in vivo *23*: 465-468 (2009)

Successful Treatment of Cryptococcal Meningitis with a Combination of Liposomal Amphotericin B, Flucytosine and Posaconazole: Two Case Reports

V. ESPOSITO, R. VIGLIETTI, M. GARGIULO, R. PARRELLA, M. ONOFRIO, V. SANGIOVANNI, D. AMBROSINO and A. CHIRIANNI

III U.O. di Malattie Infettive A.O. "Cotugno", Napoli, Italy

Abstract. *Cryptococcus neoformans CNS infection frequently affects HIV-infected patients and is often lethal, despite antifungal therapy. The most recent treatment guidelines for Cryptococcal meningitis recommend therapy with lyposomal amphotericin B and possible association with flucitosine. However, clinical response rates in HIV-infected patients are not satisfactory, with a persistent high mortality rate and long term therapy is affected by a high risk of major side effects. Posaconazole, the latest broad-spectrum azole, with both in vitro- and in vivo-documented potent activity against C. neoformans, clearly showed no antagonism with amphotericin B, echinocandins or flucytosine and it has both in vitro and in vivo agonistic activity with flucytosine against C. neoformans. We report two cases of successful salvage therapy based on the addition of posaconazole to a standard treatment based on liposomal amphotericin B and Flucytosine. In addition we used posaconazole also in a maintenance therapeutic regimen with no evidence of recurrences in the follow up of these patients. Our report confirms that posaconazole has clinical activity in the CNS against C. neoformans infection. In addition posaconazole showed no antagonism with any other currently available antifungal agent, and was in fact synergistic to some of them (flucytosine); consequently, it seems to be an ideal candidate for antimicrobial combination salvage therapies. Finally posaconazole represents a good alternative to parenteral therapy and an ideal candidate for long-term maintenance therapy due to its competent toxicity profile and oral bioavailability.*

Correspondence to: Vincenzo Esposito, MD, Ph.D., III U.O. di Malattie Infettive A.O. "Cotugno", Via T. Tasso 169/C 80127, Napoli, Italy. Tel/Fax: +39 08119565311, e-mail: esposvin@libero.it

Key Words: Cryptococcal meningitis, *C. neoformans*, HIV, combination therapy.

In the clinical practice, even if most fungal infections respond to a single antimicrobial agent, there are circumstances in which a combination of antimicrobial agents is needed to obtain a remission of the infection. At present only four classes of antifungal agents are commonly used to treat invasive fungal infections: polyenes (amphotericin B), flucitosyne, azoles (ketconazole, itraconazole, fluconazole, voriconazole, and posaconazole) and echinocadins (caspofungin).

Cryptococcus neoformans infection of the central nervous system (CNC), affects mostly immunocompromised patients, such as HIV-infected individuals, and is often lethal, despite antifungal therapy (1). In this light, antifungal combination therapy could be useful, resulting in a reduced duration of therapy, expanded spectrum of activity thus avoiding drug resistance and reduced doses of single drugs. Amphotericin B and flucytosine in association have already been shown to be superior to a single drug regimen (amphotericin B) in trials on HIV-infected patients affected by cryptococcal meningitis (2), while the combination of amphotericin B and azoles was always questioned because of their potential antagonism (3-5).

However, posaconazole, the latest broad-spectrum azole, with both *in vitro*- and *in vivo*-documented potent activity against *C. neoformans,* clearly showed no antagonism with amphotericin B, echinocandins or flucytosine and in fact has an *in vitro* and *in vivo* agonistic activity with flucytosine against *C. neoformans* (3, 6). Posaconazole was also reported to significantly reduce fungal burden in brain tissue when associated to amphotericin B, with improved survival in a murine model (3).

Posaconazole is structurally similar to itraconazole but with fluorine substituents in place of chlorine, and a furan ring in place of the dioxolane ring. It exerts antifungal activity by blocking ergosterol synthesis through inhibition of the enzyme lanosterol 14αdemethylase. Ergosterol depletion together with the accumulation of methylated sterol precursors results in inhibition of fungal cell growth and/or death. Interestingly, posaconazole is less affected by the most

0258-851X/2009 $2.00+.40

common mechanisms of secondary resistance (efflux pumps, point mutations) compared to the other azoles (6, 7).

