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Short Report

In Vitro Antifungal Activities of Sulfa Drugs against Clinical Isolates of *Aspergillus* and *Cryptococcus* Species

Ahmed Hanafy, Jun Uno, Hiroki Mitani,
Yingqian Kang, Yuzuru Mikami

Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University,
1-8-1 Inohana, Chuo-ku, Chiba 260-8673, Japan

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Abstract

In vitro susceptibilities of ten clinical isolates, including five strains of *Cryptococcus neoformans* var. *grubii* and five strains of *Aspergillus fumigatus*, were determined against nine sulfa drugs using a microdilution method. Among the five tested media, minimum inhibitory concentration (MIC) values were observed only in YNB medium: no detectable level MIC value of less than 125 μg/m*l* was observed in the four remaining media against *Cryptococcus* species. Of the nine sulfa drugs, of which sulfaphenazole showed the highest antifungal activity, the MIC values for *A. fumigatus* and *C. neoformans* var. *grubii* were, respectively, 64 μg/m*l* and 4-8 μg/m*l*, suggesting high susceptibility of *C. neoformans* to sulfa drugs.

Key words: *in vitro*, susceptibility test, sulfa drugs, *Aspergillus*, *Cryptococcus*

Introduction

Opportunistic infections are becoming common because of the growing number of immunocompromised individuals[1]. Recently, opportunistic fungal infections have also become a problem[2, 3]. Particularly, invasive fungal infections attributable to *Aspergillus fumigatus* and *Cryptococcus neoformans* have increased considerably in frequency among immunocompromised hosts, engendering excessive morbidity and mortality[4−7]. The high mortality of aspergillosis and cryptococcosis is partly attributable to shortage of highly useful drugs with lower side effects and to the emergence of drug resistance at high rates[8−10].

Sulfa drugs are metabolic inhibitors of folic acid in microorganisms. The drug combination of sulfamethoxazole (SXT)-trimethoprim (TMP), is an antimicrobial agent that is frequently used for prophylaxis to prevent *Pneumocystis carionii* (*Pneumocystis jeroveci*) pneumonia in AIDS patients and in other immunocompromised individuals[11−13]. In addition, the chemotherapeutic usefulness of sulfa drugs against infections caused by fungus *Paracoccidioides brasiliensis* has been reported[14, 15]. Nevertheless, only a few reports have addressed *in vivo* as well as *in vitro* antifungal activity of sulfa drugs. Therefore, in this paper, we report antifungal activities of nine sulfa drugs against clinical isolates of *Aspergillus* and *Cryptococcus* species in Japan.

In the first experiment for determination of an appropriate medium for MIC value measurement, the following nine strains of *C. neoformans* var. *grubii* (IFM 5817, 40215, 40216, 46660, 46501), *C. gattii* (IFM 5880, 5882), and *C. neoformans* var. *neoformans* (IFM 46089, 46139) were used. The fungi were maintained on PDA slants at an interval of 2-7 days at 27℃.

Sulfa drugs act as a competitive antagonist of *p*-aminobenzoic acid (PABA), which is an integral component of the folic acid structure. Therefore, medium ingredients were considered to play an important role for exhibition of antifungal activity by sulfa drugs. As test media, potato dextrose broth (PDB; Difco Laboratories, USA), Mueller Hinton broth (MHB, Difco

Corresponding author: Yuzuru Mikami
Research Center for Pathogenic Fungi and Microbial Toxicoses, Chiba University,
1-8-1 Inohana, Chuo-ku, Chiba 260-8673, Japan.

Laboratories), RPMI 1640 (Nissui Seiyaku Co., Ltd., Japan), yeast nitrogen base broth with glucose (YNB; Difco Laboratories), and yeast extract peptone dextrose broth (YPD; Difco Laboratories) were used. The RPMI 1640 medium was buffered to pH 7.0 with 0.165 M morpholinepropanesulfonic acid (MOPS; Wako Pure Chemical Industries, Ltd., Japan). Sulfamethoxazole (SMX; Sigma-Aldrich Corp.) was selected as a sulfa drug. The sulfa drugs and the fungal inocula were prepared in accordance with M27-A and M38-P recommendations of the National Committee for Clinical Laboratory Standards (NCCLS)[16, 17]. The MIC value was determined visually in comparison with broth control after 48 h of incubation at 37°C; it was defined as the lowest drug concentration resulting in 90% reduction of turbidity when compared with the drug-free control.

Table 1 shows that the antifungal activities of sulfamethoxazole were comparatively high in YNB medium, and the MIC values of most of the strains were 31-62 μg/ml for *Cryptococcus* species, except for one strain. However, MIC values in the remaining four tested media were greater than 125-250 μg/ml. The higher activity in YNB was considered to be due to the synthetic media that does not contain PABA or PABA related compounds while the other four tested media are organic ones rich in PABA or related compounds. These studies indicated the usefulness of YNB medium for measurement of MIC values in sulfa drugs. An interesting observation was the markedly lower susceptibility of *C. gatti* strains to sulfamethoxazole than those of *C. neoformans* var. *grubii* and *C. neoformans* var. *neoformans* strains. Because few *C. gatti* strains were used (two strains), further detailed studies of susceptibility differences among these different varieties and species in *Cryptococcus* using numerous strains would be of interest. YNB medium was also used as a MIC determination medium in the subsequent experiment because of reproducible MIC values obtained in our first experiment.

