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Micafungin: In-Vitro Activity to *Candida* and *Aspergillus* and In-vivo Activity to *Candida parapsilosis*

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Abstract

Candida and *Aspergillus*, are two important fungi that can cause systemic or invasive mycoses in human. Micafungin is an antifungal agent belongs to echinocandin group with antifungal activity against *Candida* and *Aspergillus*. In this study in vitro activity of micafungin against *Candida* and *Aspergillus*, and in vivo activity to *Candida parapsilosis* were presented. A study on the in-vitro activity of micafungin for *Candida* and *Aspergillus* was conducted at our laboratory in August-September 2010. We used E-test on Mueller Hinton agar supplemented by 2% glucose and methylen blue as indicator. The strains tested were isolated from blood, bronchial lavage, urine, stool and skin. *Candida albicans* (ATCC 90028), and *Candida parapsilosis* (ATCC 22019) were use for quality control of the experiments. The minimum inhibitory concentration (MIC) was read at 24-48 hours after incubation for *Candida* and 48 hours for *Aspergillus*. According to MIC the response to micafungin was determined as susceptible (S), susceptible dose dependent (SDD)/ intermediate and resistant (R). Quality control was done in the same method. To support the laboratory work we present an in vivo activity of micafungin as a case report of *C. parapsilosis* candidemia. All *Candida* and *Aspergillus* isolates were within the range of susceptible. From *Candida* group, *C. parapsilosis* even though susceptible but shows the highest MIC (0.75 – 1.5). *Aspergillus niger* showed highest MIC (0.32), and the most frequent causative agent of aspergillosis *Aspergillus fumigatus* shows low MIC value. The result of quality control is within expected range. Micafungin shows a good antifungal activity to all *Candida* and *Aspergillus* Jakarta isolates. In vivo activity of micafungin against *C. paraspilosis* is inline with the in-vitro result.

Key words: *Candida*, *Aspergillus,* neonate, candidemia.

Mikafungin: Aktivitas In-vitro terhadap *Candida* dan *Aspergillus* dan Aktivitas In-vivo terhadap *Candida parapsilosis*

Abstrak

Candida dan *Aspergillus*, adalah dua jamur penting penyebab mikosis invasif atau sistemik pada manusia. Mikafungin diketahui sebagai antijamur golongan echinocandin yang mampu mengeliminasi *Candida* dan *Aspergillus*. Dalam penelitian ini kami ingin meneliti kemampuan in vitro mikafungin terhadap *Candida* dan *Aspergillus*, serta menyampaikan laporan kasus aktivitas in vivo mikafungin terhadap *Candida parapsilosis*. Studi untuk mengetahui aktivitas invitro dari mikafungin terhadap *Candida* dan *Aspergillus*, dilakukan di laboratorium mikologi departemen Parasitologi FKUI pada bulan Agustus-September 2010. Kami menggunakan *E-test* pada media agar *Mueller Hinton* dengan indikator glukosa 2% dan metilen biru. Strain yang kami periksa diisolasi dari darah, cairan bronkial, urin, tinja dan kulit. Sebagai kontrol kualitas uji kepekaan, kami gunakan *Candida albicans* (ATCC 90028), dan *Candida parapsilosis* (ATCC 22019). Konsentrasi hambat minimal (KHM) dibaca setelah inkubasi 24-48 jam untuk *Candida* dan 48 jam untuk *Aspergillus*. Respons terhadap mikafungin dibaca sebagai *susceptible* (S), *susceptible dose dependent* (SDD) / *intermediate* and *resistant* (R). Kontrol kualitas uji kepekaan juga dibaca dengan metode yang sama. Guna memperkuat hasil laboratorium, kami laporkan aktivitas in vivo mikafungin terhadap *C. parapsilosis* pada kasus kandidemia. Seluruh isolat *Candida* dan *Aspergillus* sensitif terhadap mikafungin. Dari golongan

Candida, *C. parapsilosis* walaupun masih sensitif namun menunjukkan KHM tertinggi (0,75 - 1,5). *Aspergillus niger* menunjukkan KHM tertinggi (0,32), dan strain *Aspergillus fumigatus* sebagai penyebab tersering aspergillosis menunjukkan nilai KHM rendah. Aktivitas Mikafungin bagus sebagai antijamur terhadap semua strain *Candida* dan *Aspergillus* di Jakarta. Aktivitas in vivo mikafungin terhadap *C. paraspilosis* sesuai dengan in-vitro.

