ISSN: 2475-6296

Research Article

Journal of Clinical & Experimental Immunology

Voriconazole is Less Effective than Other Triazoles for Treatment of Histoplasmosis

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Submitted: 24 Sept 2021; **Accepted**: 29 Sept 2021; **Published**: 05 Oct 2021

Citation: *Melinda Smedema, Michelle Durkin, Heather Largura, Deborah Blue, Andrew Hanzlicek, et al. (2021) Voriconazole is Less Effective than Other Triazoles for Treatment of Histoplasmosis. Clin Exp Immunol, 6(5): 362-366.*

Abstract

Background: *Voriconazole is used to treat patients with histoplasmosis who are intolerant to itraconazole because of potential drug interactions. Hendrix reported higher mortality in patients treated with voriconazole than itraconazole raising the question if the Histoplasma isolates became less susceptible to voriconazole during treatment in those who failed treatment. Methods: Primary and failure isolates from a patient who failed treatment with fluconazole were incubated with increasing concentrations of voriconazole.*

Results: *In vitro exposure of the patient's primary isolate to voriconazole increased its MIC from 0.007 mcg/mL to 1.0 mcg/mL (128-fold). Exposure of the failure isolate increased the MIC from 0.125 mcg/mL to 4 mcg/mL (32-fold). Exposure to voriconazole did not increase the MIC of the primary isolate to itracunazole but did increase the MIC of the failure isolate from 0.007 to 0.030 mcg/mL (4-fold).*

Conclusion: *In vitro exposure Histoplasma capsulatum increased MICs to voriconazole 32 to 128-fold which could be a cause of treatment failure reported by Hendrix.*

Keywords: Histoplasma, Voriconazole, MIC, Susceptibility

Introduction.

A prospective study evaluated fluconazole for treatment of disseminated histoplasmosis in HIV/AIDS patients. Fifty-seven percent failed treatment [1] and relapse isolates were less susceptible to fluconazole and voriconazole [2]. To determine whether the changes in susceptibility to fluconazole were caused by alterations in the active site of the CYP51 protein a matched pair of initial and relapse isolates were analyzed [3]. A single amino acid substitution at the tyrosine 136 was identified [3]. Freifeld reported MICs in primary and relapse isolates of 17 patients who failed fluconazole treatment [4]. Concentrations ranged from undetectable to 8 mcg/mL but were >0.125 mcg/mL in 6 of the 17 (35.3%) patients.

Freifeld reported that three patients improved and six remained stable in a retrospective study of voriconazole. Eight patients were previously treated with itraconazole or fluconazole and one was initially treated with voriconazole [5]. Reasons for treatment with voriconazole included intolerance of itraconazole (N = 4), noncompliance (N = 1), increasing antigen (N = 1), low itraconazole level (N = 1), and treatment failure (N = 1). The ninth patient received voriconazole and amphotericin B for 6 weeks as initial therapy and was classified as improved.

Our concern about the effectiveness of voriconazole for treatment of histoplasmosis is supported by a clinical study [6]. Hendrix reported that previously treated patients with histoplasmosis who received "stepdown" therapy with voriconazole experienced higher mortality than those treated with itraconazole [6]. Two hundred sixty-one patients were identified from 2002-2017 and 86 (32%) were excluded who were not treated with itraconazole or voriconazole. One hundred seventy-five were analyzed, including 158 (90%) who received itraconazole and 19 (10%) voriconazole. Underlying conditions including HIV/AIDS in 25% and none in 41%. Fifty-five percent presented with disseminated, 71% pulmo-

nary, 41% gastrointestinal and 13% skin involvement. Likelihood of treatment with voriconazole or itraconazole or being immunocompromised were similar. Death occurred in 32% of patients treated with voriconazole (6/19) and 23% treated with itraconazole (41/175). Death during the first 42 days of treatment was 4.3 times higher with voriconazole and itraconazole (P = 0.015) but was not different beyond 42 days. The authors concluded "that voriconazole was associated with increased mortality in the first 42 days compared to itraconazole". They acknowledged the small number of subjects and retrospective design were limitations of the study.

