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Resistance to Voriconazole in HIV/AIDS Patients with Histoplasmosis Treated with Fluconazole

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Abstract

Background: Voriconazole is often used as an alternative to itraconazole in patients with histoplasmosis because of intolerance or drug interactions to itraconazole. A recent retrospective study showed mortality to be higher in patients treated with voriconazole. Other studies have shown higher MICs to voriconazole than itraconazole raising concern about its effectiveness for treatment of histoplasmosis.

Methods: Primary and failure isolates from 17 patients with HIV/AIDS treated with fluconazole were evaluated for susceptibility to other triazoles. Primary and failure isolates from one of the patients were subjected to *in vitro* induction of resistance to voriconazole.

Results: At least 4-fold increases in MIC occurred to fluconazole in isolates from 10 patients and to voriconazole in 7 patients. Induction of resistance by *in vitro* exposure to voriconazole causes significant increase in MIC of primary and failure isolates.

Conclusion: These findings suggest that higher early mortality in patients treated with voriconazole may be caused by *in vivo* increases in MICs induced during treatment with voriconazole.

Keywords: Itraconazole; Voriconazole; Fluconazole; Posaconazole; Isavuconazole; Histoplasmosis; Resistance

Introduction

Itraconazole is the treatment of choice for mild to moderate of histoplasmosis and step-down treatment following a 1-2 weeks course of amphotericin B in severe disease [1]. The first prospective study of itraconazole for treatment of histoplasmosis in patients with HIV/AIDS reported successful outcomes in 85% [2]. The next prospective study evaluated HIV/AIDS patients with histoplasmosis treated with fluconazole and reported that 49% failed treatment with 59% of whom developed resistance to fluconazole [3,4].

Voriconazole is active *in vitro* against Histoplasma capsulatum but clinical experience establishing its effectiveness is meager. A retrospective study reported the experience using voriconazole as "salvage" therapy in patients who were intolerant of itraconazole (5 patients), failed treatment with other antifungal agents (2 patients) or other reasons (2 patients) [5]. Six of the patients were immunocompromised. Three patients showed improvement and six remained stable. Treatment was subsequently changed to itraconazole in another because of persistent antigenuria. A study evaluating voriconazole as salvage therapy for histoplasmosis included two patient who failed treatment [6].

A recent study comparing outcome of treatment for histoplasmosis with itraconazole or voriconazole reported higher mortality during the first 42 days in those treated with voriconazole [7]. The authors concluded that "itraconazole remains the mainstay of treatment and is considered the standard of care for the treatment of histoplasmosis". The authors also stated, "until more is known about outcomes when voriconazole is used to treat histoplasmosis, other azoles should be used preferentially."

Antifungal susceptibility testing has been performed on the primary and failure isolates from HIV/AIDS patients in prospective treatment studies [8,9]. The initial and failure isolates from one of these patients were evaluated for development of increasing resistance by *in vitro* exposure to voriconazole.

Materials and Methods

The isolates had been stored in liquid nitrogen at Mira Vista since 1996 [2]. One patient's primary and failure isolates were chosen to try to induce resistance by exposing them to increasing concentrations of voriconazole in HMM (Histoplasma Minimal Media). The primary isolate was exposed to concentrations ranging from 0-1.0 mcg/ml and the failure isolate to 0-8 *mcg/ml*. Flasks were incubated at 37ºC in shakers at 150 rpm. First each H. capsulatum yeast isolate was grown in HMM without antifungals for 2-3 days (until turbid). At that point 10 milliliters (mL) of culture was removed and centrifuged at 4000 rpm. Cell pellets were resuspended in 0.5 mL media and inoculated into a flask containing 50 mL HMM with Histoplasma growth factor and 0.007 *mcg/mL* voriconazole. One mL of this suspension was removed and used for a spectrophotometric reading. The flask was then incubated at 37ºC in a shaker incubator until an increase in turbidity was observed at which time additional spectrophotometric readings were obtained and compared to initial readings. Growth was observed microscopically to ensure that turbidity was caused by

actively growing and dividing H. capsulatum yeast and that no contamination was seen.

This same process was repeated stepwise with doubling concentrations of voriconazole in each subsequent flask. At each step, 0.1 mL of yeast cultures were inoculated on HMM agar plates containing the same doubling concentrations of voriconazole used in liquid media to assess for resistance development. In addition, yeast from each step was frozen in liquid nitrogen and were subjected to simultaneous MIC testing for voriconazole, fluconazole, itraconazole, isavuconazole and posaconazole. Paired T-test was used to compare the MICs from the same isolates pre- and post- fluconazole failure.

Results

MICs to voriconazole for the primary and failure isolates from 17 patients who failed fluconazole therapy are presented in Table 1. After failing fluconazole therapy, the average MIC to fluconazole increased from 1.397+1.338 to 15.867+19.977 (p=0.008). Similarly, MIC to voriconazole increased from 0.039+0.080 to 0.139+0.240 (p=0.04). In contrast primary and failed isolates had comparable MICs to itraconazole (0.159+0.018v. 0.014+0.017, p=0.332). At least 4-fold increases in MIC occurred to fluconazole in 10 and voriconazole in 7 but not to itraconazole. No increase in MICs to posaconazole or isavuconazole was observed. The increases in fluconazole MICs ranged from 4-64 mcg/mL and to voriconazole from 4-16 mcg/mL.

