

## The Efficacy of Voriconazole Eye Drops Reconstituted in Sterile Water for Injection

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### Abstract

**Purpose:** To investigate the efficacy of voriconazole 1% eye drops reconstituted in sterile water for injection and stored in the fridge or room conditions and correlate the results to bibliographic data.

**Methods:** Voriconazole powder was reconstituted with water for injection, in order to prepare a 1% solution. The solution was separated in sterile vials stored in the fridge or in the cupboard. Malt extract agar solutions were prepared with the addition of this voriconazole solution to achieve concentrations of 1 µg/ml, 2 µg/ml and 4 µg/ml and the same procedure was repeated after one and after two weeks. Petri plates were inoculated with standardized solutions of *Candida albicans*, *Aspergillus fumigatus*, *Alternaria alternata*, *Fusarium solani* and *Fusarium oxysporum*. The fungal growth was compared to the control plates, which contained only malt extract agar without voriconazole.

**Results:** The voriconazole solutions remained effective for more than two weeks (18 days), independently of the storage conditions. The concentration of 1µg/ml was effective against *Candida albicans* and *Aspergillus fumigatus*, the 2 µg/ml against *Alternaria alternata* (and until the 17<sup>th</sup> day also against *Fusarium oxysporum* but not on the 18<sup>th</sup> day) and the 4 µg/ml against *Fusarium oxysporum* but not against *Fusarium solani*.

**Conclusion:** The reconstituted with sterile water for injection voriconazole eye drops are effective against various fungi. *Fusarium solani* was resistant up to the concentration of 4 µg/ml. The susceptibility of the examined fungi varies and thus, considerations should be raised about the combination with other routes of administration in order to achieve the appropriate drug concentrations in the eye. There was no significant difference in the effectiveness of voriconazole solutions in relation to the storage conditions.

**Keywords:** Voriconazole; Eye drops; Endophthalmitis; Keratitis; Fungal infections; Candida; Aspergillus; Alternaria; Fusarium; Storage

### Introduction

Voriconazole is a newer triazole antifungal medication (a synthetic derivative of fluconazole), which inhibits the synthesis of ergosterol in cellular membranes, and consequently the growth of the microorganism. It has a wide spectrum of activity (wider, in comparison to other older triazoles) with low minimum inhibitory concentration (MIC<sub>90</sub>) and it is the drug of choice for various mycoses [1,2].

Voriconazole is active not only against systemic infections, but also against local ocular infections. Ophthalmic mycoses can cause loss of vision, and also can be life-threatening [1]. For this reason, voriconazole has been used recently, not only for the treatment of keratitis caused by several fungal species, but also for endophthalmitis [3,4]. There is evidence, that voriconazole eye drops are effective in the treatment of fungal keratitis, potentially due to its broad spectrum of coverage, good tolerability, excellent bioavailability and high drug concentrations in the ocular surface. Administration of voriconazole

eye drops is successful, safe and well tolerated by the eye. Corneal penetration into the aqueous humor of the eye is adequate, but its penetration into the vitreous humor is not adequately evaluated and more studies are needed [1,5]. Furthermore, the intravitreal injection of voriconazole seems to be safe and effective in some cases of fungal endophthalmitis [6,7,8].

The systemic use of voriconazole can present complications (e.g. drug interactions and side-effects) and is also costly [9]. The topical application might be preferable in many cases.

Voriconazole is provided only in intravenous or oral formulations, while there is not a commercially available eye drop solution [3]. Eye drops can be prepared by using the branded product for systemic use.

This study was focused on the in vitro efficacy of voriconazole against various fungi. The effectiveness and the storage conditions of voriconazole eye drop solutions, reconstituted in sterile water for injection, in various concentrations, are evaluated in this open-label study.

## Materials and Methods

Voriconazole powder (Vfend; Pfizer Limited, Sandwich, Kent, UK) was reconstituted with water for injection, so as to produce a 1%, voriconazole solution (10 mg/ml). The solution was separated in sterile vials and was preserved in the fridge or in the cupboard under ambient room conditions (20°C-24°C).