Kata kunci: *Candida*, *Aspergillus,* neonatus, kandidemia

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Introduction

Candida and *Aspergillus*, are two important fungi that can cause systemic or invasive mycoses in human. Invasive mycoses is a life threatening infection, and to overcome the infection appropriate antifungal is needed.[1,2]

Micafungin is an antifungal agent belongs to echinocandin group with antifungal activity against *Candida* and *Aspergillus*. The mechanism of action is by inhibition of (1-3)-β-D-glucan synthase an enzyme necessary for the production of (1-3)-β-D-glucan an integral part of fungal cell wall.[3] Currently, in some countries micafungin was approved for the treatment of invasive infection caused by *Candida* and *Aspergillus* both in adult and pediatric patients.[4] In Indonesia micafungin was already use for several years, but the data on susceptibility pattern of Indonesian strains to micafungin is not available.

In this study we would like to present a preliminary report on the susceptibilitiy pattern of *Candida* and *Aspergillus* of Jakarta strains against micafungin. In vivo activity of micafungin to *Candida parapsilosis* which is known less susceptible to micafungin, will be presented as case report.

Material and Methods

Susceptibility study

Isolates

All isolates used in this study are collection of Mycology Division, Department of Parasitology, Universitas Indonesia, Faculty of Medicine. A panel consisiting of 23 clinical isolates belonging to 23 different *Candida* strains were tested. Each isolates was obtained from a various clinical specimens which was sent to our laboratory for diagnosis, identification and susceptibility testing. The clinical specimens from which the fungi isolated were blood, bronchial lavage, urine, stool and skin. The *Candida* isolates tested consist of seven isolates of *C. albicans,* one isolate of *C. glabrata, C. parapsilosis,* (three isolates), *C. tropicalis* (five isolates)*, C. krusei* (two isolates) dan *C. kefyr* (five isolates).

For *Aspergillus* a panel consist of one isolate of *A. niger*, two isolates of *A. flavus* and two isolates of *A. fumigatus* were tested. Each strain was obtainned from different clinical materials such as tumor biopsy, skin biopsy and sputum.

Candida albicans (ATCC 90028)*,* and *C. parapsilosis* (ATCC 22019) were included as quality control in the experiments.

Inocculum Preparation

The isolates were refreshed by re-culture on sabouraud dextrose agar (SDA). For *Candida* incubation period was 24 hour, while for *Aspergillus* due to its

maturation of sporulation, the incubation period was a week. Both were incubated at room temperature (29°C). After incubation, the fungi were harvested and the spores were suspended into sterile distilled water then adjusted until the final concentration equivalent to 0.5 McFarland turbidity standard and used for susceptibility test

Susceptibility Testing

Susceptibility was conducted by using E-test strip for micafungin (AB-BIODISK, Solna Sweden), on Müeller Hinton agar supplemented by 2% glucose and 0.5µg/ml methylene blue as indicator.[5-6] Using a sterile cotton swab the suspension was rubbed carefully onto the surface of Müeller-Hinton agar, and lets 15 minutes for the liquid to seep into the medium before addition of E-test strip, then incubated at 35° C. For *Candida.* Results were read at 24 and 48 hours after incubation whereas for *Aspergillus* after 48 hours.

