VOL. 53 NO. 10, OCT. 2000 THE JOURNAL OF ANTIBIOTICS pp. 1175 — 1181

In Vitro Antifungal Activity of a Novel Lipopeptide Antifungal Agent, FK463, Against Various Fungal Pathogens

K. UCHIDA, Y. NISHIYAMA, N. YOKOTA and H. YAMAGUCHI

Teikyo University Institute of Medical Mycology,
359 Otsuka, Hachioji, Tokyo 192-0395, Japan

(Received for publication May 30, 2000)

The antifungal activities of FK463 against various pathogenic fungi were tested by standard broth microdilution methods, and compared with the activities of five currently available antifungal agents; *viz.*, fluconazole (FLCZ), itraconazole, miconazole, amphotericin B and flucytosine. Fourteen clinical isolates of *Candida albicans* categorized as FLCZ susceptible, FLCZ susceptible-dose dependent and FLCZ resistant were similarly susceptible to FK463 with geometric (GM) MIC values of 0.010, 0.011 and 0.015 μg/ml, respectively. All of 17 clinical isolates of *Aspergillus fumigatus* were inhibited by FK463 at 0.0078 μg/ml or lower concentrations. The antifungal activity of FK463 against a wider range of medically important yeasts and filamentous fungi were studied using stock fungal strains. While *Cryptococcus*, *Trichosporon*, *Fusarium*, *Pseudallescheria* and *Alternaria* species or zygomycetes were scarcely or not inhibited by 16 μg/ml of FK463, two *Candida* species (*C. albicans*, *C. glabrata*), as well as all species of *Aspergillus*, *Paecilomyces* and *Penicillium*, were highly susceptible with GM-MICs of $\leqq$0.008 μg/ml. The other fungal species including several non-*albicans Candida* were less susceptible with GM-MICs ranging between 0.016 and 2 μg/ml. MICs of the reference drugs were within the range thus previously reported. These results suggest that FK463 be of use in the treatment of serious fungal infections.

The antifungal agents currently available for clinical use do not necessarily possess adequate antifungal activities against serious systemic fungal infections, or their clinical usefulness is hampered by untoward side effects. Therefore, there is an urgent need for the development of novel antifungal agents with superior therapeutic effect and high safety.

FK463 (Fig. 1) is a new echinocandin-like lipopeptide antifungal agent discovered by Fujisawa Pharmaceutical Co. which is currently being investigated in phase II/III clinical studies against *Candida* and *Aspergillus* species. Although these fungal pathogens are generally susceptible *in vitro* and *in vivo* to FK463[1~3], data on *in vitro* activity against a wider range of medically important fungi is limited. In this study, we examined the *in vitro* activity of FK463 compared with that of five existing antifungal drugs including FLCZ and amphotericin B against clinical isolates of *C. albicans* and *A. fumigatus* and also stock strains of various other medically important yeasts and filamentous fungi.

Material and Methods

Preparations of Drugs and Reagents

FK463 (Lot No. 003062L) was supplied by Fujisawa Pharmaceutical Co. Ltd. as pure powder. Itraconazole (ITCZ) was supplied by Janssen-Kyowa Co., Ltd., FLCZ by Pfizer Pharmaceutical Co. Ltd., and amphotericin B (AMPH) by Bristol-Myers Squibb Inc. all as pure powders. Flucytosine (5FC) and miconazole (MCZ) were purchased from Sigma Chemical Co. FK463 was dissolved in sterile physiological saline, and all other drugs were dissolved in dimethyl sulfoxide (DMSO) to the required concentrations. RPMI 1640 medium was used to prepare serial dilutions of each drug at twice the concentrations of the final concentrations to be tested. One hundred μl of each drug solution was dispensed into each well of a microplate. The

Dedicated to the memory of Sir EDWARD ABRAHAM.

Fig. 1. Chemical structure of FK463.