In all, ten clinical isolates including five strains of *C. neoformans* var. *grubii* (IFM 51976, 49624, 49722, 40216 and 40215) and five strains of *A.*

Table 1. Comparison of MIC values of *Cryptococcus* species against sulfamethoxazole in five susceptibility test media

Fungal species and IFM strain No.	Media [a]	MIC values (μg/ml) [b]			
	PDB	MHB	RPMI	YNB	YPD
C. neoformans var. *grubii* IFM 40215	250	250	250	31.3	>250
C. neoformans var. *grubii* IFM 40216	62.5	250	125	31.3	250
C. neoformans var. *grubii* IFM 5817	125	125	250	62.5	250
C. neoformans var. *grubii* IFM 46660	250	125	250	62.5	>250
C. neoformans var. *grubii* IFM 46501	250	250	125	31.3	>250
C. gattii IFM 5880	>250	>250	>250	250	>250
C. gattii IFM 5882	>250	>250	>250	>250	>250
C. neoformans var. *neoformans* IFM 46089	125	250	250	62.5	>250
C. neoformans var. *neoformans* IFM 46139	250	250	250	125	>250

[a] see explanation of medium in text.
[b] MIC values were determined after 48 h at 37°C.

Table 2. MIC value profiles of five strains of *Aspergillus fumigatus* and five strains of *Cryptococcus neoformans* var. *grubii* against nine sulfa drugs in YNB medium

Fungal species and IFM strain No.	MIC values (μg/ml) [b]								
	No. 1 [a]	No. 2	No. 3	No. 4	No. 5	No. 6	No. 7	No. 8	No. 9
A. fumigatus IFM 53412	≥256	≥256	≥256	64	128	64	≥256	≥256	≥256
A. fumigatus IFM 51126	64	128	64	64	128	64	≥256	≥256	≥256
A. fumigatus IFM 48051	64	128	64	64	128	64	≥256	≥256	≥256
A. fumigatus IFM 47450	32	128	64	32	128	64	≥256	≥256	≥256
A. fumigatus IFM 41362	64	128	64	64	128	64	≥256	≥256	≥256
C. neoformans var. *grubii* IFM 51976	≥256	≥256	≥256	32	128	8	≥256	≥256	128
C. neoformans var. *grubii* IFM 49624	≥256	≥256	≥256	32	64	8	≥256	≥256	≥256
C. neoformans var. *grubii* IFM 49722	≥256	≥256	≥256	32	128	8	≥256	≥256	≥256
C. neoformans var. *grubii* IFM 40216	≥256	≥256	≥256	32	64	≤ 4	≥256	≥256	≥256
C. neoformans var. *grubii* IFM 40215	≥256	≥256	≥256	32	64	≤ 4	≥256	≥256	128

[a] Sulfa drugs, No. 1; sulfamono methoxine, No. 2; sulfadimethoxine, No. 3; sulfadiazine, No. 4; sulfamethoxazole, No. 5; sulfisoxazole, No. 6; sulfaphenazole, No. 7; sulfisomidine, No. 8; sulfamethizole, and No. 9; sulfamethoxypyridazine
[b] MIC values were determined after 48 h at 37°C.

fumigatus (IFM 53412, 51126, 48051, 47450, and 41362), were used in this experiment.

The following nine sulfa drugs were used: sulfamonomethoxine (SMM; Daiichi Pharmaceutical Co., Ltd., Tokyo), sulfadimethoxine (SDM; Daiichi Pharmaceutical Co., Ltd.), sulfadiazine (SDZ; Daiichi Pharmaceutical Co., Ltd.), sulfamethoxazole (SMX; Sigma-Aldrich Corp.), sulfisoxazole (SSX; Sigma-Aldrich Corp.), sulfaphenazole (SPZ; Sigma-Aldrich Corp.), sulfisomidine (SSM; Kanto Kagaku, Tokyo), sulfamethizole (SMT; Sigma-Aldrich Corp.), and sulfamethoxypyridazine (SMP; Sigma-Aldrich Corp.). The final concentrations of the sulfa drugs used for antifungal activity tests were 4-256 μg/m*l*.

The susceptibility profiles of five isolates for *A. fumigatus* and *C. neoformans* var. *grubii* are summarized in Table 2, which shows that five strains of *A. fumigatus* were moderately susceptible to sulfamonomethoxine, sulfadimethoxine, and sulfadiazine, but that *C. neoformans* was not susceptible. Sulfisomidine, sulfamethizole and sulfamethoxypyridazine showed lower activity, and their MIC values against *A. fumigatus*, in addition to *C. neoformans* var. *grubii* are 128-256 μg/m*l*. Sulfamethoxazole and sulfisoxazole showed moderate activity against both strains of *C. neoformans* var. *grubii* and *A. fumigatus*: their MIC values were 32-128 μg/m*l*. Among nine sulfa drugs, sulfaphenazole showed the highest activity; the drugs' MIC values for *A. fumigatus* and *C. neoformans* var. *grubii* were, respectively, 64 μg/m*l* and 4-8 μg/m*l*. Among five *C. neoformans* var. *grubii* strains, strains IFM 40216 and 40215 showed the highest susceptibility against sulfaphenazole, with MIC values of ≤ 4 μg/m*l*. To our knowledge, this is the first report of the demonstration of *in vitro* antifungal activity of sulfa drugs against *C. neoformans*. Our preliminary studies show *in vitro* synergistic effects of sulfaphenazole and azole type antifungals. Coupled with *in vitro* antifungal activity of sulfa drugs and their possible synergistic effect with azole-type antifungals, sulfa drugs are inferred to be worthy of further *in vivo* evaluation as antifungal agents, especially against infections of *C. neoformans* var. *grubii* because sulfa drugs have a different action mechanism against fungi than azole type antifungals, whose action site is an ergosterol biosynthesis.

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