Malt extract agar solutions were prepared with the addition of voriconazole, in order to achieve three different final concentrations of the drug at day 0 of the study:

Solution A: 1 µg/ml (15 µl in 150 ml)

Solution B: 2 µg/ml (30 µl in 150 ml)

Solution C: 4 µg/ml (60 µl in 150 ml)

Malt extract agar is considered an effective mycological culture medium, permitting an easy and rich fungal growth.

Petri plates were inoculated with a standardized solution of 0.5 McFarland of *Candida albicans* in water for injection (further diluted at 1:2000) or solutions of 70% light transmittance (at 530 nm wavelength) of either, *Aspergillus fumigatus*, *Alternaria alternata*, *Fusarium solani* and *Fusarium oxysporum* in water for injection and

further diluted at 1:100. The inoculum solutions were prepared according to the CLSI proposals for the sensitivity testing of yeasts and moulds [9,10].

A week later, new malt extract agar solutions were produced from the same stored voriconazole vials (stored in the fridge and the cupboard) and new Petri plates were inoculated under the same conditions.

The same procedure was followed once more, two weeks after the beginning of the experiment.

Fungal growth on each plate was compared to control plates, containing only malt extract agar without voriconazole. In order to evaluate the culture results they were graded with - when there was not any growth, with ± when there was a small or mild growth and with + when there was a full growth. The plates were read for three consequent days at each week after incubation at 30°C.

## Results

The results were the same for both storage conditions (refrigerated or ambient) of the reconstituted eye drop solutions and are expressed in Table 1.

Fungus	Voriconazole concentration	1 <sup>st</sup> week 2 <sup>nd</sup> day	1 <sup>st</sup> week 3 <sup>rd</sup> day	1 <sup>st</sup> week 4 <sup>th</sup> day	2 <sup>nd</sup> week 2 <sup>nd</sup> day	2 <sup>nd</sup> week 3 <sup>rd</sup> day	2 <sup>nd</sup> week 4 <sup>th</sup> day	3 <sup>rd</sup> week 2 <sup>nd</sup> day	3 <sup>rd</sup> week 3 <sup>rd</sup> day	3 <sup>rd</sup> week 4 <sup>th</sup> day
<i>Candida albicans</i>	1 µg/ml	-	-	-	-	-	-	-	-	-
	2 µg/ml	-	-	-	-	-	-	-	-	-
	4 µg/ml	-	-	-	-	-	-	-	-	-
<i>Aspergillus fumigatus</i>	1 µg/ml	-	-	-	-	-	-	-	-	-
	2 µg/ml	-	-	-	-	-	-	-	-	-
	4 µg/ml	-	-	-	-	-	-	-	-	-
<i>Alternaria alternata</i>	1 µg/ml	-	±	+	-	±	+	-	±	+
	2 µg/ml	-	-	-	-	-	-	-	-	-
	4 µg/ml	-	-	-	-	-	-	-	-	-
<i>Fusarium solani</i>	1 µg/ml	+	+	+	+	+	+	+	+	+
	2 µg/ml	±	+	+	+	+	+	+	+	+
	4 µg/ml	-	±	+	-	±	+	-	+	+
<i>Fusarium oxysporum</i>	1 µg/ml	-	±	+	-	±	+	-	±	+
	2 µg/ml	-	-	-	-	-	-	-	-	±
	4 µg/ml	-	-	-	-	-	-	-	-	-

no growth: -, mild growth: ±, full growth: +

**Table 1:** Strength of fungal growth accordingly to the drug concentration and the time.

Voriconazole in concentration of 1 µg/ml (solution A) was effective against *Candida albicans* and *Aspergillus fumigatus* for more than two weeks (18 days).

The 2 µg/ml solutions (solutions B) were also effective against *Alternaria alternata* and *Fusarium oxysporum*, however on the 18<sup>th</sup>

day the efficacy of the solutions was questionable against *Fusarium oxysporum*.