The minimum inhibitory concentration (MIC) was determined on the concentration where the elliptical inhibition zone intersect with the scale on the strip. The interpretation of their susceptibility pattern is based on the *susceptibility breakpoint.*[7-9] Quality control with *C. albicans* (ATCC 90028) , and *C. parapsilosis* (ATCC 22019) was done by the same method.

Case Report

In completion of susceptibility study, a case report on an invasive infection caused by *C. parapsilosis* will be presented.

Results

Susceptibility Study

As summarized in Table 1, a total of 23 isolates of *Candida* and five isolates of *Aspergillus* were tested. The isolates were obtained from various clinical materials and for *Candida* mostly were derived from blood whereas the origin of *Aspergillus* is distributed from sputum, bronchial lavage, and tissue biopsy.

Table 1: The Origin of Isolates Used in Susceptibility Study

isolates	clinical materials					
	blood	bronchial washing	urine	stool	skin	biopsy
***Candida* spp**						
C. albicans	4	3	–	–	–	–
C. tropicalis	4	–	1	–	–	–
C. parapsilosis	2	–	–	–	1	–
**C. glabrata*	–	–	–	–	–	–
C. kefyr	5	–	–	–	–	–
C. krusei	–	1	–	1	–	–
***Aspergillus* spp.**						
A. fumigatus	–	1 (sputum)	–	–	–	1
A. flavus	–	–	–	–	1*	1
A. niger	–	–	–	–	1	–

* not known; *A. flavus* from the skin was obtained by touch biopsy;

The results of susceptibility study is summarized in Table 2, as MIC and result of quality control using *C. albicans* (ATCC 90028), and *C. parapsilosis* (ATCC 22019), is within the expected range.

Table 2. Results of Susceptibility Test of *Candida* spp. And *Aspergillus* spp. Against Micafungin.

isolates	n (total)	S	SDD	R	MIC – range µg/ml
C. albicans	7	7	–	–	0.016 – 0.25
C. tropicalis	5	5	–	–	0.014 – 0.25
**C. parapsilosis*	3	2	–		0.75 – 1.5
C. krusei	2	2	–	–	0.064 – 0.094
C. glabrata	1	1	–	–	0.0175
C. kefyr	5	5	–	–	0.094 – 0.38
A. niger	1	1	–	–	0.32
A. fumigatus	2	2	–	–	0.006-0.047
A. flavus	2	2	–	–	0.002-0.003
Total	28	28	0	0	

*one strain is not growing well; S, susceptible; SDDS, susceptible dose dependent; R, resistant; MIC, minimum inhibitory concentration.

All *Candida* isolates tested are susceptible and *C. parapsilosis* showed the highest MIC (0.75 – 1.5µ ml) but still in a the range of susceptible. *Candida parapsilosis* grows slowly and the result can only be read after 48 hours of incubation period. Three isolates of *C. parapsilosis* including one quality control (*C. parapsilosis* ATCC 2201), two of which managed to be read after 48 hours of incubation, whereas one isolate could not be assessed because there was absolutely no growth.

All of filamentous fungi were susceptible to micafungin and *A. niger* showed highest MIC (0.32), while *A. fumigatus* and *A. flavus* were susceptible in low concentration.

In Vivo Activity Of Micafungin Against C. parapsilosis (*Case Report*)

