

의치환자에서 *Candida albicans* 분리와 amphotericin B 및 miconazole에 시험관내 감수성

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=Abstract=

Isolation of *Candida albicans* from Denture Patients and in Vitro Activities of Amphotericin B and Miconazole

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Candida albicans is now well recognized among the denture stomatitis patients. The broth macrodilution test is the most widely used technique for antifungal susceptibility testing. The purpose of this study was to determine the *C. albicans* carrier rate of the denture patients in Iksan, Chonbuk. To determine the *C. albicans* carrier rate of denture patients, culture were made from 227 sample taken in Iksan, Chonbuk during July 1997 to August 1997. Also activities of amphotericin B and miconazole against isolates of denture patients of *C. albicans* were tested by broth macrodilution test using RPMI medium 1640. The results were as follows : First *C. albicans* was isolated from 6.6% of denture patients samples and the frequency of isolation fo *C. albicans* was highest(50%) in the age group of 71-year-old to 80-year-old denture patients. Second, against *C. albicans*, the MIC range of amphotericin B was 0.06 - 0.25 µg/ml. MIC50 and MIC90 were 0.13 µg/ml and 0.25 µg/ml, respectively. Third, the MIC range of miconazole was 10- 20 µg/ml and MIC50 and MIC90 were 20 µg/ml and 20 µg/ml, respectively.

It was concluded from this study that *C. albicans* acriages from healthy denture individuals only over 60-year-old ages were isolated, they remain susceptible to amphotericin B and not rarely resistant to miconazole.

Key Words : *Candida albicans*, Denture Patients, In-Vitro Activity, Amphotericin B, Miconazole

차 례

가 , C. albicans가 가
 . C. albicans

Rams Slots

1. Candida biotypes
- 2.
3. (In vitro antifungal susceptibility testing of C. albicans)
 - 1) (inoculation preparation) . Amphotericin B
 - 2) (procedure) ketoconazole . Miconazole

1. C. albicans C.
2. C. albicans amphotericin B albicans가
3. C. albicans miconazole RPMI medium 1640 amphotericin B miconazole

II. 재료 및 방법

I. 서 론

1. 실험균주

Candida albicans , , 1997 7 8
 (normal flora) 227

(thrush), , Stuart 37 24 48
 (Acquired Candida sp.가
 immune deficiency syndrome) Sabouraud dextrose agar
 (candidiasis) (germ tube test)
 (chlamydo spore)
 C.albicans (identification)

2. 항균제

60% amphotericin B (AMB)
 C. albicans miconazole sigma
 , AMB stock 1,660 µg/ml ,
 가 0.5ml (13 × 100mm) -20

Miconazole (MON) stock 2 µg/ml
 MON 2mg 100% DMSO 1ml
 30
 MON 2,000 µg/ml 200 µg/ml
 MON stock 0.5ml RPMI

4.5ml
 RPMI Medium 1640 Gibco Laboratories
 Technologies, INC(Gibco BRL), USA, Cat No.
 31800-014 , RPMI 10.4g
 sterile distilled reagent-grade water 1,000ml
 0.45µm-pore-size Millipore filter

3. 항균제 감수성시험(In vitro antifungal
 susceptibility testing of *C. albicans*)
 Broth macrodilution National
 Committee for Clinical Laboratory Standards
 (NCCLS)

1) (inoculation preparation)
 Sabourand dextrise agar(SDA) *C.*
albicans 35
 purity 0.85% NaCl 5ml
 3 5 vortex
 mixer 15 20 McFarland 0.5

(resulting suspension = 1×10^6 5 × 10⁶CFU/ml) 1ml

RPMI 9ml 1:10
 (resulting suspension 1×10^6 5 × 10⁶CFU/ml)

2) (procedure)

(1) AMB
 MIC dilution tube
 AMB <Table A1>
 inoculum 0.9ml AMB 0.1ml
 (12 × 75mm)
 Growth control final inoculum
 0.9ml ROMI 0.1ml , negative
 growth control RPMI 1ml
 35 24 48
 MIC
 (score 0) 가

Table A1. Dilution scheme for AMB MIC dilution tubes

Amt (ml) :		10 × Concn (µg/ml)	Final concn(µg/ml) in tube (after a 1 : 10 dilution)
Initial concn	RPMI		
160 µg/ml		160	16
1.0	1.0	80	8
0.5	1.5	40	4
0.5	3.5	20	2
20 µg/ml		10	1
1.0	1.0	5	0.5
0.5	1.5	2.5	0.25
2.5 µg/ml		1.25	0.125
1.0	1.0	0.62	0.06
0.5	1.5	0.31	0.03

(2) MON 0.9ml RPMI 0.1ml , negative
 MIC dilution tube growth control RPMI 1ml
 MON <Table A2> 35 24 48
 inoculum 0.9ml MON 0.1ml MIC
 (12×75ml) (score 0) 가
 Growth control final inoculum