We report results of an experiment to determine effects of in vitro exposure of H. capsulatum to voriconazole from a single patient who was previously described [3] on susceptibility and mechanisms for reduced susceptibility to fluconazole and voriconazole [3] .

Case Report

A 39-year-old patient with HIV/AIDS infection presented with 4 weeks of fever, cough, dyspnea, and weight loss [7]. Chest radiograph showed diffuse interstitial infiltrates. Organisms resembling Histoplasma capsulatum were seen by fungal stain of bronchoalveolar lavage fluid and H. capsulatum was isolated from lavage fluid and blood.

Treatment with fluconazole 600 mg daily was administered in an investigational protocol (AIDS Clinical Trials Group 174/Mycoses Study Group 23) and the patient improved. Monthly fungal blood cultures were required by the protocol and were positive for low numbers of organisms at weeks 8 and 12.

At week 16 fever recurred and examination revealed splenomegaly. Blood cultures grew numerous colonies of H. capsulatum. Fluconazole serum concentration was 37.4 mcg/mL.

Fluconazole was discontinued and amphotericin B was administered daily for two weeks, every other day for six weeks and then weekly for four months for treatment of relapsing histoplasmosis. Stepdown treatment was administered with itraconazole 200 mg twice daily for two years without recurrence or isolation of H. capsulatum fungal blood cultures.

Methods

Induction of resistance: The yeast isolate from this patient had been stored in liquid nitrogen since 1996. The primary and failure isolates were removed from the liquid nitrogen freezer, thawed, and grown on Brain Heart Infusion (BHI) agar slants. They were inoculated into Histoplasma Minimal Media (HMM) and incubated in a shaker incubator at 37° for 3-4 days after which turbidity was measured using a Spec 20 spectrophotometer.

50 mL of HMM was added to nine 250 mL flasks and voriconazole was added to achieve doubling concentrations from 0 to 1.0 mcg/mL for the primary isolate and 0 to 16 mcg/mL for the failure isolate. The flask was then incubated at 37ºC in a shake incubator until increase in turbidity was observed by comparison of the OD in the negative control flask.

Growth was observed microscopically to ensure turbidity was due to actively dividing H. capsulatum yeast and that contamination was not observed. Ten mL was removed from each flask, centrifuged, cultured, resuspended in freezing media and frozen at -80°C until the isolates from all 18 flasks could be tested together.

Susceptibility testing

The NCCLS method was modified for use to accommodate slow growth of H. capsulatum. First, the H. capsulatum inoculum was standardized by comparison to a McFarland standard of 5.0 at 530 nm, then diluted 1:100. Candida parapsilosis ATCC 90018 was prepared according to the NCCLS method, and diluted 1:2000. The second modification of the NCCLS protocol was to increase incubation from 96–120 hours. Growth was scored by comparison to controls grown without voriconazole. At least 80% inhibition compared with the no-drug control was defined as the MIC.

Results

Results of susceptibility testing of isolates from 17 patients who failed fluconazole therapy to other triazoles are presented in table 1. Increase in MICs were seen to fluconazole and voriconazole but not itraconazole, isavuconazole, or posaconazole.

The mean MIC to voriconazole increased from 0.039 + 0.080 to 0.139 + 0.240 (p=0.04). At least 4-fold increases in MIC occurred to voriconazole in 7 and fluconazole in 10 patients. Results of the induction experiment are shown in table 2. The MIC for voriconazole was 0.015 mcg/mL in the primary isolate and 0. 250 mcg/mL in the failure isolate. The MIC of the primary isolate to fluconazole was 1 mcg/mL compared to 16 mcg/mL for the failure isolate. The MIC of the primary isolate to itraconazole was 0.007 and 0.03 mcg/mL for the failure isolate.