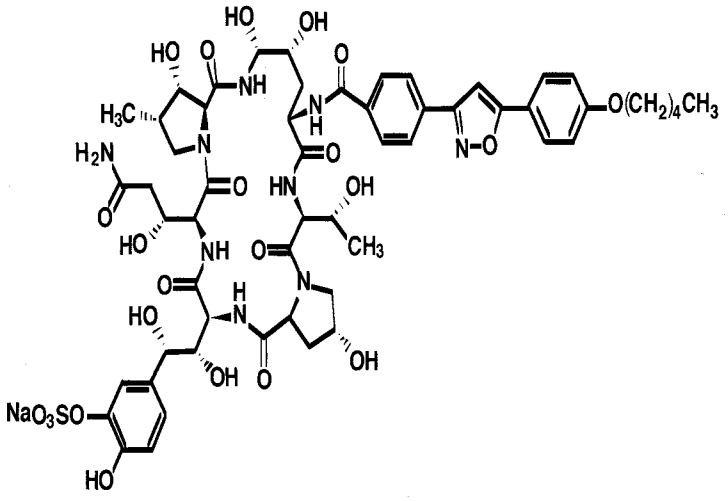

plate was stored at −80°C until used.

Concentration Range Tested for Each Drug

FK463 was tested at a concentration range of 0.0078 to 128 μg/ml, FLCZ and 5FC both at 0.125 to 64 μg/ml, AMPH at 0.031 to 16 μg/ml, ITCZ at 0.016 to 8 μg/ml, and MCZ at 0.063 to 32 μg/ml.

Test Strains

Ninety-two stock strains of 42 fungal species preserved in our institute, 19 clinical isolates of *C. albicans*, and 17 clinical isolates of *Aspergillus fumigatus* were tested. *C. albicans* ATCC90028 and *C. parapsilosis* ATCC22019 were used for quality control during performance of minimum inhibitory concentrations (MIC) determinations.

MIC Determination for Yeasts

Two related antifungal susceptibility testing methods for yeasts were used: one was the National Committee for Clinical Laboratory Standards (NCCLS) M27-A method[4] and the other the standard method for yeasts proposed by the Japanese Society for Medical Mycology (JSMM method for yeasts)[5].

In the latter method, MIC was measured by a broth microdilution method using RPMI 1640 medium (with L-glutamine, without $NaHCO_3$, and without phenol red; Gibco BRL, Lot No. 1011653) buffered with 0.165 M 3-morpholinepropansulfonic acid (MOPS; Sigma Chemical Co.). A test fungal strain was cultured on YM agar (Bacto peptone 0.5%, Bacto yeast extract 0.3%, Bacto malt extract 0.3%, glucose 1%, Bacto agar 1.5%) slant at 30 to 35°C for 1 to 3 days. The colonies were suspended in physiological saline, adjusted to a turbidity of McFarland 0.5 (equivalent to 2 to 5×10^6 cells/ml), and used for inoculation.

A microplate with frozen drug solutions was thawed at ambient temperature. The fungal suspension for inoculation was diluted 1,000 times in RPMI 1640, and 100 μl was dispensed into each well of the microplate (final concentration of inoculum; 1.0 to 2.5×10^3 cells/ml). A growth control and a negative control were included in the same plate, and the plate was cultured at 35°C. The absorbance at 620 nm was measured every 24 hours using a microplate reader (Labsystem Multiscan®; Biochromatic Inc.). Results were read when the absorbance of the growth control reached 0.2. The end-point for FK463 and AMPH was the concentration of complete growth inhibition, and that for the azole agents and 5FC was the concentration of 80% growth inhibition (IC_{80}) compared with the growth control. These values were expressed as MICs.

MIC Determination for Filamentous Fungi

Two related antifungal susceptibility testing methods for filamentous fungi were used: one was the NCCLS M38-P method[6] and the other the JSMM method for filamentous fungi[7].

MICs for filamentous fungi were measured by a broth microdilution method using RPMI 1640 medium buffered with 0.165 M MOPS according to the standard antifungal

susceptibility testing method for filamentous fungi by JSMM.