The most recent treatment guidelines for cryptococcal meningitis recommend therapy with lyposomal amphotericin B with the possibility for combination with flucytosine (8). However, clinical response rates in HIV-infected patients are not satisfactory, with a persistent high mortality rate, and long-term therapy is affected by a high risk of major side-effects. Fluconazole is reported to be less effective than amphotericin B for induction therapy, but it is recommended as a maintenance therapy to prevent recurrences (8).

Case Report 1

A 45-year old HIV-1 infected Italian black male, was admitted to our department complaining of headache and neck stiffness not responsive to non-steroidal anti-inflammatory drugs. The patient was unaware of his HIV infection and did not report any risk factor for HIV transmission nor any previous significant illness. HIV ELISA test was confirmed by RIBA test and 101,697 copies/ml of HIV RNA were detected in serum. The CD4 cell count was very low (4 cell/ml; 2% of total lymphocyte cell count) with a marked inversion of the CD4/CD8 ratio (0.03). Serum cryptococcal antigen was positive and lumbar puncture showed clear cerebrospinal fluid (CSF) with reduced glucose and 30 cells/ml count, with isolation of *C. neoformans* spp. Brain computed tomography (CT) scan and magnetic resonance imaging (MRI) did not show any significant evidence. Therefore liposomal amphotericin B (5 mg/kg daily dose) was started on day 2 together with steroids and diuretics as a symptomatic therapy to reduce intracranial hypertension.

A 30-day therapy did not reach any clinical or laboratory significant result, with persistence of *Cryptococci* in CSF and positive serum cryptococcal antigen. On the other hand, major amphotericin B side-effects developed, mostly consisting of very relevant reduction of serum potassium level (1.5 mg/ml) and a slight neutropenia. Consequently 40 mEq of potassium were infused daily to support the antimicrobial therapy that was implemented by using fluconazole (800 mg/qd). However, even after the changed therapeutic management, no relevant clinical or laboratory improvement was obtained after three weeks, while neutropenia became severe and granocyte, macrophage colony-stimulating factor (GM/CSF; lenograstim) was administered for a five day period until a satisfying blood white cell count was achieved. Fluconazole was then substituted by flucytosine (150 mg/kg/qd). This further therapy change also failed to obtain a clinical or laboratory remission during a one-month period of treatment and was followed by severe anaemia that needed whole blood transfusion twice. As a consequence of this last unsuccessful management, it was decided that posaconazole at a full daily dosage of 800 mg (200 mg/qid) would be used together with reduced flucytosine (100 mg/kg/qd) and liposomal amphotericin B (3 mg/kg/qd) total dosage, because the persistent low serum potassium level still needed a substitutive potassium infusion of 20 mEq/qd and considering the still present moderate anaemia.

A further brain MRI performed at the time of the last therapy change revealed signs of diffuse vasculitis. Therefore, steroid treatment that already had been discontinued after the acute presentation of the meningeal syndrome was again added to the treatment management.

During a 100-day, period of this last treatment, the patient displayed a progressive clinical improvement, and serum cryptococcal antigen together with culture of CNS fluid were finally negative, with a CNS fluid that did not show any pathological features and brain MRI showing only evidence of residual post-vasculitis inactive evidences (gliosis). Consequently amphotericin B, flucytosine and steroid therapy was discontinued. When discharged the patient still presented a very low CD4 cell count (19 cell/ml 2.36% of total lymphocytes).

At present the patient follows a highly active antiretroviral therapy (HAART) regimen including emtricitabine, tenofovir, enfuvirtide and fosamprenavir with undectectable serum HIV RNA level but with a persistent very low CD4 cell count, constantly under 50 cells/ml. Posaconazole (400 mg/td) is administered as a maintenance therapy without any clinical or laboratory signs of recurrence of Cryptococcus infection after an eight-month period of follow-up.