Discussion

Results of a retrospective study of voriconazole are shown in table 3 [5]. Voriconazole was given following treatment with other antifungal agents in eight patients as initial treatment in one patient. Nine patients treated with voriconazole were improved (N=3) or stable (N=6). All patients improved in response to amphotericin B or itraconazole before starting voriconazole [6]. None relapsed while receiving voriconazole despite low blood levels. The authors did not comment on the role of voriconazole for treatment of histoplasmosis.

We show MICs to voriconazole and fluconazole increased following exposure to voriconazole, table 2. No increase occurred to itraconazole in the primary isolate but a fourfold increase from <0.007 mcg/mL to 0.03 mcg/mL occurred in the failure isolate, which remains within therapeutic range of >1-2 mcg/mL [8]. Concentrations >0.5-2 mcg/mL are recommended for treatment of endemic mycoses [8].

The mechanism of resistance of H. Capsulatum to fluconazole is known [3, 7]. PCR fingerprinting with three random primers showed identical amplification patterns for the primary and failure isolate in the patient whose isolates were used in the induction experiment.

Comparison of the CYP51Ap amino acid sequences from a flu-

conazole-susceptible primary (MIC 1 mg/L) and relapse isolate exhibiting reduced susceptibility to fluconazole (MIC 16 mg/L) identified a single substitution in the relapse isolate. Cytochrome P450-dependent enzymes 14 alpha demethylase (CYP51p) and 3-ketosteroid reductase caused by the Y136F mutation were reduced [7]. Tyrosine at position 136 was replaced by phenylalanine (Y136F) [8]. The same mechanism is presumed to be responsible for increasing voriconazole MICs.

Isavuconazole also may be used for treatment of histoplasmosis [9, 10]. Some suggested drug level monitoring is unnecessary with isavuconazole [11]. A study of serial monitoring of isavuconazole levels during prolonged treatment reports gastrointestinal toxicity at levels above 4.9 mcg/mL and that target levels should be between 2.5 and 5 mcg/mL, supporting the need for drug level monitoring. Another study proposed a therapeutic range between 2 and 5 mcg/mL [12, 13].

In summary, MICs to voriconazole increase during *in vitro* exposure into a range that may not be effective, but the results are based isolates from a single patient. Therapeutic drug level monitoring is recommended when using itraconazole, posaconazole, voriconazole or isavuconazole for treatment of histoplasmosis [8, 14].

Table 1: Susceptibility in primary and failure isolates from patients treated with fluconazole

Patient	Flu2 MIC Primary	Flu MIC Failure	Fold Increase MIC	Vori MIC Primary	Vori MIC Failure	Fold increase MIC	Itra MIC Primary	Itra MIC Failure	Fold Increase MIC	Isav MIC Primary	Isav MIC Failure	Fold Increase MIC	Posa MIC Primary	Posa MIC Failure	Fold Increase MIC
1	0.50	16.0	32	0.015	0.125	8	0.007	0.007	0	0.007	0.007	0	0.007	0.007	0
23	1.0	16.0	16	0.015	0.250	16	0.007	0.030	3	0.007	0.007	0	0.007	0.007	0
3	1.0	16.0	16	0.007	0.007	0	0.007	0.007	0	0.007	0.007	0	0.007	0.007	0
4	2.0	64.0	32	0.250	1.000	4	0.007	0.007	0	0.015	0.015	0	0.007	0.007	0
5	1.0	16.0	16	0.015	0.125	8	0.007	0.007	0	0.007	0.015	0.008	0.007	0.007	0
6	0.50	32.0	64	0.007	0.250	32	0.007	0.007	0	0.015	0.015	0	0.007	0.007	0
7	2.0	64.0	32	0.250	0.250	0	0.007	0.007	0	0.007	0.007	0	0.007	0.007	0
8	0.50	4.0	8	0.015	0.030	2	0.007	0.007	0	0.007	0.007	0	0.007	0.007	0
9	0.50	2.0	4	0.015	0.060	4	0.007	0.007	0	0.007	0.007	0	0.007	0.007	0
10	1.0	1.0	0	0.015	0.015	0	0.007	0.007	0	0.007	0.007	0	0.007	0.007	0
11	0.50	0.5	0	0.007	0.015	2	0.007	0.007	0	0.007	0.007	0	0.007	0.007	0
12	0.50	2.0	4	0.007	0.007	0	0.007	0.007	0	0.007	0.007	0	0.007	0.007	0
13	4.0	16.0	4	0.004	0.060	16	0.007	0.007	0	0.007	0.007	0	0.007	0.007	0
14	4.0	8.0	2	0.030	0.150	4	0.007	0.007	0	0.007	0.007	0	0.007	0.007	0
15	4.0	4.0	0	0.004	0.015	4	0.007	0.007	0	0.007	0.007	0	0.007	0.007	0
16	0.50	8.0	16	0.004	0.007	2	0.007	0.007	0	0.007	0.007	0	0.007	0.007	0
17	0.25	0.25	0	0.004	0.004	0	0.007	0.007	0	0.007	0.007	0	0.007	0.007	0
Mean	1.40	15.87	14.47	0.04	0.14	6.00	0.007	0.008	0.00	0.01	0.01	0.00	0.01	0.01	0.00
Median	1.00	8.00	8.00	0.02	0.06	3.00	0.007	0.007	0.00	0.01	0.01	0.00	0.01	0.01	0.00
S.D.	1.34	19.98	17.23	0.08	0.24	8.40	0.02	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00

