Revista da Sociedade Brasileira de Medicina Tropical 46(1):79-83, Jan-Feb, 2013
http://dx.doi.org/10.1590/0037-868216432013

Major Article

Efficacy of voriconazole in experimental rat paracoccidioidomycosis

Daniela Silva Granzoto[1], *Lúcia Helena Vitali*[2] *and Roberto Martinez*[2]

[1]. Programa de Clínica Médica, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP. [2]. Departamento de Clínica Médica, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP.

ABSTRACT

Introduction: Amphotericin B, azole or sulfamide drugs are used for treatment of patients with paracoccidioidomycosis. Among the azole drugs, voriconazole was active *in vitro* against *Paracoccidioides brasiliensis* and showed efficacy in the treatment of patients infected with this fungus.In the present study the antifungal activity of voriconazole and of other drugs was compared in a rat model of paracoccidioidomycosis. **Methods:** Wistar rats were inoculated intravenously with the BOAS strain of *P. brasiliensis* and antifungal drugs were administered to the animals by gavage at the following doses (mg/kg weight/day): voriconazole (5 to 20), ketoconazole (12 to 15), fluconazole (6), itraconazole (4), and sulfamethoxazole-trimethoprim (120 to 150). The antifungal activity of the drugs was assessed by determining the *P. brasiliensis* colony forming units in the lungs and spleen of the animals at the end of treatment and by a survival study. **Results:** Voriconazole reduced the total tissue fungal burden of *P. brasiliensis,* particularly at doses of ≥10mg/kg weight/day but its antifungal activity was less intense than that of fluconazole, itraconazole and sulfamethoxazole-trimethoprim. The mean survival of animals treated with the last three drugs, 29.1±10.7, 26.1± 10.1 and 28.4±9.6 days, respectively, was higher than that achieved with voriconazole 10mg/kg weight/day (18.5±8.3 days) and that observed in untreated animals (15.7±3.6 days). **Conclusions:** At doses similar to those used for clinical treatment, voriconazole showed lower antifungal activity in experimental rat paracoccidioidomycosis than that obtained with itraconazole and sulfamethoxazole-trimethoprim.

Keywords: Paracoccidioidomycosis. *Paracoccidioides brasiliensis.* Voriconazole. Itraconazole. Fluconazole. Sulfamethoxazole-trimethoprim.

INTRODUCTION

Orally administered itraconazole and sulfamethoxazole-trimethoprim[1] are drugs recommended for the treatment of patients with paracoccidiodomycosis of low or moderate severity[1]. Previous studies have shown the clinical efficacy of itraconazole[2] and sulfamides[3] as well as ketoconazole[4] and fluconazole[5] for the control of systemic disease caused by *Paracoccidioides brasiliensis*. Comparative studies of antifungal agents for the treatment of paracoccidioidomycosis have detected similar efficacy of ketoconazale and amphotericin B[6] and also of itraconazole, ketoconazole and sulfadiazine[7]. A more recent controlled open label comparative clinical investigation showed that itraconazole and voriconazole were equally successful in controlling this fungal disease after six to 12 months of treatment[8].

Voriconazole is a second-generation triazole currently recommended for the treatment of patients with acute invasive aspergillosis. This drug has a broad spectrum of antifungal activity including dimorphic fungi, *Cryptococcus* spp., *Candida* spp., *Trichosporon* spp. and *Fusarium* spp.[9]. Voriconazole shows good bioavailability, providing maximum plasma concentrations of 2 to 3μg/mL after the oral administration of a 200mg dose[10]. A minimum inhibitory concentration of < 0.03 to 2μg/mL voriconazole against *P. brasiliensis* has been observed[11], suggesting that this triazole drug could also be a therapeutic alternative for the control of paracoccidioidomycosis.

Voriconazole has been studied in only a few patients with paracoccidioidomycosis[8,12] and its *in vivo* efficacy against *P. brasiliensis* is still little known. Experimental models of infection can provide relevant data about the comparative efficacy of antifungal drugs against this dimorphic fungus[13].

The objective of the present study was to assess the effectiveness of voriconazole, ketoconazole, itraconazole, fluconazole and sulfamethoxazole-trimethoprim in the control of experimental paracoccidioidomycosis of the rat.

METHODS

Animals

The study was conducted on female Wistar rats (*Rattus novergicus)* supplied by the University of São Paulo, Ribeirão Preto Campus, SP, Brazil. At the time of inoculation the animals weighed 45 to 60g and were housed 6 to 8 to a cage, with free access to food and water.

