Medical Mycology Case Reports 19 (2018) 30–32

Contents lists available at ScienceDirect

Medical Mycology Case Reports

journal homepage: www.elsevier.com/locate/mmcr

Emerging fluconazole resistance: Implications for the management of cryptococcal meningitis

Edward Mpoza[a], Joshua Rhein[a,b], Mahsa Abassi[a,b,*]

[a] Infectious Diseases Institute, Makerere University, Kampala, Uganda
[b] University of Minnesota, 420 Delaware Street SE, Minneapolis 55455, USA

ARTICLE INFO

Keywords:
Cryptococcus
Cryptococcal meningitis
Antifungal susceptibility
Fluconazole resistance
Cryptococcal meningitis relapse

ABSTRACT

We present the case of an HIV-seropositive individual with cryptococcal meningitis who was found to have a fluconazole resistant strain of *Cryptococcus neoformans*. The individual required multiple rounds of amphotericin and fluconazole 800–1200 mg after several episodes of clinical relapse. Cerebrospinal fluid sterilization was achieved and maintained with high doses of fluconazole. This case demonstrates the emerging dilemma of increasing rates of fluconazole resistance in *Cryptococcus* and the clinical difficulties in meningitis management.

1. Introduction

Cryptococcus neoformans is an opportunistic pathogen that gives rise to most cases of AIDS-related fungal meningitis worldwide [1]. Initial infection occurs after respiratory inhalation with pulmonary infiltration, followed by subclinical disease with detectable antigenemia by latex agglutination assays for a median of at least 3 weeks, prior to dissemination into the central nervous system (CNS) [1]. The global prevalence of cryptococcal antigenemia, in HIV-seropositive persons with CD4 cell count < 100 cells/μL, is estimated at 6%, with 223,100 (95% CI, 150, 600-282, 400) incident cases of cryptococcal meningitis (CM) occurring annually [2]. Annual deaths from cryptococcal meningitis are estimated at 181,100 (95% CI 119, 400-234, 300), accounting for 10–15% of AIDS-related mortality [2].

The mainstay therapy for cryptococcal meningitis, presently, begins with induction therapy with amphotericin and flucytosine for 2-weeks [3]. In Sub-Saharan Africa, where most cryptococcal cases occur, flucytosine is not licensed, available, or affordable [4].

Therefore, therapy options are reduced to amphotericin and fluconazole, or when amphotericin is not available, fluconazole monotherapy. At the end of induction therapy, consolidation therapy typically consists of fluconazole 400–800 mg daily for 8 weeks, followed by maintenance therapy with fluconazole 200 mg daily for greater than 1 year [3].

Antifungal susceptibility of Cryptococcus isolates in primary cryptococcal infection is not routinely recommended because antifungal resistance has not yet been a clinically significant problem. However, in cases of either persistent or relapsed cases of cryptococcal meningitis, isolates are recommended to be checked for antifungal susceptibility [3]. Established Cryptococcus breakpoints for fluconazole susceptibility have minimum inhibitory concentrations (MIC) of ≤ 8 μg/mL as susceptible, MIC 16–32 μg/mL as dose-dependent susceptible, and MIC ≥ 64 μg/mL as resistant [5]. We present a case of cryptococcal meningitis in the setting of fluconazole resistance; highlighting factors that give rise to fluconazole resistance as well as difficulties in the management of fluconazole resistant cryptococcal meningitis in settings where alternative antifungals are not available.

2. Case

A 50 y/o HIV-seropositive man presented to an HIV clinic in Kampala, Uganda on day 0 with persistent headaches, stiff neck, and vomiting. He was diagnosed with cryptococcal meningitis based on symptoms and a positive serum cryptococcal antigen (CrAg+). A lumbar puncture (LP) was not performed and he was given 2-weeks of fluconazole 400 mg/daily as monotherapy for the treatment of cryptococcal meningitis and initiated on antiretroviral therapy (ART) with tenofovir, lamivudine, and nevirapine. He had a temporary resolution of his symptoms.