In the latter method, a test fungus was cultured on a potato dextrose agar slant at 27°C for 2 to 7 days. Next, a conidial suspension was prepared for the matured cultures by the conventional method using physiological saline supplemented with 0.05% Tween 80. The conidial concentration was enumerated using a hemocytometer. The suspension was adjusted to a conidial concentration of 1.2×10^4 cells/ml with RPMI 1640 medium, and was used as the inoculum.

A microplate containing frozen drug solutions and the freeze was thawed at ambient temperature. Eighty μl of the inoculum together with 20 μl of a redox indicator, Alamar Blue™ (Alamar Bioscience Inc.) solution, were dispensed into each well of the microplate. A growth control and negative control were included, and the plate was incubated at 30°C. Results were read when the color of the growth control had changed to a definite red. The end-point was determined visually as the lowest concentration at which the blue color just turned reddish, or by measurement using a microplate reader at 570 nm, as the lowest concentration showing 20% or less of the absorbance of the growth control (calculated IC_{80}). These values were expressed as MICs.

Calculation of Geometric Mean of MIC

The mean MIC of all the strains of a given species was calculated geometrically. If the MIC exceeded the highest concentration tested, the MIC was taken as 2 times the maximum concentration. If the MIC was below the lowest concentration tested, then it was taken as the lowest concentration. After the MIC for each strain was determined, MIC_{50}, MIC_{90} and/or geometric mean MIC (GM-MIC) values for each species or subgroup of species was calculated.

Results

Comparison of Four Different Testing Methods to Determine MIC of FK463

Fourteen yeast strains: 8 strains of 5 *Candida* species (3 strains of *C. albicans*, 1 strain of *C. glabrata*, 1 strain of *C. krusei*, 2 strains of *C. parapsilosis*, and 1 strain of *C. tropicalis*) and 2 strains of *Cryptococcus neoformans*, along with 3 filamentous fungal strains (1 strain each of *Aspergillus fumigatus*, *Paecilomyces variotii* and *Pseudallescheria boydii*) were used as test fungi to compare MIC values of FK463 for the testing of fungal strains. Both of the susceptibility testing methods for yeasts and the two for filamentous fungi gave virtually the same results with both yeast strains and filamentous fungus strains. With each testing method, all fungal strains exhibited sharp MIC endpoints to FK463.

Activity of FK463 Against Clinical Isolates of *C. albicans* with Different FLCZ-susceptibility

The MICs of FK463 and 5 reference drugs (FLCZ, ITCZ, MCZ, AMPH and 5FC) for 19 clinical isolates of *C. albicans* were determined by the JSMM for yeasts. According to NCCLS guidelines (M27-A)[4], 19 isolates were categorized as 5 FLCZ-susceptible (S), as 2 FLCZ-susceptible-dose dependent (S-DD), and 12 as FLCZ FLCZ-resistant (R) isolates.

The results are shown in Table 1. The FLCZ susceptibility of S-DD isolates (GM-MIC, 32.00 μg/ml)

Table 1. *In vitro* antifungal activities of FK463 and five reference drugs against clinical isolates of *C. albicans* with different susceptibities to fluconazole.

FLCZ susceptibility categories group[1] (No. of isolates tested)	Geometric mean MIC (μg/ml)					
	FK463	FLCZ	ITCZ	MCZ	AMPH	5FC
FLCZ susceptible (5)	0.010	1.32	0.142	0.287	0.500	0.223
FLCZ susceptible-dose dependent (2)	0.011	32.00	1.000	2.000	0.500	0.354
FLCZ resistant (12)	0.015	107.60	7.127	7.810	0.472	0.454

[1]: According to NCCLS guidelines (M27-A).
FLCZ; fluconazole, ITCZ; itraconazole, MCZ; miconazole, AMPH; amphotericin B, 5FC; flucytosine.

Table 2. *In vitro* antifungal activities of FK463 and five reference drugs against 17 clinical isolates of *A. fumigatus*.