Paracoccidioides brasiliensis and inoculums

The rats were infected with the BOAS strain of *P. brasiliensis* isolated from a patient and kept in the laboratory by subculture in Sabouraud medium. The fungus was cultured at 35ºC for 7 to 15 days and yeast-like cells were collected in isotonic saline. The suspension was homogenized, adjusted to

Address to: Dr. Roberto Martinez. Deptº de Clínica Médica /FMRP/USP. Av. Bandeirantes 3900, 14048-900 Ribeirão Preto, SP, Brasil.
Phone: 55 16 3602-2468; 55 16 3602-2464
e-mail: rmartine@fmrp.usp.br
Received in 05/03/2012
Accepted in 28/05/2012

a content of $1x10^7$ to $2x10^7$ yeast cells/mL in a hemocytometer and 0.2mL amounts were injected into the lateral vein of the rat tail. Fungus viability was confirmed by culturing the yeast cell suspension on plates containing brain-heart-infusion agar supplemented with 4% horse serum and 5% of a culture filtrate of the B339 strain of *P. brasiliensis.*

Drugs and treatment

The following drugs, manufactured for clinical use, were employed to treat the animals: voriconazole (Vfend®, Pfizer), ketoconazole (Nizoral®, Jansen-Cilag), fluconazole (Zoltec®, Pfizer), and sulfamethoxazole-trimethoprim (Bactrim®, Roche). The tablets or capsules of each drug were weighed and macerated and added to a 3% aqueous solution of gelatin (Vetec®, Brazil) immediately before being administered to the animals. The drugs suspended in gelatin were administered by gavage once a day (ketoconazole, fluconazole and itraconazole) or twice a day with an 8 hour interval between doses (voriconazole and sulfamethoxazole-trimethoprim). The antifungal treatment was started seven days after *P. brasiliensis* inoculation.

Paracoccidioides brasiliensis colony forming units

The rats were sacrificed 48 or 78 hours after the last dose of the antifungal agents. The lungs and spleen were placed in a solution of penicillin G (200U/mL) and gentamicin (48µg/mL) and triturated with a tissue homogenizer (Marconi®, Brazil). Serial dilutions of the lung and spleen homogenates were cultured on plates containing brain-heart-infusion agar supplemented with 4% horse serum and 5% of a culture filtrate of the B339 strain of *P. brasiliensis.* The colony forming units (CFU) of *P. brasiliensis* were counted after 15 days of culture at 35ºC and the total fungal burden of each organ was estimated.

Experimental design

A) To assess the efficacy of different doses of voriconazole, 5, 10 or 20mg/kg weight/day of the drug was administered to the animals for three weeks. The number of *P. brasiliensis* CFU in the lungs and spleen of rats was compared between the treated animals and also to the CFU of rats that received only gelatin (controls); B) In two independent experiments, the CFU of *P. brasiliensis* in tissues were analyzed in rats respectively receiving for three weeks voriconazole (7 or 10mg/kg weight/day), ketoconazole (12 or 15mg/kg weight/day), fluconazole (6mg/kg weight/day), itraconazole (4mg/kg weight/day), sulfamethoxazole-trimethoprim (120 or 150mg/kg weight/day of sulfamethoxazole), or only 3% gelatin (control); C) For the survival study, the animal groups received the same drugs for 12 days and were followed up together with the controls for 36 days after inoculation with *P. brasiliensis.*

Statistical analysis

The mean numbers of *P. brasiliensis* CFU in the tissues of the different animal groups were compared by analysis of variance (ANOVA) with pairwise comparisons in according to Bonferroni correction and using the PROC GLM feature of the SAS software, version 9.0. Survival was evaluated by the Kaplan-Meier method using a parametric model based in log-normal distribution; this analysis employed the SAS software, version 9.0 (PROC LIFEREG) and R software with a library survival. Differences were considered to be significant when $p<0.05$.

Ethical considerations

The research project was approved by the Animal Research Ethics Committee of the Faculty of Medicine of Ribeirão Preto, University of São Paulo (n 033/2006).

RESULTS

Voriconazole significantly reduced the number of *P. brasiliensis* CFU in the lungs and spleen of infected animals. No difference in efficacy was observed between the doses of 5, 10 or 20 mg/kg weight/day, except for a tendency to a greater reduction of the number of CFU in the lungs with increasing doses **(Table 1)**. In independent experiments, voriconazole 7 or 10mg/kg weight/day reduced the fungal burden in the spleen of rats, but only the higher dose reduced the pulmonary CFU **(Table 2)**.