Two months later (day 60) he was referred to our HIV clinic with a recurrence of headaches, neck stiffness, and vomiting. At this time, workup revealed a CD4 cell count of 32 cells/μL and a viral load of 34 copies/mL. A LP was performed and opening pressures were elevated to 38 cm H_20. Cerebrospinal fluid (CSF) analysis was pertinent for WBC 45 cells/μL, CrAg+, and 80 colony-forming units (CFU) of *Cryptococcus*/mL CSF. He received 10 days of amphotericin B and fluconazole

* Corresponding author at: Infectious Diseases Institute, Makerere University, Kampala, Uganda.
E-mail address: abass004@umn.edu (M. Abassi).

https://doi.org/10.1016/j.mmcr.2017.11.004
Received 31 October 2017; Accepted 24 November 2017
Available online 26 November 2017

800 mg/daily. He had three subsequent therapeutic LPs and CSF sterilization was documented on the 10th day of amphotericin. He continued fluconazole 800 mg/day for one month, followed by 400 mg/day for 10 weeks, and finally 200 mg/day as maintenance therapy.

He returned to the clinic on day 180 (6 months from his initial encounter) with recurrent symptoms of meningitis reporting poor adherence to fluconazole. A repeat LP was performed and CSF analysis demonstrated WBC 25 cells//μL with CSF culture growing 93,000 *Cryptococcus* CFU/mL. He received another 14 doses of amphotericin, placed on fluconazole 1200 mg/day, and had 3 therapeutic LPs. At the end of 14 doses of amphotericin, his CSF culture continued to grow 21,000 *Cryptococcus* CFU/mL. After discharge, he was lost to follow up but returned to the clinic on day 240 (8 months from initial encounter) when his symptoms again recurred. Another LP was performed demonstrating WBC 220 cells/μL with 58,000 *Cryptococcus* CFU/mL on culture. He refused hospital admission and received only 3 doses of amphotericin as an outpatient prior to again being lost to follow-up. Fluconazole resistance testing performed on the cryptococcal isolates demonstrated a (MIC) > 64 μg/mL with high-level resistance to fluconazole.

He once again returned to the clinic on day 330 (approximately 1 year from initial encounter) complaining of worsening meningitis symptoms. At this point, his CD4 cell count had dropped to 2 cells/μL with the most recent HIV-1 viral load of 38,605 copies/mL. He received an additional 14 doses of amphotericin and remained on fluconazole 1200 mg/day. At the end of the 14 doses of amphotericin, his CSF cultures continued to grow 110 *Cryptococcus* CFU/mL. He received another 8 doses of amphotericin and CSF cultures at the end of this had sterilized. He continued high-dose fluconazole as an outpatient, and his ART regimen was switched to abacavir, lamivudine, and ritonavir-boosted atazanavir due to HIV virologic failure. Doses of fluconazole included 1200 mg/day for 10-weeks of consolidation therapy and 800 mg/day thereafter for secondary prophylaxis. On day 480, now 16 months from time of initial diagnosis, he presented with possible paradoxical immune reconstitution inflammatory syndrome vs. severe sepsis secondary to pneumonia. CSF cultures taken at that time were found to be sterile. He passed away 4 days into this hospitalization (Table 1).

3. Discussion

This case not only highlights the complexity and challenges in managing fluconazole resistant cases of cryptococcal meningitis, but also highlights important factors that facilitate the rise of fluconazole resistance. Without antimicrobial susceptibility testing on initial presentation, it is difficult to determine whether fluconazole resistance, in this case, was intrinsic or acquired. However, several risk factors may have put him at risk for acquired resistance, including: 1) monotherapy with inadequate, FDA-approved doses of fluconazole for cryptococcal meningitis and 2) medication non-adherence and interruptions to fluconazole during both the consolidation and maintenance phases of therapy. The case also raises several important questions. If fluconazole resistance is an emerging threat, does in vitro susceptibility testing translate into clinical outcomes? If so, what are the implications for treatment when fluconazole is the only antifungal therapy option and alternatives are not widely available?

Globally, there has been an increase in the percentage of *Cryptococcus* isolates found to have some degree of fluconazole resistance. Over a 10-year period, the ARTEMIS DISK Global Antifungal Surveillance Study found that fluconazole resistance in *Cryptococcus* has been progressively increasing from 7.3% from 1997 to 2000, to 10.9% from 2001 to 2004, and 11.7% from 2005 to 2007 [6]. In the same surveillance study, fluconazole resistant isolates of *Cryptococcus* from the United Kingdom and the United States were rare, while isolates from Africa, Cambodia, and Spain demonstrated increasing fluconazole resistance [6]. Individual studies from Taiwan and Uganda have seen similar increases in fluconazole non-susceptibility and increasing MICs among *Cryptococcus* isolates, all within the last decade [7,8]. While low rates of fluconazole resistance seen in North America and Europe may be attributed to decreasing rates of cryptococcal disease and fluconazole use with widespread antiretroviral access, increasing rates of fluconazole resistance in Asia, Africa, and Latin America may be attributed to both the increasing uses of fluconazole in clinical setting as well as agricultural settings. The increasing use of triazole fungicides for preventing fungal diseases in crops (e.g. Black Banana Rot Disease) may also be responsible for the rise in fluconazole resistance in *Cryptococcus* isolates [8].