Case Report 2

A 44-year-old Italian white male was seen for headache. The patient, a former intravenous drug user and HIV/HCV co-infected, had already been followed up for six months at our department and was being treated by antiretroviral therapy including emtricitabine, tenofovir and efavirenz. At this time the CD4 cell count was 311 cells/ml with a CD4/CD8 ratio of 0.4% and HIV RNA was undetectable. At the time of the visit, a slight neck stiffness was oserved. Brain CT scan did not reveal any pathological findings. Serum cryptococcal antigen was positive and lumbar puncture was performed, with evidence of clear CNS fluid and *C. neoformans* spp. Therefore, therapy with liposomal amphotericin B (5 mg/kg/qd), fluconazole (800 mg/qd) and flucytosine (150 mg/kg/qd) was started. After fifteen days of treatment, the patient still complained of headache and a second lumbar puncture was performed with a persistent isolation of *C. neoformans* spp. In light of our previous experience, posaconazole at a daily dosage of 800 mg (200 mg/qid) was added to the therapy. Following a 30-day period of therapy, complete symptomatic and microbiological remission was observed. The patient was

then discharged with posaconazole 400 mg bid as a maintenance therapy.

No recurrences have been observed during a three months follow up period.

Discussion

In the last decade the introduction of HAART has significantly changed the clinical course of HIV disease, with prolonged survival and better quality of life for HIV-infected patients. However, a relevant number of patients still come to clinicians' attention with opportunistic infections, mostly being unaware of their HIV status or anyway immunocompromised to a level sufficient to acquire an opportunistic infection in the first six months of therapy when the immune system is not yet reconstituted. One of these very frequent opportunistic infections is meningeal cryptococcosis. This disease is reported to have a clinical success rate in HIV positive patients ranging between 40% and 60% when induction therapy with amphotericin B and flucytosine is administered (9, 10). Fluconazole, although effective for maintenance therapy is considered less useful for induction therapy (8). Therefore, almost 50% of these patients require salvage therapy being affected by a disease refractory to standard therapies. A small number of studies evaluated salvage therapy for refractory cryptococcal meningitis in HIV-infected patients, reporting a clinical success rate of approximately 50% by using liposomal formulations of amphotericin B and a complete or partial response in 39% of those treated with Voriconazole (11, 12). Pitisuttithum *et al*. (8) for a cohort of 28 HIV positive patients with cryptococcal meningitis refractory to amphotericin B or fluconazole therapy, reported a clinically successful outcome in approximately 50% of cases using posaconazole as a monotherapy with a mean treatment period of 81 days (range 4-195 days).

We described two cases of successful salvage therapy based on addition of posaconazole to a standard treatment based on liposomal amphotericin B and flucytosine. In addition, having observed the inefficacy of fluconazole addition to the management in the first clinical case we describe, we decided to use posaconazole also in the management of the maintenance therapeutic regimen, being very well tolerated and available as an oral formulation. This choice is also supported by other published case reports of patients with invasive fungal infections, describing the long-term safety and tolerability of posaconazole treatment (8, 13,14).

These very encouraging results bear out the previous observation that posaconazole, displayed a consistent satisfying pharmacokinetics profile in several different animal models with a very well documented activity in the CNS (6). In addition, to date, no relevant evidence of antagonism with other antifungal agents has been described, highlighting the suitability of this molecule as a perfect candidate for antifungal combinations (15). Furthermore the association of posaconazole with flucytosine is described to significantly enhance their antifungal activity, with the geometric mean minimum inhibitory concentration (MIC) of both agents dramatically dropping when co-administered. This mechanism by which one drug enhances the efficacy of the other is not yet well described, but some authors speculated that posaconazole acts by inhibiting ergosterol intake into the fungal cell membrane which would facilitate the uptake of flucytosine (3). It is notable that the addition of posaconazole to an amphotericin B-based regimen in a murine model led to a significant reduction of fungal burden, with prolonged overall survival (8).

Our report, thus, confirms that posaconazole has clinical activity in the CNS against *C. neoformans* infection. It is also useful in patients already treated with other azoles in light of the broad spectrum of activity and the reduced susceptibility to secondary cross-resistance. In addition, posaconazole was reported to show no antagonism with any other actually available antifungal agents, being indifferent to some of them (amphotericin B, echinocandin) (3) and synergistic to others (flucytosine); it would seem to be an ideal candidate for antimicrobial combination salvage therapies. Finally, its good toxicity profile (6, 7) and its oral bioavailability makes posaconazole a good alternative to parenteral therapy and an ideal candidate for use in long term maintenance therapy.

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Received August 7, 2008
Revised January 15, 2009
Accepted February 13, 2009