Journal of Clinical & Experimental Ophthalmology
ISSN: 2155-9570

Solaiman et al., J Clin Exp Ophthalmol 2015, 6:4
DOI: 10.4172/2155-9570.1000469

Research Article Open Access

# Topical Voriconazole Drops With and Without Intrastromal Voriconazole Injection for Treatment of Deep or Resistant Fungal Keratitis

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**Received date:** Jun 12, 2015, **Accepted date:** Aug 26, 2015, **Published date:** Aug 31, 2015



## Abstract

**Purpose:** To compare the safety and efficacy of topical voriconazole drops with and without intrastromal voriconazole injection for treatment of deep and/or resistant fungal keratitis.

**Patients and methods:** A prospective, randomized and comparative study performed on eyes with deep and/or resistant fungal keratitis. Eyes were randomly allocated into two groups according to their order of presentation. Group (A) included eyes treated with intrastromal injection(s) of voriconazole (50 µg/0.1 ml) plus voriconazole eye drops 1% and group (B) included eyes treated with topical voriconazole eye drops 1% alone. Healing of the fungal keratitis was considered as the primary outcome measure. Secondary outcome measures included any reported complication as well as the visual outcome.

**Results:** Each group included 20 eyes with deep and/or resistant fungal keratitis. Complete healing of fungal keratitis was higher in group A (85%) than in group B (55%) and the difference was statistically significant ($P<0.05$). The duration of healing ranged between 2-4 weeks in group A and between 2-6 weeks in group B ($P>0.05$). Laboratory studies showed increased prevalence of filamentous fungi (70% in group A and 65% in group B) more than yeasts (30% in group A and 35% in group B), and the difference between both groups was statistically not significant ($P>0.05$).

**Conclusion:** Voriconazole eye drops might be effective for treatment of deep and/or resistant fungal keratitis. Adding intrastromal injection to topical drops could significantly raise the healing rate and hasten the resolution period without significant complications related to injection.

## Introduction

Fungal keratitis is an important cause of corneal blindness and usually carries an unfavorable prognosis due to its prolonged course and requirement of a specific therapy [1]. Therapeutic failure rates in fungal keratitis are very high, and the poor outcome is related to several factors including: late diagnosis, reduced ocular drug penetration and low antifungal susceptibility of certain etiologic agents [2].

Voriconazole, a triazole antifungal, is available commercially for systemic administration in the form of oral and intravenous formulations. It has an excellent broad spectrum antifungal activity and is active against species that are known to be resistant to the other antifungal agents commonly used in fungal keratitis. Topical voriconazole eye drops, used in an off-label manner, have also been prescribed for the treatment of keratitis with promising results. With topical administration, voriconazole demonstrated good penetration through the cornea into the aqueous humor, without compromising intraocular safety [3].

Ayala-Lugo [4] has used targeted delivery of voriconazole by intrastromal injection to treat cases of deep-seated recalcitrant fungal keratitis responding poorly to conventional treatment modalities. He injected voriconazole 50 micrograms/0.1 ml circumferentially around the fungal abscess in the corneal stroma as an adjunctive to the topical antifungal therapy with good results.

The aim of this study is to compare the safety and efficacy of topical versus intrastromal injection plus topical voriconazole for treatment of deep and/or resistant fungal keratitis.

## Patients and Methods

A prospective randomized study that included eyes with deep and/or resistant fungal keratitis. Eyes were randomly allocated between two groups in their order of presentation: group A included eyes which received intrastromal injection of voriconazole plus topical voriconazole eye drops and group B included eyes which received topical voriconazole eye drops without intrastromal injection. An informed consent was obtained after detailed explanation for the patients. The inclusion criteria were proven fungal keratitis by direct smear and/or culture, infiltrates involving ≥ 1/2 depth of corneal thickness (up to midstromal level or deeper) and/or evidence of resistance to other conventional antifungal therapy for at least 2 weeks. The exclusion criteria included perforated corneal ulcers, impending corneal perforation, total corneal involvement, corneal melting, associated endophthalmitis and any known allergy to voriconazole.

All patients were subjected to a full history taking which included risk factors for fungal keratitis such as history of trauma (mainly

vegetable matter trauma), contact lens wear (type, duration and solution), prolonged use of topical steroids and history of surgical procedures, mainly corneal surgeries. Duration of keratitis and the previous drugs used for treatment were also reported. Complete ophthalmic examination was performed especially the cornea and anterior segment. B-scan ultrasonography was done if posterior segment could not be visualized to exclude associated endophthalmitis.