A seven days old neonate was reffered to the hospital, suspected of sepsis and respiratory distress. His gestation period was 41 weeks. During pregnancy, her mother was admitted to the hospital when her pregnancy was ca. 32 weeks because of oligo-hydramnion. He was born by caesarean section with oligo-hydramnion as the indication, APGAR score 2/7, body weight is 2700 g; height is 48 cm; circumference of head is 33 cm and no congenital abnormalities were recognized. During pregnancy his mother was not smoking, no alcohol consumption, not a drug user and did not take jamu (Indonesian herbs) as supplementary. On admission, a severely ill neonate, dyspnea (using O_2, 2L/ minute and saturation 59%), with unstable temperature, heart rate is 158/min, respiratory rate 60/min, epigastric retraction present, multi organ dysfunction, electrolyte imbalance, his body weight was 2454g and there was decrease of consciousness. The hematology data are: Blood: Hemaglobin 15; Hematocryte 44.3; Leucocyte 9 430; Thrombocyte 10 000; CRP: 121, Prothrombin time 19.9; albumine 2.20; blood culture: *Enterobacter gergoviae,*that susceptible to meropenem; Glucose 68 mg/dl; electrolytes imbalance (Sodium 122, Potassium 6.60, Chloride 96); APTT 35.5; Calcium 1.02; pH 7.39; PCO_2 35.0; PO_2 65; Base excess 3.9; TCO_2 21.5; HCO_3 20.4; O_2 Saturation 94; IT ratio 0.11. The emergency condition was handled and he was sent to neonates intensive care unit (NICU). During his stay in NICU, nutrition was given via deep vein catheter on his right

groin. He was given 2x75mg meropenem with a good response, but his temperature was always a little bit above 37°C; amikacin 2x15 mg was added and the temperature became normal (< 37°C) and the baby is more active, and able to take nutrition orally. Antibiotics were planned to be given for 21 days but, on the day 12 there was an increase of temperature (38.8°C) and a second blood culture was taken. While waiting for the laboratory results mycostatin was given to prevent translocation of *Candida* from the bowel system. The result of blood culture is *C. parapsilosis* and the diagnosis of candidemia caused by *C. parapsilosis* was established. Antifungal agent was considered and we checked liver and renal function to choose suitable antifungal agent. Amphotericin B was not given because of the decrease of renal function (blood urea 172.5 & creatinine 1.5); azole derivatives did not come in to consideration because there were increased of ALT/SGOT and AST/SGPT (350 & 281). Based on the condition of both renal and liver function, micafungin was chosen. In addition, the patient was hemodynamically unstable. Then all antibiotics were stopped and micafungin was started with the dose 2 mg/ kgBW/ day (according to monograph released by Astellas), diluted in saline and given for 12 days. On the day-3 of treatment another blood culture was conducted and again *C. parapsilosis* was isolated; the diagnosis is proven candidemia caused by *C. parapsilosis*. The use of micafungin improved patient's condition, clinically the patient was getting better but his temperature was still around 37°C with some spikes, maximum 38.2°C at the day-3 of treatment. During micafungin treatment liver and renal function were returned to normal (ALT from 350 to 40 and AST from 281to 38.8). On the day 13 micafungin was replaced by fluconazole orally (12 mg/day) for 14 days. The reason to give fluconazole were persistent *C. parapsilosis* infection (two times blood culture positive, one before and one during treatment and temperature always above 37°C). During micafungin treatment liver function returned to normal, patient was able to have treatment orally and hemodynamically stable).

Discussion

Isolates tested were obtained from various clinical materials. *Candida* strains mostly isolated from blood and other body parts (Table 1). In human body, digestive tract, respiratory tract, and sometimes skin are known as the sites where *Candida* lives as saprophyte. But, in certain condition such as alteration of micro-environmental balance due to many causes, they are able to cause invasive infection, and candidemia is the most frequent clinical manifestation.[10-11] Thus, we used the strains isolated from blood and also from other sites represent the source of infection. Nucci and Annaisie[10] analysed 21 papers on the association of candidemia and its possible source of infection. They ended on conclusion that the major source of infection is colonization of *Candida* in the gut. Whereas for *C. paraspilosis* infection mostly originated from skin colonization especially patients who use deep vein/central venous catheter as happened in this patient.