Drug	MIC range(μg/ml)	MIC_{50}	MIC_{90}	Geometric mean MIC*(μg/ml)
FK463	≦0.0078	≦0.0078	≦0.0078	0.008
Fluconazole	64<	64<	64<	128.000
Itraconazole	0.03~0.5	0.13	0.5	0.121
Miconazole	0.25~2	1	1	0.752
Amphotericin B	0.5~2	1	2	0.923
Flucytosine	4~64<	16	64	18.834

*: Geometric mean MICs were calculated by taking the following approximations: MIC≦0.0078 μg/ml was taken as 0.0078 μg/ml, MIC>64 μg/ml as 128 μg/ml, and all figures were corrected to the nearest 4th decimal place.

and that of R isolates (GM-MIC, 107.60 μg/ml) decreased by 24 and 82 times, respectively, as compared with that of S isolates (GM-MIC, 1.32 μg/ml). Partly consistent with this, the susceptibility to other azoles, ITCZ and MCZ, of S, S-DD and R isolates also decreased in this order. In contrast, these three categories of isolates showed virtually the same susceptibility to AMPH and 5FC. FK463 was highly active against all of S, S-DD and R isolates and its GM-MICs for them were 0.010, 0.011 and 0.015 μg/ml, respectively.

Activity of FK463 and Reference Drugs Against Clinical Isolates of *A. fumigatus*

Table 2 shows the antifungal activities of FK463 and reference drugs against 17 clinical isolates of *A. fumigatus* determined by the JSMM method for filamentous fungi. Of 5 reference drugs ITCZ was the most active (MIC range, 0.03~0.5 μg/ml; GM-MIC, 0.121 μg/ml) and FLCZ the least (MIC range, >64 μg/ml; GM-MIC, 128.00 μg/ml). The activity of FK463 exceeded that of ITCZ and all isolates were completely inhibited by FK463 at a concentration of 0.0078 μg/ml or lower.

Activity of FK463 and Reference Drugs Against Stock Strains of Various Species of Yeasts and Filamentous Fungi

Table 3 summarizes the results of experiments in which MICs of FK463 and 5 reference drugs against 38 stock strains of 17 species of medically important yeast were determined by the JSMM method for yeasts. Although most strains tested were susceptible to some degree to all of the 5 reference drugs, their MIC values appreciably varied among strains of the same species, as well as among different species. Moreover, some strains were resistant to FLCZ. The MIC of FK463 for *Candida* species and other ascomycetous and related imperfect yeast were distributed within a low and narrow concentration range of ≦0.0078 to 0.25 μg/ml, except one strain each of *C. krusei*, *C. parapsilosis* and *C. guilliermondii* with relatively high MIC (>4, 2 and 1 μg/ml, respectively). The MIC range of FK463 for every ascomycetous or related imperfect yeast was at the lowest level among the 6 tested drugs. On the other hand, FK463 had no activity against the basidiomycetous yeast species such as *Cryptococcus* species and *Trichosporon asahii*.

Table 4 shows the MICs of FK463 and the 5 reference drugs against 54 strains of 25 species of filamentous fungi of medical importance, including hyaline hyphomycetes, dematiaceous fungi and zygomycetes, determined by the JSMM method for filamentous fungi. AMPH inhibited all fungal species at 4 μg/ml or lower concentration, except *P. lilacinus* (MIC, 8~>16 μg/ml), while 5FC showed moderate activity only against *Aspergillus* and *Penicillium* species, and FLCZ was scarcely or not at all active against any filamentous fungi. The antifungal spectrum of ITCZ and MCZ was intermediate; they were weakly or not at all active against *P. variotii*, *Fusarium* spp. and *M. circinelloides*. MICs of FK463 against all strains of *Aspergillus*, *Paecilomyces* and *Penicillium* species were at

Table 3. *In vitro* antifungal activities of FK463 and five reference drugs against stock strains of yeasts.