Compared to other drugs, voriconazole 7mg/kg weight/day was less effective in reducing the pulmonary fungal burden than fluconazole, itraconazole and sulfamethoxazole-trimethoprim. At the dose of 10mg/kg weight/day it was also less efficient in reducing pulmonary CFU than ketoconazole, fluconazole and itraconazole **(Table 2)**. The last two drugs were also more effective than voriconazole in reducing the fungal burden of the spleen **(Table 2)**.

TABLE 1 - Colony forming units in the lungs and spleen of rats inoculated with* Paracoccidioides brasiliensis *according to the dose of voriconazole administered by gavage for three weeks.

	Dose		CFU/organ (mean±SD)	
Experimental group	**mg/kg/day**	**Number**	**lungs (CFUx10^6)**	**spleen (CFUx10^3)**
Control	-	15	6.7±3.5	2.7±5.2
Voriconazole 1 (V1)	5	16	3.7±3.5[a]	0.2±0.5[b]
Voriconazole 2 (V2)	10	16	3.1±3.0[a]	0.1±0.1[b]
Voriconazole 3 (V3)	20	16	2.9±2.5[a]	0.2±0.2[b]

CFU: colony forming units; **SD:** standard deviation; [a](CxV1) p=0.0142, (CxV2) p=0.0021 and (CxV3) p=0.0014; [b](C x V1) p=0.0041, (CxV2) p=0.0005 and (CxV3) p=0.0456.

Figure 1 and **Table 3** presents the survival data for infected animals treated with different antifungal agents for 12 days. Fluconazole, itraconazole and sulfamethoxazole-trimethoprim increased the mean survival of the animals, while the survival of animals treated with voriconazole and ketoconazole was similar to that of the untreated group.

TABLE 2 - Colony forming units in the lungs and spleen of rats inoculated with **Paracoccidioides brasiliensis** *who received different antifungal drugs by gavage for three weeks.*

Experimental group	Dose mg/kg/day	Number	CFU/organ (mean±SD) lungs (CFUx10^6)	CFU/organ (mean±SD) spleen (CFUx10^3)
		Experiment 1		
Control	-	14	105.1±153.1	68.8±123.7
Voriconazole (V)	7	12	72.8±68.9	36.9±77.0[b]
Ketoconazole (K)	12	12	27.7±14.8[a]	17.0±24.8[b]
Fluconazole (F)	6	12	13.1±11.3[a]	1.6±1.6[b]
Itraconazole (I)	4	12	2.9±3.2[a]	0.3±0.3[b]
SMX-TMP (ST)	120	12	17.7±12.1[a]	7.5±5.6[b]
		Experiment 2		
Control (C)	-	15	96.5±133.1	108.9±183.8
Voriconazole (V)	10	13	20.1±23.8[c]	9.1±15.3[d]
Ketoconazole (K)	15	13	5.1±3.6[c]	3.4±3.0[d]
Fluconazole (F)	6	13	2.5±2.4[c]	1.4±1.8[d]
Itraconazole (I)	4	13	2.3±2.2[c]	1.6±1.7[d]
SMX-TMP (ST)	150	13	7.3±8.7[c]	4.7±2.9[d]

CFU: colony forming units; **SD:** standard deviation; **SMX-TMP:** sulfamethoxazole-trimethoprim; **NS:** non significant [a] (C xV) p- NS ; (CxK) p=0.0187, (CxF) p<0.0001, (CxI) p<0.0001, (CxST) p=0.0003, (IxV) p<0.001, (F x V) p = 0.0002, (ST x V) p= 0.0115; [b](CxV) p=0.0291, (CxK) p=0.0897, (CxF) p<0.0001, (CxI) p<0.0001, (CxST) p=0.0020; (I x V) p<0.0001, (FxV) p= 0.0016; [c](CxV) p<0.0001, (CxK) p<0.0001, (CxF) p<0.0001, (CxI) p<0.0001, (CxST) p<0.001, (KxV) p= 0.0155, (FxV) p<0.0001 and (IxV) p< 0.0001; [d](CxV) p=0.0003, (CxK) p<0.0001, (CxF) p<0.0001, (CxI) p<0.0001, (CxST) p=0.0003, (FxV) p= 0.0233 and (IxV) p =0.0349.