Corneal scraping from the base and edge of the ulcer was done, under topical anesthesia, to all patients after stoppage of all antimicrobials for at least 48 hours. The scraped material was subjected to direct smear and Sabouraud dextrose agar culture. The fungal growth was used to form a film stained with Giemsa and Gram stains for visualization of the different types of fungi microscopically (direct film).

Voriconazole (VFEND; Pfizer Inc, New York, USA) is available as 200 mg of white lyophilized powder in a glass vial. Voriconazole is stable under regular storage temperatures (+4°C and room temperature), and exposure to light does not cause its degradation [5]. Voriconazole, intrastromal injection and/or eye drops , was started as soon as the diagnosis was confirmed both clinically and by smears and/or cultures, along with topical antibiotic eye drops (moxifloxacin hydrochloride 0.5%) to treat any associated bacterial infection and topical mydriatic cycloplegic eye drops (atropine sulphate 1%) along with systemic non-steroidal anti-inflammatory drugs.

### Preparation and injection of intrastromal voriconazole

Under complete aseptic conditions, voriconazole powder was divided in Epindorf tubes; each containing 2 mg of dry lyophilized powder and was reconstituted in the laminar flow apparatus (Micro Safety Cabinet) with 4 ml of lactated Ringer solution to obtain a concentration of 500 µg/ml (50 µg/0.1 ml). The reconstituted solution was loaded in one ml tuberculin syringes with 27-gauge needles where each syringe contained 0.5 ml of the reconstituted solution which was kept under complete aseptic conditions.

After topical and local anesthesia, the preloaded voriconazole was injected into cornea under the operating microscope according to the technique described by Prakash et al. [6]. With the bevel down, the needle was inserted obliquely from the uninvolved, clear cornea to reach just flush to the ulcer at the mid-stromal level (as the intended level for drug deposit). Then, the drug was injected and the amount of corneal hydration was used as a guide to assess the area covered. After achieving the desired amount of hydration, the plunger was withdrawn slightly to ensure discontinuation of the capillary column and thus prevent back-leakage of the drug. Three to five divided doses were given around the ulcer to form a deposit of the drug around the circumference of the lesion to form a barrage of intrastromal voriconazole around the entire ulcer. The total amount of drug injected intrastromally ranged from 0.05 ml to 0.1 ml for each injection. Intraoperative complications; if any, were reported.

So long as there was clinical improvement no more injections were given. If no signs of clinical improvement were observed within three days, another injection was given with three successive injections as a maximum. If no response occurred after 3 injections, the treatment was considered as a failure and no more injections were given. On the other side, the infection was considered resolved and treatment was successful when there were complete healing of epithelial defects, resolution of stromal infiltrations and scar formation.

### Preparation of voriconazole eye drops

The 200 mg lyophilized powder in the voriconazole glass vial is reconstituted with 19 ml of sterile distilled water for injection to produce a 20 ml aqueous voriconazole solution with a concentration of 10 mg/ml (1%). Aseptic preparation of voriconazole eye drops (1%) was done in the laminar flow apparatus (Micro-Safety Cabinet). Reconstituted voriconazole eye drops were aseptically instilled in sterile droppers which were kept under complete aseptic conditions. Voriconazole 1% eye drops were given once hourly and were continued for at least 2 weeks after complete resolution of the infection.

After initiation of treatment, follow up was done on daily basis for one week and then twice weekly until either complete resolution (success of treatment) or stoppage of treatment and failure.

Statistical analyses were made using the Statistical Package for Social Science version 18 (SPSS Inc., Chicago, IL, USA). Testing the differences between the two groups was done by the Chi square test and Fisher's exact test for qualitative data. Quantitative data were expressed as mean and standard deviation (SD) and t-test was used for the differences between the two groups. P value less than 0.05 was considered significant.

## Results

The study included 40 eyes with deep and/or resistant fungal keratitis, that were randomly distributed between the two groups according to their order of presentation: group (A) included 20 eyes which received intrastromal injection of voriconazole plus topical voriconazole eye drops and group (B) included 20 eyes which received topical voriconazole eye drops alone. Patients' demographic data are shown in Table 1 with no statistically significant difference between both groups as regards age, sex and residency. Table 2 shows the risk factors in all patients with no statistically significant differences between both groups of the study as regards all risk factors. The most common risk factor in both groups was ocular trauma; either with vegetable or non-vegetable trauma (11/20 in group A and 8/20 in group B). Three eye in group A and four eyes in group B had more than one risk factor. No risk factor could be detected in 3 eyes in each group.

