

Original Articles

Antifungal Activity of Itraconazole and Voriconazole against Clinical Isolates Obtained from Animals with Mycoses

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Abstract

Animal mycosis, particularly deep mycosis, is one of the most challenging conditions encountered by veterinarians. Pathogens causing mycotic infections in animals include fungi such as *Cryptococcus neoformans*, *Candida* spp., and *Aspergillus* spp. The antifungal drugs used for the treatment of deep mycoses in animals as well as humans are polyenes and azoles. However, the sensitivity of clinical isolates obtained from animals toward these drugs has rarely been assayed. In this study, the antifungal activities of itraconazole and voriconazole against clinical isolates of *C. neoformans*, *Candida* spp., and *A. fumigatus* isolated from animals with mycoses were examined using the broth microdilution method performed according to the guidelines provided by the Clinical and Laboratory Standards Institute. The minimum inhibitory concentrations (MICs) of itraconazole toward the *C. neoformans*, *Candida* spp., and *A. fumigatus* isolates were 0.125-1, 0.125-2, and 0.25-2 $\mu\text{g/ml}$, respectively, and those of voriconazole were 0.0625-0.5, ≤ 0.0313 -0.0625, and 0.0625-1 $\mu\text{g/ml}$, respectively. The results of the MIC analyses implied that the fungal isolates obtained from infected animals exhibit an equivalent degree of susceptibility to itraconazole and voriconazole, as is observed in the case of isolates obtained from humans. The appropriate antifungal therapeutic strategy for the treatment of mycoses in animals must be selected taking into consideration the host immune status and organ function as well as the *in vitro* sensitivity of the pathogens to antifungal drugs.

Key words : itraconazole, voriconazole, *Cryptococcus neoformans*, *Candida* spp., *Aspergillus fumigatus*

Introduction

The incidence of cases of deep mycosis caused by yeasts and filamentous fungi in animals has gradually been increasing. Similar to mycosis in humans, mycotic infection in animals is caused by various pathogens, including fungi such as *Cryptococcus neoformans*, *Candida* spp., and *Aspergillus* spp. *C. neoformans* is an encapsulated fungus and a common pathogen causing systemic fungal infection in humans as well as various

animal species, particularly cats¹⁾. *C. neoformans* can be classified into 5 serotypes, A, B, C, D, and AD, and most cases of cryptococcosis in humans and other mammals in Japan are caused by serotype A^{2,3)}. The genus *Candida* includes approximately 200 species of asexual yeasts, some of which inhabit the gastrointestinal tract and external genitalia as a part of the normal flora. Most cases of candidiasis in humans, dogs, and cats are due to *C. albicans*, with *C. tropicalis*, *C. glabrata*, and *C. parapsilosis* also occasionally causing this condition^{4,5)}. Aspergillosis is a major cause of morbidity and mortality in birds; it is predominantly caused by *A. fumigatus* and less commonly by *A. flavus* and *A. niger*^{6,7)}.

Antifungal drugs that are routinely used for the

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Table 1. Antifungal activity of itraconazole and voriconazole against clinical isolates obtained from animals with mycoses

Species	Origin	n	Itraconazole MIC ($\mu\text{g/ml}$)	Voriconazole MIC ($\mu\text{g/ml}$)
<i>Cryptococcus neoformans</i>	Dog	1	1	0.125
	Cat	14	0.125 – 1	0.0625 – 0.5
<i>Candida albicans</i>	Dog	2	0.125 – 0.5	≤ 0.0313 – 0.0625
	Cat	2	0.25	≤ 0.0313
<i>Candida glabrata</i>	Dog	1	2	0.0625
<i>Candida tropicalis</i>	Dog	1	0.125	≤ 0.0313
<i>Candida parapsilosis</i>	Dog	1	0.5	≤ 0.0313
<i>Aspergillus fumigatus</i>	Cat	1	0.25	0.125
	Pig	1	0.5	0.5
	Bird	9	0.25 – 2	0.0625 – 1

treatment of mycoses in animals include polyenes and azoles, which are also used in the case of humans. Azoles belong to a broad class of synthetic compounds that inhibit the biological activity of many medically significant fungi by blocking the biosynthesis of ergosterol⁸⁾. Various azole derivatives have been characterized on the basis of their chemical structure, solubility, and formulation. Itraconazole, a triazole derivative, is more potent than imidazole and is highly lipophilic⁸⁾. It exhibits a broad range of activity against many important fungi, including dermatophytes, and is used as an antifungal agent in veterinary medicine⁸⁾. Further, itraconazole is one of the most reliable drugs for the treatment of deep mycoses in animals, such as cryptococcosis, candidiasis, and aspergillosis. Voriconazole is a second-generation triazole derived from fluconazole⁹⁾, and it exhibits a wide spectrum of antifungal activity against opportunistic fungal pathogens, including *C. neoformans*, *Candida* spp., and *A. fumigatus*. However, reports on the antifungal activity of voriconazole against clinical isolates obtained from animals with mycoses have been scarce.