TABLE 3 - Survival of rats treated 12 days with antifungal agents and monitored for 36 days after inoculation of **Paracoccidioides brasiliensis.**

Type of Treatment	Dose mg/kg/day	Number	Mean survival (days)	Days for death of 50% of the animals n	Survivors on the 36th day n	Survivors on the 36th day %
Control (C)	-	14	15.7±3.6	16	0	0.0
Voriconazole (V)	10	13	18.5±8.3	17	1	7.7
Ketoconazole (K)	12	13	18.3±7.1	14	1	7.7
Fluconazole (F)	6	13	29.1±10.7*	>36	8	61.5
Itraconazole (I)	4	13	26.0±10.1*	25	5	38.5
SMX-TMP (ST)	120	13	28.4±9.6*	32	6	46.1

SMX-TMP: sulfamethoxazole-trimethoprim; **NS:** non significant *Mean survival: (CxF) p=0.0002, (CxI) p=0.0054, (CxST) p=0.0007, (CxK) p-NS; (CxV) p-NS.

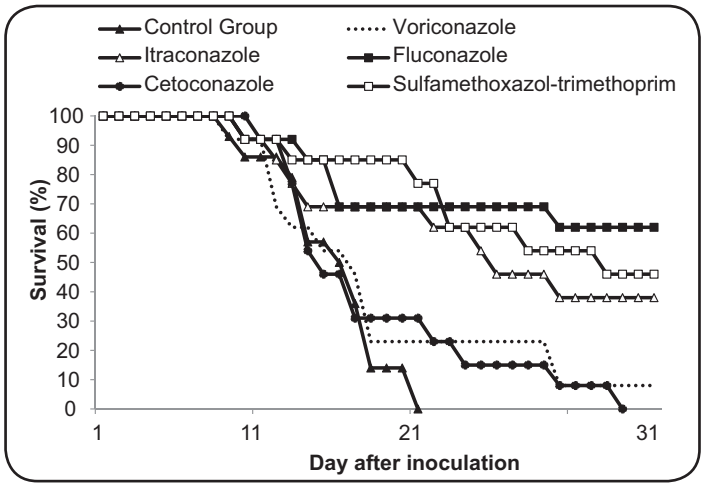

FIGURE 1 - Survival of rats infected i.v. (intravenous) with* Paracoccidioides brasiliensis *and treated during 12 days with drugs administered by gavage.
Gelatin-control group; voriconazole (10mg/kg weight/day); itraconazole (4mg/kg weight/ day); fluconazole (6mg/kg weight/day); (ketoconazole: 12mg/kg weight/day) and sulfamethoxazole-trimethoprim (120mg/kg weight/day of sulfamethoxazole).

DISCUSSION

This was the first study to assess simultaneously the antifungal efficacy of voriconazole, other azole drugs and sulfamethoxazole-trimethoprim in experimental paracoccidioidomycosis. We used a model of intravenous animal infection in which granulomas containing *P. brasiliensis* are formed, being more numerous in the lungs, spleen and liver. After the occurrence of established fungal infection in tissues, the animals were treated with medications available for clinical use and at doses similar to those recommended for patient treatment.

Voriconazole was effective against *P. brasiliensis,* as demonstrated by the reduction of the fungal burden in the lungs and spleen of the animals. Three separate experiments have suggested a dose-dependent action of voriconazole. The dose-dependent efficacy of this drug was detected in mice infected with *Aspergillus* spp. and treated with 10,

20 and 30mg/kg weight/day[14]. In the present study, doses of 5 and 7mg/kg weight/day had a lesser impact in the reduction of number of CFU in the lungs. In another preliminary experiment in which voriconazole 7mg/kg weight/day was administered in a single daily dose, again no reduction of the pulmonary fungal burden occurred (data not shown).

These results suggest that the dose of voriconazole recommended for clinical treatment (4 to 6mg/kg weight/twice daily)[9] does not have a sufficient antifungal activity to control the paracoccidioidomycosis induced in rats. This is probably due to an accelerated metabolism self-induced by voriconazole in rats, leading to a half-life of about one to two hours[15].

Voriconazole 10mg/kg weight/day significantly reduced the fungal burden in the lungs and spleen after three weeks of treatment. However, it failed to prolong the life of rats treated for 12 days, a period sufficient to demonstrate the anti-*P. brasiliensis* action of other drugs. The survival of mice infected with *Blastomyces dermatitidis* and treated for 23 days with voriconazole 1, 5 and 20mg/kg weight/day was increased compared to untreated animals[16]. Voriconazole 5 and 10mg/kg/day prolonged the survival of guinea pigs with invasive trichosporonosis and reduced the number of CFU in the liver and kidney of these animals[17]. Mice infected with *Cryptococcus neoformans* showed 100% survival after receiving voriconazole 60mg/kg weight/day, but doses of 10 and 40mg/ kg weight/day had a lower impact on animal survival[18]. In addition to the quantity of voriconazole administered daily, other variables may influence the results of different studies, such as time and route of administration of the drug, number of CFU inoculated, and the animal model employed.

