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BRIEF COMMUNICATION

DEVELOPMENT OF SECONDARY RESISTANCE TO FLUCONAZOLE IN *Cryptococcus neoformans* ISOLATED FROM A PATIENT WITH AIDS

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Cryptococcus neoformans is the fifth most common opportunistic agent of infection in patients with AIDS in the USA, exceeded only by *Candida* species, *Pneumocystis carinii*, cytomegalovirus and *Mycobacterium avium*[1, 2, 6, 10, 11]. In Brazil is the sixth, exceeded by *Candida* species, *P. carinii*, *Mycobacterium* species, *Toxoplasma gondii*, and herpes simplex virus (AIDS, Boletim Epidemiológico, set/nov 96, Ministério da Saúde, Brasil). During 30 years, the treatment of *C. neoformans* meningitis was based on the use of amphotericin B with or without flucytosine[13]. Nowadays, with the immunodepression caused by human immunodeficiency virus (HIV) infection and the availability of new antifungal drugs as the triazoles, the concept related to cure and relapses of cryptococcosis has been altered[7, 20]. Patients are treated with amphotericin B with or without flucytosine as initial therapy, but maintenance therapy is always necessary in AIDS patients with *C. neoformans* infections.

We communicate a case of *C. neoformans* meningitis in a patient with AIDS whose successive isolations during maintenance therapy with fluconazole showed reduction on susceptibility to the drug.

A 30 year-old white man with HIV infection since 1987, related to intravenous drug abuse, was hospitalized at the Santa Maria University Hospital on January 13, 1995. He has suffered from tuberculosis since 1994, and on that occasion complained of headache, nausea, projectile vomiting and fever (39°C). Cerebrospinal fluid (CSF) examination revealed *C. neoformans*, isolated on Sabouraud dextrose agar and characterized as var. *neoformans* on canavanine-glicine medium. The patient was treated with intravenous amphotericin B 50 mg/day during six weeks, and later with oral fluconazole 200 mg/day as maintenance therapy.

On May 19, 1995, he was hospitalized again with similar clinical history, and *C. neoformans* was isolated from CSF. Broth dilution susceptibility testing[16] revealed a MIC of 0.5 µg/ml for fluconazole, itraconazole, ketoconazole and amphotericin B, and the dosage was maintained without improvement of clinical symptoms. On May 30, CSF was collected again and *C. neoformans* isolation revealed a MIC of 1.0 µg/ml. Even with dosage of 400 mg/day, *C. neoformans* was isolated again on the 17th day. A MIC of 32 µg/ml for fluconazole and 0.5 µg/ml for other azoles and amphotericin B was detected, and the drug was then substituted by amphotericin B.

C. neoformans isolate was tested for *in vitro* susceptibility by standard method proposed by NCCLS (proposed standard M27-P)[16]. Antifungal agents were provided by manufacturers as pure powder form. Isolates were tested by a broth macrodilution technique with RPMI 1640 medium containing L-glutamine but no sodium bicarbonate and buffered to pH 7.0 with 0.165M morpholinopropane-sulphonic acid (MOPS). An inoculum of 10^3 cells/ml was prepared by a spectrophotometric method, and incubation was at 35°C for 72h. Final drug concentrations ranged from 64 to 0.125 µg/ml. The MICs were determined after incubation: for amphotericin B the MIC was the lowest concentration that inhibited the growth. Azoles MICs were defined as the lowest drug concentrations which resulted in a visual turbidity less than or equal to 80% inhibition compared with that produced by growth control which was obtained by diluting at 1:5 the drug-free control growth, with RPMI broth.

The treatment of *C. neoformans* meningitis remains a frustrating problem, considering limitations and effectiveness on therapy. In AIDS patients cure has not been achieved, and

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studies indicated that cryptococcosis is highly refractory to therapy and associated with a relapse rate of more than 50% after a primary treatment[7, 22]. The treatment includes an initial therapy with amphotericin B, followed by a maintenance therapy with an azole derivative, such as fluconazole or itraconazole[7, 20]. Therapy with amphotericin B or fluconazole alone is inefficient[14], but the association has been an alternative therapeutic option[7]. In addition, the possibility of resistance of *C. neoformans* to antifungal drugs make the management of these patients more problematic. *C. neoformans* resistance to amphotericin B related to alterations on the quality of cytoplasmic membrane sterol has been reported[3, 11, 12, 21].

Primary resistance of yeast to azoles derivatives, specially fluconazole, is an emerging phenomenon, well described in *Candida krusei* and *C. glabrata*[23] and may represent the tip of an iceberg[17]. Alterations on susceptibility of *C. neoformans* to fluconazole have been described[6, 8, 18, 20] and have been attributed mainly to efflux phenomenon in which drug loss from cellular interior occurs by active transport and is mediated by glicoprotein P or by other proteins such as MFS (major facilitator superfamily)[5]. Another resistance mechanism to azole agents include: a) reduced permeability of the membrane resulting from changes in membrane sterol composition with consequent minor capture of the drug by the fungus; b) a mutation in the target fungal enzyme (sterol 14-a demethylase, a cytochrome P450 enzyme) resulting in decreased binding affinity for the azole drugs; c) an overproduction of the target cytochrome P450 enzyme; d) amplification of CYP 51 gene; e) $\Delta^{5,6}$ desaturase alterations and $\Delta^{8,7}$ isomerase lesions, among others[17, 23].

In this report we must emphasize that neither azoles cross resistance was observed nor resistance among amphotericin B and azoles. In view of these facts we may suppose that ergosterol biosynthesis pathway remained unaltered, reinforcing the idea that efflux can be the main mechanism of fluconazole-resistance.

In Brazil, FRANZOT & HAMDAN[9] studied the susceptibility of 53 strains of *C. neoformans* from clinical and environmental isolates and showed that the fluconazole MIC range for clinical strains were 0.5-16 µg/ml which in our opinion encompass resistant strains. So, the present fluconazole-resistant strain, the elevated MICs showed by those authors[9] and the frequent fluconazole failures on cryptococcosis meningitis in AIDS patients[1, 6, 7, 8, 14, 15] support the idea that even in Brazil *C. neoformans* fluconazole-resistant strains may be more frequent than has been supposed.

Chemotherapy alone is not adequate for all patients with cryptococcosis[7]. In the present report, relapse of cryptococcal meningitis reflects deterioration on immune defenses, incapable of eradicate the infection[4, 15], since *C. neoformans* strains remained susceptible to amphotericin B. Alternative treatment regimens have been developed or are under investigation, including induction-consolidation regimen using several drugs[4, 7]. On the other hand, additional studies are necessary on prophylactic therapy with the purpose to detect interference on initial susceptibility of *C. neoformans* or other fungi[20].

RESUMO

Desenvolvimento de resistência secundária ao fluconazol em *Cryptococcus neoformans* isolado de paciente com AIDS

Relatamos um caso de meningite por *Cryptococcus neoformans* em paciente com Síndrome de Imunodeficiência Adquirida (SIDA). A terapia de manutenção com fluconazol não evidenciou melhora clínica e micológica, ao mesmo tempo em que o teste de suscetibilidade *in vitro* revelou aumento progressivo da concentração inibitória mínima (CIM). Estes resultados sugerem o desenvolvimento de resistência secundária ao fluconazol, todavia, resistência cruzada com outros derivados azólicos não foi constatada.

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