In this study, we examined the antifungal activities of itraconazole and voriconazole against clinical isolates of *C. neoformans*, *Candida* spp., and *A. fumigatus* obtained from animals with mycoses using the broth microdilution method according to the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI; formerly known as the National Committee for Clinical Laboratory Standards).

Materials and methods

Strains

In this study, 33 fungal strains that were clinically isolated from animals with mycoses in Japan were used (Table 1), and *C. parapsilosis* ATCC 22019 was used as the quality control strain. Of the 33 isolates, 15 were isolates of *C. neoformans*; 7, of *Candida* spp.; and

11, of *A. fumigatus*. *C. neoformans* strains of serotype A were isolated from the lymph nodes, serum, sputum, cerebrospinal fluid, nasal mucosa, and spleen tissues of 14 domestic cats and 1 dog and were maintained on sunflower seed agar¹⁰⁾. Further, 4 strains of *C. albicans* were isolated from the nasal discharge and spleen tissues of 2 cats and from urine samples of 2 dogs and strains of *C. glabrata*, *C. tropicalis*, and *C. parapsilosis* were isolated from the urine samples of each of 3 dogs. All these strains were maintained on diluted Sabouraud dextrose agar containing 0.2% glucose, 0.1% peptone, 0.1% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1% KH_2PO_4 , and 2% agar¹¹⁾. Eleven *A. fumigatus* strains were isolated from the lungs and air sacs of 9 birds during autopsy, from the orbital tissue of 1 cat, and from the turbinate tissue of 1 pig, and these strains were maintained on diluted Sabouraud dextrose agar.

Antifungal drug susceptibility

The procedures used for the broth dilution antifungal susceptibility testing of yeasts and of filamentous fungi were standardized according to the M27-A2¹²⁾ and M38-A¹³⁾ reference method, respectively, which have been proposed by the CLSI.

Itraconazole was obtained from Janssen Research Foundation (Beerse, Belgium), and voriconazole from Pfizer Central Research (Sandwich, UK). The antifungal drug stock solutions were prepared by dissolving the drugs in dimethyl sulfoxide (DMSO). The stock solutions of itraconazole and voriconazole were diluted to a final concentration of 0.0313–16 $\mu\text{g/ml}$ in modified RPMI 1640 medium, which had been buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS) buffer.

The *C. neoformans* and *Candida* spp. strains were subcultured on Sabouraud dextrose agar at 35°C, while the *A. fumigatus* strains were subcultured on potato dextrose agar at 35°C in order to induce conidium for-

mation. Cells of *C. neoformans* cultured for 48 hours, *Candida* spp. cultured for 24 hours, and *A. fumigatus* cultured for 7 days were suspended in 0.85% saline. The *C. neoformans* and *Candida* spp. suspensions were adjusted to concentrations of 5.0×10^3 to 2.5×10^3 cells/ml with the modified RPMI 1640 medium at the above mentioned concentration, while the *A. fumigatus* suspension was adjusted to a concentration of 0.4×10^4 to 5×10^4 colony-forming units (CFU)/ml with the modified RPMI 1640 medium at the final concentration. Next, the *C. neoformans* suspension was incubated at 35°C for 72 hours, while the *Candida* spp. and *A. fumigatus* suspensions were incubated at 35°C for 48 hours. Growth of the cultures was assessed visually by comparing the turbidity of the fungal suspension with that of the control culture that was grown in a medium lacking antifungal drugs. For *C. neoformans* and *Candida* spp., the end points were defined as an 80% reduction in turbidity as compared to the control culture. The minimum inhibitory concentration (MIC) was defined as the lowest drug concentration that produced a prominent decrease in turbidity.