| | Group A | Group B | P Value |
|---|---|---|---|
| Age (years): Range | 19-70 | 10-72 | 0.22 |
| Mean ± SD | 47.3 ± 17.3 | 40.2 ± 18.6 | |
| Sex : Male | 14 (70%) | 12 (60%) | 0.51 |
| Female | 6 (30%) | 8 (40%) | |
| Residency : Rural | 18 (90%) | 15 (75%) | 0.41 |
| Urban | 2 (10%) | 5 (25%) | |

| | Group A | Group B | P Value |
|---|---|---|---|
| Vegetable trauma | 8 | 4 | 0.16 |
| Non vegetable trauma | 3 | 4 | 1.0 |
| Diabetes mellitus | 2 | 3 | 1.0 |
| Prolonged topical steroid | 2 | 0 | 1.0 |
| Contact lens wear | 1 | 4 | 0.34 |

| | | | |
|---|---|---|---|
| Ocular surgery | 3 | 3 | 1.0 |
| Recurrent herpetic keratitis | 1 | 1 | 1.0 |
| Trichiasis | 0 | 1 | 1.0 |
| Immunosuppression | 0 | 1 | 1.0 |
| More than risk factor | 3 | 4 | 1.0 |
| No risk factor | 3 | 3 | 1.0 |
| P>0.05=non-significant | | | |

**Table 2:** Risk factors in patients of both groups of the study.

The mean time from the onset of symptoms till start of voriconazole treatment in both groups of the study was 20.48 ± 4.22 days in group A and 18.82 ± 3.65 days in group B (P>0.05). All eyes of the study had corneal ulceration with deep stromal infiltrates before initiation of voriconazole treatment. Findings of anterior chamber on presentation were reported in table 3 with no statistically significant difference between all findings in both groups (P>0.05). Hypopion with level was found in 4 eyes in group A and in 6 eyes in group B, while peaked hypopion was found in 7 eyes in group A and in 4 eyes in group B. Anterior chamber granuloma was detected in 1 eye only in group A. The results of direct films and cultures (Table 4) were Aspergillum species (12 eyes in group A and 11 eyes in group B), Candida species (6 eyes in group A and 7 eyes in group B) and Fusarium species (2 eyes in group A and 2 eyes in group B) with no statistically significant difference between both groups (P>0.05).

| | Group A | Group B | P Value |
|---|---|---|---|
| Hypopion with level | 4 | 6 | 0.46 |
| Peaked hypopion | 7 | 4 | 0.28 |
| Granuloma | 1 | 0 | 1.0 |
| P>0.05=non-significant | | | |

**Table 3:** Anterior chamber findings on presentation in patients of both groups.

| | Group A | Group B | P Value |
|---|---|---|---|
| *Aspergillus* | 12 | 11 | 0.74 |
| *Candida* | 6 | 7 | 0.73 |
| *Fusarium* | 2 | 2 | 1.00 |
| P>0.05=non-significant. | | | |

**Table 4:** Results of direct films among study groups.

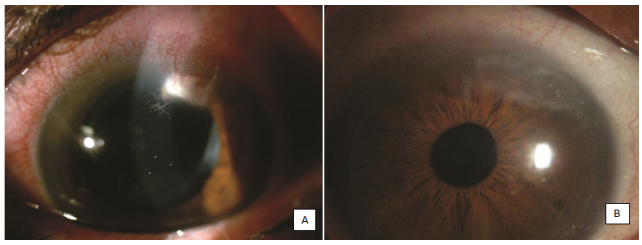

**Figure 1**: (A) Fungal infection (*Candida* keratitis) in the corneal tunnel following phacoemulsification. (B): Complete resolution of fungal keratitis 2 weeks after intrastromal injections of voriconazole.

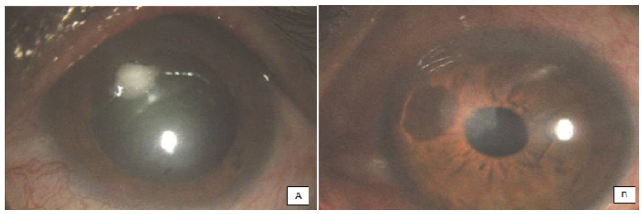

**Figure 2:** (A) Fungal (*Candida*) keratitis and stromal infiltrates (2 × 2 mm) after trauma. (B) Three weeks after 2 intrastromal injections one week apart with complete resolution of stromal infiltrates and healing of epithelial defect.