In our study (Tabel 2), all *Candida* strains tested were within the range of susceptible to micafungin. The highest MIC was shown by *C. parapsilosis* (MIC range is 0.75 – 1.5µg/ml). This result was generally consistent with the result of other studies.[7, 12-14] Pfaler et al conducted a global surveilance for in vitro activity of micafungin and caspofungin. They find out that *C. albicans, C. tropicalis, C. kefyr, C. glabrata,* and *C. krusei* were belong to the group of highly susceptible, while *C. parapsilosis* was less susceptible.[14] *Candida krusei* known as intrinsically resistent to fluconozale,

a widely use antifungal agent, while *C. glabrata* is less sensitive to fluconazole,[15] but in our study both fungi show low MIC which is in line with high eficacy. Further more, *C. albicans,* and *C. tropicalis* are the most common causes of *Candida* invasive infection in Jakarta[2] and were susceptible to micafungin.

Our in vitro study showed a less activity against *C. parapsilosis*, it needs longer time to show its efficacy and the result showed highest MIC compare with other species. Other studies conducted in Italy[13] also support our finding, and even rare, the development of resistant to micafungin is more likely to occur in *C. parapsilosis*.[16-17] *Candida parapsilosis* is unusual cause for candidemia and known as less susceptible to micafungin.[7]

Our result on in-vitro study confirmed by in vivo observation in a neonate who suffered invasive candidiasis caused by *C. parapsilosis.* This drug is not really potent for *C. parapsilosis,* but only inhibit the growth of fungi. But on the other hand micafungin is more safe for liver and kidney, so it could be used for the patients with mild-moderate liver and kidney dysfunction.[18]

Its inhibitory power of the fungus provides an opportunity for the homeostasis system to improve the functioning of both organs. So when both organs were returned to normal, anti-fungal drugs which could eradicate *C. parapsilosis* such as fluconazole can be given. Micafungin is also excellent for use in unstable homeostasis conditions. All of the above conditions can be found in the neonates reported in this report. Dosage of 2 mg/ BW later on known as a small dose since in neonates the clearance of micafungin is faster then adult.[19]

Filamentous fungi tested in our study is *Aspergillus* spp., consisting of *A. fumigatus, A. flavus* and *A. niger*. The result showed that the MIC of the Aspergilli tested were within the range of susceptible with the highest MIC is *A. niger* (0.32µg/ml). This result is consistent to Watanabe *et al.,* [20] that micafungin has killing activity to *Aspergillus*.

Aspergillosis, an infection caused by *Aspergillus* associated with wide clinical spectrum, among others invasive aspergillosis a lethal sino-pulmonary infection. Invasive aspergillosis is a disease in compromised individual e.g. as patients with hematology malignancy, patients admitted to the intensive care and patient under steroid treatment. Without proper treatment the disease can be fatal.[21] This study is a preliminary study with limited number of strains. So, it has not been able to have a complete susceptibility pattern of *Candida* and *Aspergillus* against micafungin and it is limitations of our study.

According to this result micafungin *Aspergillus* and *Candida* used in this study were susceptible to micafungin and can be used for the treatment of invasive candidasis and invasive aspergillosis.

In Indonesia, antifungal armamentarium are quite limited, even for such a grave infection as invasive fungal infection, only amphotericin B and azoles derivatives (fluconazole and voriconazole) are available. Thus, the availability of echinocandin group in this case micafungin which has different mechanism with azole and poly-en will be beneficial.

In this case we choose micafungin because of patient condition that did not permit the use of azole derivatives such as fluconazole. We have to be careful with the increasing number of *C. parapsilosis* infection due caspofungin usage,[22] although other (Le Pera *et al* 2011-Asbtract) mentioned that *C. parapsilosis* can be eradicated in the *C. parapsilosis* break through infection during fluconazole prophylaxis.[23]

Conclusion

In vitro, all the *Candida* and *Aspergillus* strains tested were susceptible to micafungin. In *Candida* group *C. parapsilosis* showed the highest MIC while in the Aspergilli group was *A. niger*. In vivo, even though micafungin treatment in the dose 2mg/kg/bw/day could not eradicate *C. parapsilosis*, but it restore liver and renal function, enable replacement by other more suitable antifungal agent.

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