 15 years, and 2 patients had rheumatoid arthritis and were under systemic steroid therapy for more than 3 years. Mean corneal ulcer diameter (mean of both horizontal and vertical diameters) was 5.5mm (ranges from 3.5 to 6.5mm) and changed after treatment to 1.3mm (range from 0 to 6.5). The time range of healing of the ulcers was from 3 to 4 weeks. Mean duration of ulcer at presentation 7.6 days (ranges fro 3 to 14 days). Eight patients (73%) improved after 1 month while 1 patient had partial improvement (9%) and 2 patients (18%) did not improve and underwent amniotic membrane transplantation. One of the non improved patients progressed to perforation one month after amniotic membrane transplantation and keratoplasty was done. Mean snellen decimal notation best corrected visual acuity was 0.06 which was minimally improved to 0.08 improved at the end the study. (Table 1) As noted by patients, the eye drops exhibited stinging sensation at the beginning of its use. Healing ulcer was associated with heavy dense scarring in some patients.

The response to the treatment was divided into 3 categories:

1- Complete improvement: Complete healing with scar formation at the end of one moth.

2- Partial improvement: Decrease in the ulcer size at the end of one month.

3- No improvement: Includes extension of ulcer size and or perforation which necessitates other approaches of management.

The patients were admitted to the hospital and were examined daily over a 30-day period by slit lamp for signs of improvement the hypopyon and the size of the ulcer. Best corrected visial acuity in decimal notation was measured before starting of the treatment and after one month. Photography was done before starting of the treatment and one month after to document the signs of improvement. The occurrence of complications such as desmatocele, corneal perforation, resistant corneal ulcer, atrophia bulbi and endophthalmitis were noted.
Fig. (1): This figure shows the coloured photography of the cornea before and 1 month after topical treatment by liposomal fluconazole (2mg/ml) three times daily. Patients from 1 to 4 show improvements of the proved Candida Albicans fungal ulcer. Patient 5 and 6 shows no signs of improvement at the end of one month.

Table 1: Showing demographic data, ulcer characteristics and visual changes before and after treatment.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Duration of ulcer at presentation (days)</th>
<th>Response to treatment</th>
<th>Diameter of ulcer at presentation (mm)</th>
<th>Diameter of ulcer after treatment for one month (mm)</th>
<th>Best corrected visual acuity before treatment</th>
<th>Best corrected visual acuity after treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>65</td>
<td>5</td>
<td>improved</td>
<td>3.5</td>
<td>0</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>47</td>
<td>7</td>
<td>improved</td>
<td>4</td>
<td>0</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>44</td>
<td>14</td>
<td>partial improvement</td>
<td>5</td>
<td>2</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>56</td>
<td>9</td>
<td>improved</td>
<td>4.5</td>
<td>0</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>34</td>
<td>1</td>
<td>improved</td>
<td>5</td>
<td>0</td>
<td>0.03</td>
<td>0.05</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>54</td>
<td>3</td>
<td>no improvement</td>
<td>5.5</td>
<td>6.5</td>
<td>0.05</td>
<td>0.02</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>60</td>
<td>11</td>
<td>no improvement</td>
<td>5</td>
<td>6</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>67</td>
<td>8</td>
<td>improved</td>
<td>6</td>
<td>0</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>9</td>
<td>F</td>
<td>39</td>
<td>7</td>
<td>improved</td>
<td>6.5</td>
<td>0</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>10</td>
<td>F</td>
<td>50</td>
<td>9</td>
<td>improved</td>
<td>4</td>
<td>0</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>61</td>
<td>4</td>
<td>improved</td>
<td>6</td>
<td>0</td>
<td>0.07</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Discussion

The availability of an effective and convenient treatment for fungal keratitis has become an urgent need. Several azole compounds are available for the treatment of Candida infections, including miconazole, ketoconazole, fluconazole and itraconazole [12].

Fluconazole is a new triazole compound that has certain properties suggesting its use for the treatment of fungal keratitis. It acts against all pathogenic Candida species except Candida krusei [13].

The effect of topical fluconazole in experimental Candida keratitis (in-vitro) or on human eyes which proved to have Candida keratitis were studied [12,14,15]. However, reports of its use in clinical Candida keratitis are scarce.

The fluconazole eye drops were administered every 30min or 1hr for 7-21 days depending on the severity [14,15].
To overcome the tedious process of frequent instillation, the possibility of toxicity and to accomplish patient compliance, fluconazole-loaded liposomes were prepared in the present study to exert a sustained release effect of the drug. Our previous in-vitro results showed that liposomes had encouraging results in induced Candida albican infection of the rabbits’ corneas using only 3 times instillation regimen [10]. Therefore, the liposomal formulations were instilled in this study three times daily for the period of treatment.

In current study, seventy three percent of patients showed a positive response with no local or systemic side effects. Decrease in numbers of instillation, complete healing of ulcers, emphasizes the effectiveness of the drug in liposomes against deep Candida infection of the cornea.

We think that late presentation and extensive damage to the corneal stroma at the time of presentation were responsible for partial or non improvement of ulcers in at least two of the three cases.

Although there was minimal improvement of the visual acuity because the healing process of the ulcer was associated with dense scarring in some patients but the great role of liposomes loaded with fluconazole was in controlling the fungal infection, allow healing and preserving the structural integrity of the globe.

The results obtained are in accordance with those of the many researchers who found that fluconazole applied topically produced a significant antifungal effect [14].

Our previous published comparative study proved that the antifungal activity of fluconazole in liposomal formulation was better than that of fluconazole in solution [10]. Also It was reported previously that liposomal encapsulation increases lipid solubility and hence permeability through the cell membrane. Moreover, the liposomes may enhance the binding of the drug to the active sites inside the cells [16].

In the present study, some of the most important benefits of the preparation of fluconazole in the form of liposomes were decreased instillation frequency, better patient compliance and faster recovery from candidiasis. This gives us a hope that the incidence of the other infections and complications that may occur because of the long time required for an antifungal to start working may be diminished.

The results presented here showed that the use of topical liposomes fluconazole (2mg/ml) as a drug delivery system was successful in treatment of C. albicans infection of the human cornea.

The idea of fluconazole-loaded liposomes is a new one, and although the efficacy of topically applied eye drops of liposomes containing fluconazole in human mycotic keratitis has shown encouraging results, but the limitation of this study is the small number of the included eyes and a clinical trail of a large series of patients, conducted in a randomized controlled manner, is desirable.

References