The 4 µg/ml solutions (solutions C) were effective against all the above fungi and against *Fusarium oxysporum* but not against *Fusarium solani*.

During the first week, a growth on the second day for *Fusarium solani* at solutions A and B was observed and on the third day at solution C. *Fusarium oxysporum* grew only at solution A on the third day and the same was with *Alternaria alternata*. There was not any growth for *Aspergillus fumigatus* or *Candida albicans*.

During the second week, *Fusarium solani* was presented with a full growth on the second day at solutions A and B and on the third day at solution C. *Fusarium oxysporum* had a mild growth at solution A on the third day and this growth became more eminent on the fourth day, but still only at solution A.

The same happened with *Alternaria alternata*, while *Aspergillus fumigatus* and *Candida albicans* didn't present any growth at all.

The same as above were also the results during the third week, except for *Fusarium oxysporum* that had also a growth at solution B on the fourth day.

## Discussion

The results of this study indicate that the prepared solutions of voriconazole, irrespectively of the storage conditions, are in vitro effective against *Candida albicans*, *Aspergillus fumigatus*, *Alternaria alternata* and *Fusarium oxysporum*, as it is shown in Table 1. Only *Fusarium solani* presented a full growth at all tested concentrations.

The combination of these results with the existing data from the work of other investigators, leads to the extraction of very useful treatment considerations.

Current literature provides evidence on the penetration of voriconazole into the eye and its effectiveness for the treatment of fungal ocular infections.

Dupuis D et al. have reported that voriconazole 1% eye drop solution remains chemically and visually stable, without any significant variation in the osmolarity and pH for at least 30 days in whatever storage conditions were used. Furthermore, exposure to light did not cause any degradation of the drug [3]. Amorós-Reboredo P et al. reported that frozen 1% voriconazole ophthalmic solution at -20°C was stable for 90 days, and after thawing remained stable at 5°C for 14 days. The clearness, pH and osmolarity of the solutions remained unchanged, regardless of the solution temperature [11]. Al-Badriyeh D et al. showed that voriconazole 1% solution, preserved with 0.01% benzalkonium chloride, was stable at 2-8°C for at least 14 weeks, while voriconazole 2% solution was stable for 16 weeks at 2-8°C or at 25°C and for 8 weeks at 40°C [12].

The eye drops of voriconazole 1% in our study were unpreserved, and independently of the storage conditions, in the fridge or at room conditions, they retained their efficacy for more than two weeks (18 days) after the reconstitution.

As regards the eye infections, fungal keratitis is a severe condition, which potentially can lead to blindness. The main causes of fungal keratitis are *Fusarium*, *Candida* and *Aspergillus* species as well as *Scedosporium* or *Alternaria* and the variety of effective antifungal agents and the clinical outcomes are poor [1]. Moreover, intraocular infections, such as endophthalmitis, can be treated very difficultly. Topical administration of voriconazole is potentially very useful for the treatment of fungal keratitis and perhaps for prophylaxis against the development of fungal endophthalmitis and in many cases, voriconazole eye drops are used in combination with systemic formulas [13,14,15].

Vemulakonda GA et al. studied 13 patients and obtained aqueous and vitreous samples after topical administration of 1% voriconazole every 2 hours for 24 hours before surgery. The mean  $\pm$  SD voriconazole levels in the aqueous humor were  $6.49 \pm 3.04$   $\mu\text{g/ml}$ . Furthermore, the mean  $\pm$  SD voriconazole levels in the vitreous humor were  $0.16 \pm 0.08$   $\mu\text{g/ml}$  [4]. In relation to our results, this application with eye drops only, can achieve satisfactory concentrations of the drug in the aqueous humor. However, it is not adequate to give sufficient concentrations in the vitreous cavity.