Cryptococcus demonstrates an intrinsic mechanism of survival by adapting to stress caused by stepwise increases in fluconazole concentrations [5]. Prior to the advent and widespread use of azoles, isolates analyzed demonstrated subpopulations of resistant strains able to grow at concentrations of fluconazole between 4 and 64 μg/mL [9]. Fluconazole resistant strains collected from AIDS patients have demonstrated subpopulations of resistant strains that can grow at fluconazole concentrations of 16 and 128 μg/mL [9]. Genetic sequencing has shown that during antifungal therapy, *Cryptococcus* can undergo mutations that give rise to subpopulations of cells that are drug-resistant [10]. These resistant strains of *Cryptococcus* can alter their growth rate when exposed to fluconazole, such that fluconazole resistant isolates grow slower than isolates that are susceptible to fluconazole [8]. Therefore, when exposed to fluconazole, susceptible cells are eliminated, while a subpopulation of resistant cells, which exhibit slow growth at higher concentrations of fluconazole, are selected for and can emerge to cause a relapse of clinical disease.

The clinical significance of the increasing trends of *Cryptococcus* strains exhibiting fluconazole resistance has not yet been elucidated and studies have had contradictory findings, albeit all with small sample sizes. Nasri et al. found that there was no association between elevated MIC and mortality in 13 out of 35 individuals with *Cryptococcus* strains with elevated MIC ≥ 16 μg/mL [11]. In Uganda, Smith et al. found a trend towards reduced 2-week culture positivity and lower mortality in individuals who had fluconazole resistant strains of *Cryptococcus*, although, the sample size was too small to conclude any statistically significant associations [8]. Aller et al. observed better

Table 1
Clinical summary of events.

Days	Initial fungal burden (CFU/mL)	Doses of amphotericin	Final fungal burden (CFU/mL)	Fluconazole dose (mg/day)	Diagnosis
0	Not done	0	Not done	400 mg	Primary CM
60	80	10	Sterile	800 mg for 2 wks/400 mg for 10wks/200 mg	Untreated CM
180	93,000	14	21,000	1200 mg	CM Relapse
240	58,000	3	Not done	1200 mg	Persistent CM
330	Not done	14	110	1200 mg	Persistent CM
360	Not done	8	Sterile	1200 mg	Persistent CM
480	Sterile	0	Not done	800 mg	IRIS[a] vs. Sepsis

[a] During the last hospitalization a diagnosis of either paradoxical immune reconstitution inflammatory syndrome vs. severe sepsis was made. CSF cultures at this time were sterile and CM relapse was ruled out.

clinical outcomes after maintenance therapy in *Cryptococcus* strains with fluconazole MIC < 16 μg/mL and a statistically significant association between a MIC ≥ 16 μg/mL and mortality, however, only 5 out of 25 individuals demonstrated elevated fluconazole MIC [12].

This case represents the emerging clinical dilemma that increasing rates in fluconazole resistant strains of *Cryptococcus* pose. Widespread use of fluconazole, in addition to inadequate and interruptive uses of fluconazole therapy in the treatment of both cryptococcal antigenemia and cryptococcal meningitis, has lead to an increase in strains of *Cryptococcus* having elevated MICs. Routine susceptibility testing is not currently recommended in first episodes of cryptococcal meningitis. However, in persons with prior fluconazole use or a history of medication non-adherence with frequent interruptions in therapy leading to relapsed or persistent cases of cryptococcal meningitis, susceptibility testing should be pursued. Although the clinical significance of increased fluconazole MIC is still undetermined, treatment failure and clinical relapse have been documented in cases of elevated fluconazole MIC. As demonstrated in this case, high dose fluconazole can be utilized throughout the consolidation and maintenance phases to maintain CSF sterility, especially in cases when no other antifungals are available. The increasing emergence of fluconazole resistance is becoming a widespread concern. Trends in fluconazole susceptibility should continue to be monitored and emphasis on optimal fluconazole therapy and ongoing advocacy for increasing the availability of alternative antifungals in resource-limited settings should become a priority.