Fungal groups	Fungal species (No. of strains)	MIC (μg/ml)					
		FK463	FLCZ	ITCZ	MCZ	AMPH	5FC
Ascomycetous and related important yeasts	*Candida albicans* (3)	≦0.0078	0.25~0.5	0.03~0.06	0.06~0.25	0.5	1.0~64<
	C. dubliniensis (1)	0.0156	0.5	0.13	0.25	0.13	0.13
	C. glabrata (3)	≦0.0078~0.0156	2~8	0.25~1	0.25~0.5	0.25~0.5	0.13
	C. guilliermondii (3)	0.0313~1	2~64<	0.5~2	1~2	0.5~1	0.13~0.25
	C. inconspicua (1)	0.0313	32	0.5	1	0.25	8
	C. krusei (3)	0.0625~4<	64<	0.25~1	2~4	0.5~1	16~32
	C. lambica (1)	0.0313	64<	0.25	1	0.03	4
	C. lusitaniae (3)	0.0313~0.06256	1	0.06~0.13	0.13~0.5	0.5	0.13
	C. parapsilosis (3)	0.25~2	0.5~2	0.13~0.5	1~2	0.5~1	0.13~0.5
	C. tropicalis (3)	0.0313~0.0625	4~64<	0.5~8<	4	0.5~1	0.25
	C. utilis (1)	≦0.0078	4	0.25	0.5	0.5	0.13
	C. zeylanoides (1)	0.25	16	0.13	1	0.06	0.13
	Pichia anomala (3)	0.0156~0.0313	8~16	1	4	0.5~1	0.13~64<
	Saccharomyces cerevisiae (2)	0.125	8~16	1	1	0.5	0.13~0.25
Basidiomycetous and related important yeasts	*Cryptococcus humicola* (1)	128<	32	0.13	0.5	0.13	16
	C. neoformans (3)	128<	16~32	0.015~0.25	0.06~1	0.5~1	16~64<
	Trichosporon asahii (3)	128<	2~4	0.5~1	1~2	2	64<

FLCZ; fluconazole, ITCZ; itraconazole, MCZ; miconazole, AMPH; amphotericin B, 5FC; flucytosine.

Table 4. *In vitro* antifungal activities of FK463 and five reference drugs against stock strains of filamentous fungi.

Fungal groups	Fungal pecies (No. of strains)	MIC (μg/ml)					
		FK463	FLCZ	ITCZ	MCZ	AMPH	5FC
Hyaline hypomycetes	*Aspergillus fumigatus* (4)	≦0.0078	64<	0.13	0.5	0.2~2	4~32
	A. flavus (3)	≦0.0078	64<	0.13~0.5	2~8	2	4~32
	A. niger (3)	≦0.0078	64<	0.06~0.25	0.25~0.5	0.5~1	1~4
	A. terreus (3)	≦0.0078	64<	≦0.015~0.06	≦0.06~0.5	0.25~0.5	2~8
	A. clavatus (1)	≦0.0078	64<	0.06	0.25	0.5	16
	A. japonicus (1)	≦0.0078	64<	0.13	0.5	0.5	4
	A. nidulans (1)	≦0.0078	64<	0.25	2	0.25	32
	A. oryzae (1)	≦0.0078	64<	0.5	2	0.5	8
	A. versicolor (1)	≦0.0078	64<	0.06	0.5	0.25	16
	Paecilomyces lilacinus (2)	≦0.0078	64	≦0.015~0.5	≦0.06~2	8~16<	64<
	P. variotii (2)	≦0.0078	64<	0.5~8<	1~8	1	64<
	Penicillium citeoviride (1)	≦0.0078	64<	0.06	1	2	64<
	P. decumbens (1)	≦0.0078	64	≦0.015	≦0.06	1	16
	P. expansum (1)	≦0.0078	64	0.5	2	1	16
	P. marneffei (3)	≦0.0078	1~8	≦0.015	≦0.06	0.5~1	0.25~0.5
	P. notatum (1)	≦0.0078	64<	0.06	2	1	64
	Fusarium moniliforme (3)	128<	64<	2~8<	4~16	1~2	64<
	F. oxysporum (3)	128<	64<	8<	32<	0.5~2	64<
	F. solani (3)	128<	64<	8<	32<	2	64<
Dematiaceous fungi	*Alternaria alternate* (3)	16~32	16	≦0.015	≦0.06	0.5	64<
	Pseudallescheria boydii (3)	128	8~64	≦0.015~0.25	≦0.06~0.25	2	64<
	Scedosporium prolificans (1)	0.5	64<	0.25	2	8	64<
Zygomycetes	*Absidia corymbifera* (3)	32	64<	0.03~0.13	2~4	0.25~0.5	64<
	Mucor circinelloides (3)	128<	64<	8<	4~8	0.13~0.5	64<
	Rhizopus oryzae (3)	128<	64<	0.25~0.5	2	0.13~0.25	2