The primary and failure isolate from patient 2 in Table 1 was subjected to induction of resistance to voriconazole *in vitro* in Table 2. In the primary isolate no increase in MIC occurred to itraconazole but 128-fold increases were induced to fluconazole and voriconazole. In the failure isolate, a 4-fold increase in MIC (0.007-0.030 mcg/mL) was induced to itraconazole, a 32-fold increase to voriconazole and greater than 8-fold to fluconazole.

Discussion

Whether resistance will develop during treatment to voriconazole, as with fluconazole, has not been determined. However, results of these *in vitro* studies suggest that resistance will develop and may be responsible for treatment failure, as described in several cases, and the higher early mortality in patients treated with voriconazole than itraconazole [7]. Itraconazole remains the treatment of choice for mild to moderate cases of histoplasmosis not requiring hospitalization [1].

The resistant isolates remained fully susceptible to posaconazole and isavuconazole [10]. Posaconazole was as effective as amphotericin B for treatment of histoplasmosis in a murine model of histoplasmosis in immunocompetent and immunocompromised mice [11]. Posaconazole was used successfully as salvage treatment in five patients with disseminated and one with pulmonary histoplasmosis who failed treatment with amphotericin B and other triazole antifungal agents including voriconazole [6].

Isavuconazole is highly active *in vitro* against Histoplasma capsulatum, (Table 1) [9]. It was successful in four of seven (57%) patients with histoplasmosis in a clinical trial to evaluate treatment of rare fungal infections [12]. There are no reports evaluating isavuconazole for treatment of histoplasmosis in animal models or prospective studies in humans.

Patient	Flu MIC Primary	Flu MIC Failure	Fold Increase	Vori MIC Primary	Vori MIC Failure	Fold increase	Itra MIC Primary	Itra MIC Failure	Fold Increase	Isav MIC Primary	Isav MIC Failure	Fold Increase	Posa MIC Primary	Posa MIC Failure	Fold Increase
1	0.50	16.0	32	0.015	0.125	8	0.010	0.010	0	0.007	0.007	0	0.007	0.007	0
2	1.0	16.0	16	0.015	0.250	16	0.010	0.010	0	0.007	0.007	0	0.007	0.007	0
3	1.0	16.0	16	0.007	0.007	0	0.010	0.010	0	0.007	0.007	0	0.007	0.007	0
4	2.0	64.0	32	0.250	1.000	4	0.010	0.010	0	0.015	0.015	0	0.007	0.007	0
5	1.0	16.0	16	0.015	0.125	8	0.010	0.010	0	0.007	0.015	0.008	0.007	0.007	0
6	0.50	32.0	64	0.007	0.250	32	0.010	0.010	0	0.015	0.015	0	0.007	0.007	0
7	2.0	64.0	32	0.250	0.250	0	0.080	0.080	0	0.007	0.007	0	0.007	0.007	0
8	0.50	4.0	8	0.015	0.030	2	0.010	0.010	0	0.007	0.007	0	0.007	0.007	0
9	0.50	2.0	4	0.015	0.060	4	0.010	0.010	0	0.007	0.007	0	0.007	0.007	0
10	1.0	1.0	0	0.015	0.015	0	0.010	0.010	0	0.007	0.007	0	0.007	0.007	0
11	0.50	0.5	0	0.007	0.015	2	0.010	0.010	0	0.007	0.007	0	0.007	0.007	0
12	0.50	2.0	4	0.007	0.007	0	0.010	0.010	0	0.007	0.007	0	0.007	0.007	0
13	4.0	16.0	4	0.004	0.060	16	0.010	0.010	0	0.007	0.007	0	0.007	0.007	0
14	4.0	8.0	2	0.030	0.150	4	0.010	0.010	0	0.007	0.007	0	0.007	0.007	0
15	4.0	4.0	0	0.004	0.015	4	0.010	0.010	0	0.007	0.007	0	0.007	0.007	0
16	0.50	8.0	16	0.004	0.007	2	0.010	0.010	0	0.007	0.007	0	0.007	0.007	0

17	0.25	0.25	0	0.004	0.004	0	0.040	0.010	0	0.007	0.007	0	0.007	0.007	0

Table 1: Resistance in failure isolates from patients treated with fluconazole.

Antifungal	Original MIC mcg/mL	Induced with Voriconazole	Fold increase
Primary isolate			
Itraconazole	0.007	0.007	0
Fluconazole	0.500	64.0	128
Voriconazole	0.007	1.000	128
Failure isolate			
Itraconazole	0.007	0.030	4
Fluconazole	8.0	>64.0*	>8*
Voriconazole	0.125	4.00	32
*The non-induced MIC in the failure isolate was 8 mcg/mL and increased to >64 mcg/mL, the highest concentration tested. Consequently, the exact increase could not be determined.			

Table 2: Induction of resistance by incubation with voriconazole.

CONCLUSION

In summary, itraconazole is the preferred triazole for treatment of histoplasmosis and posaconazole is the best alternative in patients unable to take itraconazole. Voriconazole is not recommended because of development of resistance observed *in vitro* and higher early mortality in patients with histoplasmosis. Isavuconazole is probably an acceptable alternative but prospective studies are required to establish its role for treatment of histoplasmosis.

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