Acknowledgements

We would like to thank Drs. Lillian Tugume, Reuben Kiggundu, Kenneth Ssebambulidde, and the ASTRO-CM team for their medical care. We also thank Andrew Akampurira, Tonny Luggya, Kizza Tadeo and the Makerere University Medical School Microbiology Laboratory team. We thank Drs. David Boulware and David Meya for their support in assisting with the drafting of the manuscript, and their input into the intellectual content. Support for this project was received via: the National Institutes of Neurologic Diseases and Stroke and Fogarty International Center (R01NS086312, K01TW010268, R25TW009345).

Conflict of interest

There are none.

References

[1] T.G. Mitchell, J.R. Perfect, Cryptococcosis in the era of AIDS–100 years after the discovery of Cryptococcus neoformans, Clin. Microbiol. Rev. 8 (4) (1995) 515–548.
[2] R. Rajasingham, R.M. Smith, B.J. Park, J.N. Jarvis, N.P. Govender, T.M. Chiller, et al., Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis, Lancet Infect. Dis. (2017).
[3] J.R. Perfect, W.E. Dismukes, F. Dromer, D.L. Goldman, J.R. Graybill, R.J. Hamill, et al., Clinical practice guidelines for the management of cryptococcal disease: 2010 update by the infectious diseases society of america, Clin. Infect. Dis.: Off. Publ. Infect. Dis. Soc. Am. 50 (3) (2010) 291–322.
[4] A. Loyse, F. Dromer, J. Day, O. Lortholary, T.S. Harrison, Flucytosine and cryptococcosis: time to urgently address the worldwide accessibility of a 50-year-old antifungal, J. Antimicrob. Chemother. 68 (11) (2013) 2435–2444.
[5] J.W. Cheong, J. McCormack, Fluconazole resistance in cryptococcal disease: emerging or intrinsic? Med. Mycol. 51 (3) (2013) 261–269.
[6] M.A. Pfaller, D.J. Diekema, D.L. Gibbs, V.A. Newell, H. Bijie, D. Dzierzanowska, et al., Results from the ARTEMIS DISK Global Antifungal Surveillance Study, 1997 to 2007: 10.5-year analysis of susceptibilities of noncandidal yeast species to fluconazole and voriconazole determined by CLSI standardized disk diffusion testing, J. Clin. Microbiol. 47 (1) (2009) 117–123.
[7] Y.C. Chen, T.Y. Chang, J.W. Liu, F.J. Chen, C.C. Chien, C.H. Lee, et al., Increasing trend of fluconazole-non-susceptible Cryptococcus neoformans in patients with invasive cryptococcosis: a 12-year longitudinal study, BMC Infect. Dis. 15 (2015) 277.
[8] K.D. Smith, B. Achan, K.H. Hullsiek, T.R. McDonald, L.H. Okagaki, A.A. Alhadab, et al., Increased antifungal drug resistance in clinical isolates of cryptococcus neoformans in Uganda, Antimicrob. Agents Chemother. 59 (12) (2015) 7197–7204.
[9] E. Sionov, Y.C. Chang, H.M. Garraffo, K.J. Kwon-Chung, Heteroresistance to fluconazole in Cryptococcus neoformans is intrinsic and associated with virulence, Antimicrob. Agents Chemother. 53 (7) (2009) 2804–2815.
[10] J. Rhodes, M.A. Beale, M. Vanhove, J.N. Jarvis, S. Kannambath, J.A. Simpson, et al., A population genomics approach to assessing the genetic basis of within-host microevolution underlying recurrent cryptococcal meningitis infection, G3 7 (4) (2017) 1165–1176.
[11] H. Nasri, S. Kabbani, M. Bou Alwan, Y.F. Wang, P.A. Rebolledo, C.S. Kraft, et al., Retrospective study of cryptococcal meningitis with elevated minimum inhibitory concentration to fluconazole in immunocompromised patients, Open Forum Infect. Dis. 3 (2) (2016) ofw076.
[12] A.I. Aller, E. Martin-Mazuelos, F. Lozano, J. Gomez-Mateos, L. Steele-Moore, W.J. Holloway, et al., Correlation of fluconazole MICs with clinical outcome in cryptococcal infection, Antimicrob. Agents Chemother. 44 (6) (2000) 1544–1548.