[1]These results were published [4, 8] and summarized here

[2]Abbreviations: Flu-Fluconazole, Vori-voriconazole, Itra-itraconazole, Isav-isavuconazole, Posa-posaconazole

[3]Isolate subjected to in vitro exposure to voriconazole $p<0.05$, paired T-test

Table 2: Effect of incubation with voriconazole on MICs of H. capsulatum

Antifungal	MIC mcg/mL before exposure	MIC mcg/mL following exposure	Fold increase
Primary isolate			
Voriconazole	0.007	1.000	128
Fluconazole	0.5	64.0	128
Itraconazole	0.007	0.007	0
Failure isolate			
Voriconazole	0.125	4	32
Fluconazole	8	>64.0	>8
Itraconazole	0.007	0.03	4

Table 3: Outcome in immunocompromised patients with disseminated histoplasmosis treated with voriconazole [5].

Prior antifungal therapy	Reason for switch to voriconazole	Response within the first 2 months	Reason vori discontinued
Itra, flu	Increasing antigen	Improved	Completed therapy (315D)1
AmB, itra	Intolerance	Stable	Completed therapy (293D)
AmB	Intolerance	Stable	Cost (22D)
Itra	Noncompliance	Improved	Completed therapy (559D)
AmB, flu, itra	Low itra level	Stable	Persistent antigenemia, switched to itra (151D)
Itra	Intolerance	Stable	Costs (243D)
AmB	Persistent signs and symptoms	Stable	Completed therapy (31D)
Itra	Toxicity	Stable	Completed therapy (91D)
AmB, vori	Primary therapy	Improved	Completed therapy (640D)

[1]D=days

Author Contributions

Melinda Smedema: performed in-vitro experiments, writing, and editing of parts of the manuscript.
Michelle Durkin: performed in-vitro experiments, writing, and editing of parts of the manuscript.
Heather Largura: study design, writing, and editing the manuscript
Deborah Blue: study design, writing, and editing the manuscript
Andrew Hanzlicek: study design, writing, and editing the manuscript
Lawrence J Wheat: study design, data analysis, writing, and editing the manuscript
Chadi A. Hage: study design, data analysis, writing, and editing the manuscript

Conflicts of Interest: Melinda Smedema, Michelle Durkin, Heather Largura, Deborah Blue, Andrew Hanzlicek, Lawrence J Wheat are employees of MiraVista Diagnostics the provider of H. capsulatum antigen test. MiraVista does not perform fungal susceptibility testing or drug level monitoring.

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