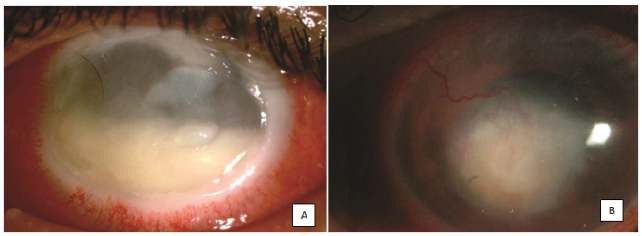

**Figure 3:** (A) Resistant Fusarium keratitis in an agricultural worker following a vegetable trauma showing diffuse and deep stromal infiltrations, central thick plaque, epithelial defects and peaked hypopion of about 2 months duration. (B): Patient response after 5 weeks and three intrastromal injections of voriconazole one week apart with adjuvant voriconazole eye drops 1 % showing complete resolution of stromal infiltrates and hypopion and healing of epithelial defects with central vascularized leucoma and a single large blood vessel which is characteristic for fungal keratitis.

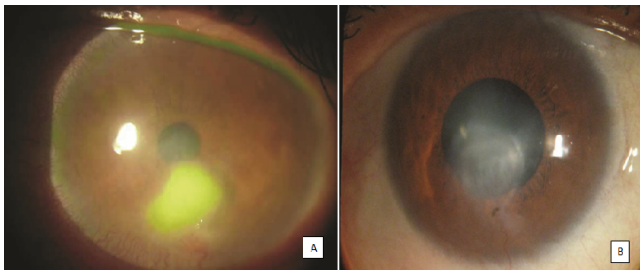

**Figure 4:** (A): candida keratitis showing ulcer and stromal infiltrates (3 × 3 mm). B): shows complete healing of fungal keratitis with the characteristic gutter 3 weeks after intrastromal voriconazole injection eye drops.

Healing of fungal keratitis was statistically significant higher in group A than in group B (P<0.05), Table 5. In group A, 17 eyes (85%) responded adequately to intrastromal injection of voriconazole plus 1% voriconazole eye drops with complete healing and resolution, including the 2 eyes with Fusarium keratitis (Figures 1-4), while 3 eyes (15%) failed to respond adequately to treatment and were considered failure. The numbers of intrastromal injections were one injection in 5 eyes (25%), two injections in 12 eyes (60%) and 3 injections in 3 eyes (15%). No complications related to injection were recorded. In group B, 11 eyes (55%) responded adequately to 1% voriconazole eye drops with complete healing and resolution, while 9 eyes (45%) did not respond adequately to 1% voriconazole eye drops and were considered failure. Of the 9 patients of group B who did not respond to voriconazole eye drops 1% alone, four eyes showed complete resolution of the infection when received intrastromal injections as adjunctive therapy. The 2 eyes with Fusarium keratitis in group B did not respond to 1% voriconazole eye drops and did not respond also after adding intrastromal voriconazole injection.

The duration of healing ranged between 2 and 4 weeks in group A and between 2 and 6 weeks in group B (Table 6), with no statistical significant difference between both groups of the study (P>0.05). The changes in the best corrected visual acuity (BCVA) on presentation and after treatment in both groups of the study are illustrated in (Table 7). The mean duration of follow up after starting voriconazole treatment was 5.75 ± 2.31 weeks in group A and was 6.92±3.02 weeks in group B (P>0.05).

| | Group A | Group B | P Value |
|---|---|---|---|
| Healing | 17 (85%) | 11 (55%) | 0.03 |
| Failure | 3 (15%) | 9 (45%) | |
| P<0.05=Significant | | | |

**Table 5:** Outcome of treatment in group A and group B.

| Duration of healing (weeks) | Group A | Group B | P Value |
|---|---|---|---|
| 2 | 7 (41.2%) | 3 (27.3%) | 0.68 |
| 3 – 4 | 10 (58.8%) | 7 (66.8%) | 0.43 |
| 6 | 0 (0%) | 1 (5.9%) | 1.00 |

**Table 6:** Duration of healing in healed eyes of both groups.

| | Group A | | Group B | |
|---|---|---|---|---|
| | BCVA on presentation | BCVA at last visit | BCVA on presentation | BCVA at last visit |
| HM | 18 (90%) | 3 (15%) | 11 (55%) | 2 (10%) |
| CF-<0.1 | 2 (10%) | 16 (80%) | 8 (40%) | 16 (80%) |
| ≥ 0.1 | 0 (0%) | 1 (5%) | 1 (5%) | 2 (10%) |
| BCVA: Best Corrected Visual Acuity; HM: Hand Movement; CF: Counting Fingers | | | | |