Fluconazole and itraconazole showed the best performance in the comparative assessment of the drugs by both reducing the tissue fungal burden in a more intense manner and by prolonging animal survival. In the same model of rat paracoccidioidomycosis, fluconazole showed efficacy comparable to that of amphotericin B in reducing the number of CFU in the lungs[13]. This triazole is little used for the treatment of paracoccidioidomycosis, but has proved to be effective even in immunosuppressed patients[5,19]. Itraconazole reduced the fungal burden of tissues and increased the survival of mice and rats infected with *P. brasiliensis*[20,21]. In two controlled clinical studies, this triazole elicited high rates of a favorable response in patients with paracoccidioidomycosis[7,8]. In the model of rat paracoccidioidomycosis, sulfamethoxazole-trimethoprim had a lower antifungal activity than itraconazole and fluconazole, but a higher activity than ketoconazole and voriconazole. This experimental result supports the use of sulfamethoxazole-trimethoprim for the treatment of patients with paracoccidioidomycosis but this drug combination is now recommended only as an alternative to itraconazole[1]. Among the drugs tested, ketoconazole showed the lowest antifungal activity, perhaps due to its lower bioavailability.

The application of the results of antifungal efficacy obtained in animal models of infection to human therapy should be considered with caution. The data of the present study validate the therapeutic use of itraconazole, fluconazole and sulfamethoxazole-trimethoprim in human paracoccidioidomycosis. Voriconazole showed *in vivo* activity against *P. brasiliensis* and has the potential for use in the treatment of some patients with paracoccidioidomycosis of low or moderate severity, especially those with damage to the central nervous system[8]. The rate of voriconazole metabolism varies from person to person[22] and patients treated with the recommended doses usually present plasma levels of less than 1μg/mL[23]. It may be necessary to monitor the levels of this drug in order to establish an adequate dose, to achieve a favorable therapeutic response and to minimize adverse effects[24,25] .

ACKNOWLEDGMENTS

The animals used in the study received professional care from Alberto V. Verceze, Maurício R. Arantes and Roni Fabbri. Prof. Edson Zangiacomi Martinez, Quantitative Methods Center, Faculdade de Medicina de Ribeirão Preto, provided the supervision for the statistical analysis.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FINANCIAL SUPPORT

Fundação de Apoio ao Ensino, Pesquisa e Assistência (FAEPA) from the *Hospital das Clínicas, Faculdade de Medicina de Ribeirão Preto*, *Universidade de São Paulo*, Brazil.

REFERENCES

1. Shikanai-Yasuda MA, Queiroz-Telles F, Mendes RP, Colombo AL, Moretti ML. Guidelines in paracoccidioidomycosis. Rev Soc Bras Med Trop 2006; 39:297-310.
2. Naranjo MS, Trujillo M, Munera MI, Restrepo P, Gomez I, Restrepo A. Treatment of paracoccidioidomycosis with itraconazole. J Med Vet Mycol 1990; 28:67-76.
3. Valle ACF, Wanke B, Wanke NCF, Lima NS, Perez M. Tratamento da paracoccidioidomicose. Estudo retrospectivo de 500 casos. II. Avaliação dos resultados terapêuticos com sulfanilamídicos, anfotericina B, associação sulfametoxazol/trimetoprim, cetoconazol e miconazol. An Bras Dermatol 1993; 68:65-70.
4. Del Negro G. Ketoconazole in paracoccidioidomycosis. A long-term therapy study with prolonged follow-up. Rev Inst Med Trop São Paulo 1982; 24:27-39.
5. Diaz M, Negroni R, Montero Gei F, Castro LGM, Sampaio SAP, Borelli D, et al. A Pan-American 5-year study of fluconazole therapy for deep mycoses in the immunocompetent host. Clin Infect Dis 1992; 14 (supl I):68-76.
6. Marques SA, Dillon NL, Franco MF, Habermann MC, Lastoria JC, Stolf HO, et al. Paracoccidioidomycosis: a comparative study of the evolutionary serologic clinical and radiologic results for patientes treated with ketoconazole or amphotericin B plus sulfonamides. Mycopathologia 1985; 89:19-23.
7. Shikanai-Yasuda MA, Benard G, Higani Y, Del Negro GMB, Hoo S, Vaccari EH, et al. Randomized trial with itraconazole, ketoconazole and sulfadiazine in paracoccidioidomycosis. Med Mycol 2002; 40:411-417.
8. Queiroz-Telles F, Goldani LZ, Schlamm HT, Goodrich JM, Espinel-Ingroff A, Shikanai-Yasuda MA. An open- label comparative pilot study of oral voriconazole and itraconazole for long-term treatment of paracoccidiodomycosis. Clin Infect Dis 2007; 45:1462-1469.
9. Donnelly JP, Pauw BE. Voriconazole - a new therapeutic agent with an extended spectrum of antifungal activity. Clin Microbiol Infect 2004; 10 (supl I):107-117.
10. Lazarus HM, Brumer JL, Yanovicjh S, Schlamm H, Romero A. Safety and pharmacokinetic of oral voriconazole in patients at risk of fungal infection: a dose escalation study. J Clin Pharmacol 2002; 42:395-402.