For *A. fumigatus*, the end points were defined as a 100% inhibition, and the MIC was defined as the lowest drug concentration that prevented any discernible fungal growth. The interpretive breakpoints of the *Candida* spp. for itraconazole were as follows: ≤ 0.125 mg/ml for susceptibility, 0.25-0.5 $\mu\text{g/ml}$ for dose-dependent susceptibility, and ≥ 1 $\mu\text{g/ml}$ for resistance¹²⁾, while for voriconazole these break points were: ≤ 1 $\mu\text{g/ml}$ for susceptibility, 2 $\mu\text{g/ml}$ for dose-dependent susceptibility, and ≥ 4 $\mu\text{g/ml}$ for resistance¹⁴⁾.

Results

The susceptibility of *C. parapsilosis* ATCC 22019 (quality control) to itraconazole and voriconazole was 0.25 $\mu\text{g/ml}$ and 0.0625 $\mu\text{g/ml}$, respectively. The antifungal activity of both drugs against the clinical isolates obtained from animals with mycoses is shown in Table 1. The *C. neoformans* strains were susceptible to itraconazole at an MIC of 0.125-1 $\mu\text{g/ml}$ and to voriconazole at an MIC of 0.0625-0.5 $\mu\text{g/ml}$. The *C. albicans* strains were susceptible to itraconazole at an MIC of 0.125-0.5 $\mu\text{g/ml}$ and to voriconazole at an MIC of $\leq 0.0313 - 0.0625$ $\mu\text{g/ml}$. The other strains of *Candida* spp., namely, one of *C. glabrata* strain, one *C. tropicalis* strain, and one *C. parapsilosis* strain, which had been isolated from each of 3 dogs, were susceptible to itraconazole at MICs of 2, 0.125, and 0.5 $\mu\text{g/ml}$, respectively, and to voriconazole at MICs of 0.0625, ≤ 0.0313 , and ≤ 0.0313 $\mu\text{g/ml}$, respectively. The *A. fumigatus* strains were susceptible to itraconazole at MICs of

0.25-2 $\mu\text{g/ml}$ and to voriconazole at MICs of 0.0625-1 $\mu\text{g/ml}$.

Discussion

For the treatment of mycoses in animals as well as humans, it is very important that the pathogens are accurately identified and that the appropriate antifungal drugs are selected accordingly. Although susceptibility testing with antifungal drugs has been performed by many investigators for pathogenic fungi isolated from human patients with mycosis¹⁵⁻¹⁸⁾, such analyses have rarely been performed for fungi isolated from animals with mycosis.

In this study, *C. neoformans* strains isolated from cats were found to be susceptible to both itraconazole and voriconazole, as was the case with strains that were isolated from humans and tested simultaneously (data not shown); these results were consistent with those of previous studies^{15,16)}. The susceptibility of the *Candida* spp. strains isolated from animals toward both the drugs was similar to that of the strains isolated from humans in Japan, as has been reported in a previous study¹⁷⁾. Our MIC data revealed this to also be true for *C. albicans* strains isolated from humans (data not shown). The interpretive breakpoints of the *Candida* spp. for itraconazole were as follows: a *C. albicans* strain isolated from a dog and the *C. tropicalis* strain exhibited susceptibility, *C. albicans* strains isolated from another dog and 2 cats as well as the *C. parapsilosis* strain exhibited dose-dependent susceptibility, and the *C. glabrata* strain exhibited resistance. With regard to the interpretive breakpoints of *Candida* spp. for voriconazole, all the strains exhibited susceptibility. The susceptibility of *A. fumigatus* strains isolated from birds in Japan to both drugs was almost identical to that reported for these strains isolated from humans in Japan¹⁸⁾ and from falcons in the United Arab Emirates¹⁹⁾.

In this study, we did not note any distinct resistance to the antifungal drugs itraconazole or voriconazole during the susceptibility testing of clinical isolates obtained from animals with mycoses. Fungal pathogens isolated from humans and animals are generally expected to exhibit equivalent susceptibility to itraconazole and voriconazole. We noted some differences in the susceptibility of the individual *Candida* spp. strains isolated in this study toward these two drugs: these strains were more susceptible to voriconazole than to itraconazole.

The appropriate antifungal therapeutic strategy for the treatment of mycoses in animals must be selected taking into consideration the host immune status and organ function as well as the *in vitro* sensitivity of the

pathogens to antifungal drugs. The results of this study provide useful information for the treatment of animal mycoses.

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