**Table 7:** Comparison between BCVA on presentation and at last visit in group A and group B.

## Discussion

Fungal infections of the cornea are usually difficult to treat. Superficial keratomycosis fairly responds to topical antifungal therapy as natamycin 5% suspension. However, treatment of deep fungal keratitis is challenging [7]. In the last few years, broad spectrum antifungal agents such as voriconazole have been tried and also alternate routes of administrasion such as intracorneal injections have been used to treat deep and/or resistance fungal keratitis. Al-Badriyeh et al. [8] have demonstrated the clinical benefit of topical voriconazole when used alone as primary and salvage therapies in two case reports. Bunya et al. [9] reported that voriconazole has been successful when amphotericin B or fluconazole have been unsuccessful in cases of drug-resistant fungal keratitis and endophthalmitis. Arora et al. [10] evaluated the efficacy of topical 1% voriconazole versus 5% natamycin in treatment of 30 patients with fungal corneal ulcers. They concluded that topical 1% voriconazole was a safe and effective drug in primary management of fungal keratitis. Its efficacy was matching conventional natamycin. Intracorneal injection of the antifungal agent provides maximum concentration of the drug at the target site and this increases its killing effect. Intrastromal injections are easy to prepare and inject and can be repeated several times safely with no learning curve [11].

The method of voriconazole reconstitution described in this study is economically very valuable as it saves the fresh lyophilized powder as it is for further reconstitution of either intracorneal injections or topical eye drops. Each 2 mg of the lyophilized powder can produce 4 ml when reconstituted and this is sufficient for 8 injections. So, each voriconazole vial of (Vfend 200 mg) can be used to prepare about 800 injections. Prakash et al. [6] and Sharma et al. [12] on the other hand, used a different method of reconstitution that was sufficient for only one injection and was very costly.

In this study, voriconazole, intrastromal injection and/or eye drops, was started as soon as the diagnosis was confirmed clinically and by smears and/or cultures. No other antifungal agent was used concurrently to detect the response to voriconazole as a single antifungal medication. However, in other studies [6,12] the other antifungal eye drops were not stopped and were maintained after intrastromal injections of voriconazole for their patients with deep and/or resistant fungal keratitis. Thus, evaluation of the efficacy of intrastromal voriconazole as a single treatment modality in their cases was doubted.

In this study, topical voriconazole eye drops in a concentration of 1% was used. This is because Al-Badriyeh et al. [8] reported that the concentration of voriconazole in the aqueous humor resulting from the 2% voriconazole eye drops was not significantly different from that

reported for the 1% solution, suggesting that the penetration of voriconazole is not concentration-dependent, at least for the concentration range studied.

Healing of fungal keratitis was statistically significantly higher ($P<0.05$) in eyes of group A, where 17 eyes (85%) showed complete healing and resolution compared to 11 eyes (55%) in group B. Of the 9 patients of group B who did not respond to voriconazole eye drops 1% alone, four eyes showed complete resolution of the infection when received intrastromal injections as adjunctive therapy. This proves that intrastromal injection is more effective than topical drops in treatment of resistant fungal keratitis especially that with deep infiltrations.

As regards the duration of healing of fungal keratitis, healing was more rapid in group A where 41.2% showed complete healing in the first 2 weeks, compared to 27.3% in the same period in group B. This proves that adding intrastromal injection to topical voriconazole drops shortens the duration of healing of fungal keratitis as well as the period of hospitalization. Jones et al. [13] reported that the average length of healing by natamycin eye drops as a topical antifungal was 39 days which is longer than the mean duration of healing in this study.

In the present study, 4 cases of Fusarium keratitis were reported; two of them were successfully treated with intrastromal voriconazole (group A) while the other two in group B failed to respond to voriconazole eye drops and the further intrastromal injections of voriconazole. Heidar et al. [14] have also reported successful treatment of 3 cases of recalcitrant Fusarium keratitis with intastromal voriconazole and topical voricoazole administration.

No complications related to intrastromal voriconazole injection were recorded during the study. Repeated intrastromal injections of voriconazole (50 µg/0.1 ml) were tolerated with no signs of ocular toxicity. No side effects caused by voriconazole eye drops were reported in this study. This matches with the clinical study by Al-Badriyeh et al. [8] who reported no side effects with 2% voriconazole eye drops. However, in the study by Vemulakonda et al. [15], only two patients reported a mild transient stinging sensation on instillation of the 1% voriconazole eye drops.

In conclusion, Voriconazole, either eye drops or intrastromal injection could be effective for treatment of deep and/or resistant fungal keratitis. Adding intrastromal injection to topical drops could significantly raise the healing rate and hasten the resolution period without significant complications related to injection. This is economically better as it shortens the period of hospitalization, with faster return of patients to their usual activity.

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