11. McGinnis RM, Pasarell L, Sutton DA, Fothergill AW, Cooper Jr CR, Rinaldi MG. *In vitro* evaluation of voriconazole against some clinically important fungi. Antimicrob Agents Chemother 1997; 41:1832-1834.
12. Mayr A, Kirchmair M, Rainer J, Rossi R, Kreczy A, Tintelnot K, et al. Chronic paracoccidioidomycosis in a female patient in Austria. Eur J Clin Microbiol Infect Dis 2004; 23:916- 919.
13. Martinez R, Malta MHB, Verceze AV, Arantes MR. Comparative efficacy of fluconazole and amphotericin B in the parenteral treatment of experimental paracoccidiodomycosis in the rat. Mycopathologia 1999; 146:131-134.
14. Murphy M, Bernard EM, Ishimaru T, Armistronng D. Activity of *Aspergillus species* and its effectiveness in an experimental model of invasive pulmonary aspergillosis. Antimicrob Agents Chemother 1997; 41:696-698.
15. Roffey SJ, Cole S, Comby P, Gibson D, Jezequel SG, Nedderman AN, et al. The disposition of voriconazole in mouse, rat, rabbit, guinea pig, dog, and human. Drug Metab Dispos 2003; 31:731-741.
16. Sugar AM, Lin XP. Efficacy of voriconazole in treatment of murine pulmonary blastomycosis. Antimicrob Agents Chemother 2001; 45:601-604.
17. Serena C, Gilgado F, Marine M, Pastor FJ, Guarro J. Efficacy of voriconazole in a guinea pig model of invasive trichosporonosis. Antimicrob Agents Chemother 2006; 50:2240-2243.
18. Serena C, Pastor FJ, Marine M, Rodriguez M, Guarro J. Efficacy of voriconazole in murine model of cryptococcal central nervous system infection. J Antimicrob Chemother 2007; 60:162-165.
19. Morejón KML, Machado AA, Martinez R. Paracoccidioidomycosis in patients infected with and not infected with human immunodeficiency virus: a case-control study. Am J Trop Med Hyg 2009; 80:359-366.
20. Restrepo S, Tabares AM, Restrepo A. Activity of two different triazoles in a murine model of paracoccidioidomycosis. Rev Inst Med Trop São Paulo 1992; 34:171-176.
21. Negroni R, Elias-Costa MRI, Finquelievich TL, Iovannitti C, Agorio L, Tiraboshi IN. Three triazoles in the treatment of experimental paracoccidioidomycosis. Rev Iberoamer Micol 1991; 8:8-12.
22. Lewis RE. What is the therapeutic range for voriconazole? Clin Infect Dis 2008; 46:212-214.
23. Myrianthefs P, Markantonis SL, Evaggelopoulou P, Despotelis S, Evodia E, Panidis D, et al. Monitoring plasma voriconazole levels following intravenous administration in critically ill patients: an observational study. Int Antimicrob Agents 2010; 35:468-472.
24. Pascual A, Calandra T, Bolay S, Buclin T, Bille J, Marchetti O. Voriconazole therapeutic drug monitoring in patients with invasive mycoses improves efficacy and safety outcomes. Clin Infect Dis 2008; 46:201-211.
25. Pasqualotto AC, Xavier MO, Andreolla HF, Linden R. Voriconazole therapeutic drug monitoring: focus on safety. Expert Opin Drug Saf 2010; 9:152-137.