FLCZ; fluconazole, ITCZ; itraconazole, MCZ; miconazole, AMPH; amphotericin B, 5FC; flucytosine.

0.0078 μg/ml or lower concentrations. These values were markedly lower than those of all the other reference drugs. However, the antifungal activity of FK463 against *Fusarium* spp. was as low as the currently available azole agents; complete inhibition was not accomplished even at the highest concentration of 128 μg/ml. In addition, FK463 had no antifungal activity against *Pseudallescheria boydii* (MIC, >128 μg/ml).

Discussion

Recently, accompanying advances in medical science, invasive or disseminated mycosis in compromised patients has recently increased and the causative fungi tend to be diversified[8]. The current clinical practice of antifungal chemotherapy for these serious or life-threatening infections is to use the limited number of available drugs proficiently and in the most efficient way. The development of novel antifungal agents with a broad antifungal spectrum, potent antifungal activity, and high safety has been anticipated.

The *in vitro* antifungal activity of the novel lipopeptide antifungal agent FK463 against *Candida* species, *Cryptococcus neoformans* and *T. cutaneum* has been reported by the research group of Fujisawa Pharmaceutical Co.[1,9,10]. With the exception of the basidiomycetous yeasts, FK463 has been demonstrated to have superior antifungal activity against *Candida* and *Aspergillus* species compared with the existing antifungal drugs FLCZ, ITCZ and AMPH. Furthermore, NAKAI *et al.*[11] have reported that FK463 is also active against several dimorphic fungi which cause endemic mycoses, such as *Histoplasma capsulatum* and *Penicillium marneffei*, particularly those grouping in a mycelial form.

The present study attempted to examine the *in vitro* activity of FK463 against a wide range of medically important fungi, with special reference to its potency and antifungal spectrum, in comparison with that of existing antifungal drugs. No antifungal susceptibility testing method for FK463 or any other lipopeptide antifungal has yet been standardized. We chose the methods recommended by NCCLS and those by JSMM to test the *in vitro* activity of existing antifungal drugs.

It was demonstrated that with a given fungal strain, both the NCCLS and JSMM methods gave virtually the identical MIC value for FK463 and were equally satisfactory for its MIC determination. However, since the JSMM method is easier to use to determine simultaneously a large number of fungal strains with diverse growth rates than is the NCCLS method, the former was adopted in subsequent experiments.

The results of the study demonstrate that FK463 inhibited growth of a wide range of fungal pathogens, including almost all *Candida*, *Aspergillus*, *Penicillium* and *Paecilomyces* species as well as several emerging fungal pathogens such as *Pichia anomala* and *S. cerevisiae* at a concentration range of 0.01 to 0.1 μg/ml. The *in vitro* potency of FK463 against susceptible fungal species was greater than any of the reference drugs. These results also show that the antifungal spectrum of FK463 is broader than that for 5FC and FLCZ, although it may be inferior to ITCZ, MCZ and AMPH. As reported previously[1], the high susceptibility to FK463 of FLCZ-resistant isolates of *C. albicans* was confirmed in the present study. The efficacy of FK463 in treating candidiasis caused by FLCZ-resistant *C. albicans* and non-*albicans Candida*, aspergillosis and several other opportunistic or endemic fungal infections due to FK463 susceptible fungi is expected to be a focus of attention in the future.