Hariprasad SM et al. obtained plasma, vitreous, and aqueous samples from 14 patients after oral administration twice of 400 mg of voriconazole, in a period of 12 hours, before surgery. The mean  $\pm$  SD voriconazole levels were  $2.13 \pm 0.93$   $\mu\text{g/ml}$ ,  $0.81 \pm 0.31$   $\mu\text{g/ml}$  and  $1.13 \pm 0.57$   $\mu\text{g/ml}$ , respectively. This study reports that orally administrated voriconazole could achieve therapeutic levels in aqueous and vitreous humors in no inflamed human eyes, for a wide spectrum of microorganisms, including *Aspergillus*, *Candida* and other fungal causes of endophthalmitis and thus, oral administration of voriconazole could be very useful and efficacious for the management and prophylaxis of fungal endophthalmitis [16]. In relation to our results, this application may achieve therapeutic concentrations in the aqueous against *Candida albicans* and *Aspergillus fumigatus*, questionably against *Alternaria alternata* and not against *Fusarium oxysporum* and *Fusarium solani*. As regards to the vitreous concentrations, they are even lower and thus are expected to be less effective.

Thiel MA et al. showed that combined oral and topical administration of voriconazole resulted in drug concentration  $>2.93$   $\mu\text{g/ml}$  in aqueous humor, which is efficacious against many fungi. On the other hand, when voriconazole is used topically, the drug concentration in aqueous humor is  $>0.61$   $\text{mg/ml}$ , which is above the MIC<sub>90</sub> for the majority of *Candida* species. Thus, the topical application of voriconazole alone is not effective for some less susceptible fungi and it is recommended a combined topical and systemic administration as initial treatment, until the causative microorganism is identified [17]. Accordingly and in relation to our results, *Alternaria alternata* and potentially *Fusarium oxysporum* are also sensitive to this combined oral and topical application. The resistance of *Fusarium solani* is a problem as has been shown in an interesting work of Oechsler RA et al. *Fusarium solani* isolates had significantly higher resistance to voriconazole than the non-solani isolates requiring a concentration of 16  $\mu\text{g/ml}$  [18].

Another important issue is the half-life of voriconazole. Shen YC et al. estimated the rapid decline of voriconazole after an intracameral injection. The half-life of the drug in the aqueous humor was 22 minutes [19] and this rapid decline raises certain considerations about the required frequency of administration. The same authors, after the intravitreal injection of 35  $\mu\text{g}/0.1$  ml voriconazole, estimated a half-life of 2.5 hours [20].

Furthermore, Wei LC et al. in an experimental study found that by increasing the frequency of drops, the drug concentration in the aqueous and in the vitreous increases significantly, especially in eyes with previous mechanical epithelium debridement (scraped corneas). The every 5 minutes application for 30 minutes schedule gave concentrations of  $33.44 \pm 5.77$   $\mu\text{g/ml}$  in the aqueous of the non-scraped and  $57.67 \pm 6.77$   $\mu\text{g/ml}$  in the aqueous of the scraped eyes. With the dosing schedule they followed after this initial installation by installing drops for four additional times every 20 minutes, the aqueous concentration ranged from 19.97 to 23.70  $\mu\text{g/ml}$  5 min after

these doses in the non-scraped eyes and from 44.44 to 49.02 µg/ml in the scraped eyes while the vitreous concentrations remained significantly lower and ranged from 0.38 to 0.49 µg/ml in the non-scraped eyes and from 0.72 to 0.94 µg/ml in the scraped eyes [21]. These results indicate that the frequent topical application may lead to significantly higher concentrations in the aqueous.

Consequently the therapeutic approach of fungal infections is rather complex. The topical application of voriconazole carries various considerations about the frequency of installation and the duration of the treatment. Combined application increases the drug efficacy. The intravitreal application, for increased concentrations of the drug in the eye, is also an interesting therapeutic approach and more experience has to be gained. The drug monitoring of voriconazole is a useful adjunct.

In conclusion, the solution of voriconazole 1% (reconstituted eye drops in sterile water for injection), proved to be effective in several concentrations, independently of the storage conditions (refrigerator or room storage), for more than two weeks (18 days). In relation to the results of the present study and according to the involved fungus, the topical administration of voriconazole should be adjusted appropriately, so as to achieve the required therapeutic levels of the drug in the eye.

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