References

1) TAWARA, S.; F. IKEDA, K. MAKI, Y. MORISHITA, K. OTOMO, N. TERATANI, T. GOTO, M. TOMISHIMA, H. OHKI, A. YAMADA, K. KAWABATA, H. TAKASUGI, K. SAKANE, H. TANAKA, F. MATSUMOTO & S. KUWAHARA: *In vitro* activity of a new lipopeptide antifungal agent, FK463 against a variety of clinically important fungi. Antimicrob. Agents Chemother. 44: 57~62, 2000
2) IKEDA, F.; Y. WAKAI, S. MATSUMOTO, K. MAKI, E. WATABE, S. TAWARA, T. GOTO, Y. WATANABE, F. MATSUMOTO & S. KUWAHARA: Efficacy of FK463, a new lipopeptide antifungal agent, in mouse models of disseminated candidiasis and aspergillosis. Antimicrob. Agents Chemother. 44: 614~618, 2000
3) MATSUMOTO, S.; Y. WAKAI, T. NAKAI, K. HATAMO, T. USHITANI, F. IKEDA, S. TAWARA, T. GOTO, F. MATSUMOTO & S. KUWAHARA: Efficacy of FK463, a new lipopeptide antifungal agent, in mouse models of pulmonary aspergillosis. Antimicrob. Agents Chemother. 44: 619~621, 2000
4) National Committee for Clinical Laboratory: Reference method for dilution antifungal susceptibility testing of yeasts. Approved standard. NCCLS document M27-A, National Committee for Clinical Laboratory Standards, Villanova, Pa., 1997
5) YAMAGUCHI, H.; K. UCHIDA, H. KUME, T. SHINODA, K. WATANABE, T. KUSUNOKI, M. HIRUMA & H. ISHIZAKI: Report of the committee of clinical laboratory standards-1994. Jpn. J. Med. Mycol. 36: 61~86, 1995
6) National Committee for Clinical Laboratory: Reference method for dilution antifungal susceptibility testing of conidium-forming filamentous fungi; Proposed standard. NCCLS document M38-P, National Committee for Clinical Laboratory Standards, Wayne, PA, 1998
7) SHINODA, T.; H. KUME, K. FUKUSHIMA, Y. SHIMADA, S. MURASE, K. UCHIDA, R. IKEDA, M. ANDO, T. MORI, T.

KATOH: Report of the standardization committee of the Japanese Society of Medical Mycology 1995–1997. Jpn. J. Med. Mycol. 40: 239~257, 1999

8) KAWAKAMI, S.; Y. ONO, Y. MIYAZAWA & H. YAMAGUCHI: Survey of fungemia case during the past seventeen years at Teikyo University Hospital. J. Jap. Assoc. Infect. Dis. 72: 105~113, 1998

9) MAKI, K.; Y. MORISHITA, Y. IGUCHI, E. WATABE, K. OTOMO, N. TERATANI, Y. WATANABE, F. IKEDA, S. TAWARA, F. GOTO, M. TOMISHIMA, H. OHKI, A. YAMADA, K. KAWABATA, H. TAKASUGI, K. SAKANE, F. MATSUMOTO, & S. KUWAHARA: *In vitro* antifungal activity of FK463, a novel water-soluble echinochandin-like lipopeptide, Abstr. F141, p. 268. *In* Program and Abstracts of the 38th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C., 1998

10) TOMISHIMA, M.; H. OHKI, A. YAMADA, H. TAKASUGI, K. MAKI, S. TAWARA & H. TANAKA: FK463, a novel water-soluble echinocandin lipopeptide: Synthesis and antifungal activity. J. Antibiotics 52: 674~676, 1999

11) NAKAI, T.; J. UNO & M. MIYAJI: *In vitro* activity of FK463, a novel antifungal lipopeptide, against clinically important, less common molds and dimorphic fungi. Abstr. 1509, p. 569, *In* Program and Abstracts of the 39th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C., 1999