Table A2. Dilution scheme for MON MIC dilution tubes

Amt (ml) :		10×Concn ($\mu\text{g}/\text{ml}$)	Final concn($\mu\text{g}/\text{ml}$) in tube (after a 1 : 10 dilution)
Initial concn	RPMI		
200 $\mu\text{g}/\text{ml}$		200	20
1.0	1.0	100	10
0.5	1.5	50	5
0.5	3.5	25	2.5
25 $\mu\text{g}/\text{ml}$			
1.0	1.0	12.5	1.25
0.5	1.5	6.25	0.625

III. 결 과

1. 의치환자에서 C. albicans의 보균율

1997 7 8 227 15
 (6.6%) C. albicans가 ,
 70 가 10 (50%) 가
 , 60 4 (40%) , 가 60
 .(Table 1)

Table 1. *Candida albicans* isolation rate from denture patients

Age group (year)	No. of specimes cultured	Positive	
		No.	%
≤30	50	0	0
31-40	51	0	0
41-50	68	0	0
51-60	28	1	3.6
61-70	10	4	40.0
71-80	20	10	50.0
Total	227	15	6.6

2. C. albicans에 대한 amphotericin B의 감수성

C. albicans amphotericin B
 MIC 0.06 0.25 $\mu\text{g}/\text{ml}$, 50%
 MIC(MIC50) 0.13 $\mu\text{g}/\text{ml}$, 90%
 MIC(MIC90) 0.25 $\mu\text{g}/\text{ml}$
 .(Table 2)

3. C. albicans에 대한 miconazole의 감수성

C. albicans miconazol MIC
 10- 20 $\mu\text{g}/\text{ml}$, 50%
 MIC(MIC50) 20 $\mu\text{g}/\text{ml}$, 90%
 MIC(MIC90) 20 $\mu\text{g}/\text{ml}$.(Table
 2)

IV. 고 찰

Candida sp.

Table 2. In vitro activities of amphotericin B and miconazole against denture isolation of *Candida albicans*

Antifungal agents	No. and percent of isolates (N=15) ^a inhibited at ($\mu\text{g/ml}$) :										
	0.03	0.06	0.13	0.25	0.5	1	2	4	8	16 ^c	$\geq 20^d$
Amphotericin B	No.	1	11	3							
	% ^b	6.7	80	100							
Miconazole	No.								3	7	5
	%								20	66.7	100

^a No. of isolates tested

^b Cumulative percent

^c Concentration of amphotericin B

^d Concentration of miconazole

(yeast fungi) , , C.
 가 , albicans 6.6%
 , 가 2/3 71 80
 stomatitis) (denture 50% Arendorf Walker
 , , ,
 , Candida albicans
 , , ,
 , 28% 70% , Amphotericin B가
 , , ,
 (mucosal mycosis), ,
 (Denture plaque) 가 Miconazole azole
 , Makila Cytochrome P450 heme moiety
 , Bergendahl (ergosterol)
 , Miconazole C. albicans
 가 C. albicans
 가
 , C. albicans
 , C. albicans
 , C. albicans 50%
 100%fh C.
 albicans 가
 , C.
 albicans Torulopsis glabrata Candida NCCLS
 tropicalis ,
 RPMI Medium 1640

(broth macrodilution method)

C. albicans 가
MIC amphotericin B
MIC 가 0.06 0.25µg/ml, MIC50 MIC90
0.13µg/ml, 0.25µg/ml breakpoint 8 µg
/ml
NCCLS QC

Miconazole MIC 10- 20µg
/ml, 20 µg/ml 0.016
100µg/ml MIC
0.39 25µg/ml MIC Isenberg
0.6-2.5µg/ml MIC
, breakpoint 8µg/ml MIC
가

60
C.
albicans
amphotericin B, miconazole
fuconazole, 5-fluorocytosine
ketoconazole Candida sp.

V. 결론

1997 7 8

C. albicans

1. C. albicans
6.6% , 71 80
가 50% 가 , 61 70 가
40% , 60 3.6%
가

2. C. albicans MIC
amphotericin B 0.06 0.25 µg/ml
, MIC90 0.25 µg/ml
breakpoint

3. Miconazole C. albicans MIC
10- 20µg/ml, MIC90 20µg/ml
breakpoint

C.
albicans가 , 61
, amphotericin B
, miconazole

참고문헌

1. 張世洪, 薛盛用, 趙東擇, 全燾基, 口腔內 *Candida*의 分布 및 抗真菌劑에 대한 感受性. 대한화학요법학회지 3 : 45, 1985.
2. 고춘명, 박진환 : 임상검사물에서 분리한 *Candida* sp.의 항진균 ketoconazole, 5-fluorocytosine 및 amphotericin B의 단독 혹은 복합처리에 의한 항진균력에 대한 연구. 대한미생물학회지 21 : 63, 1986.
3. 대한미생물학회편 : 의학미생물학, 서흥출판사 P. 644, 1997.
4. Bodey G(ed) L Candidiasis : A growing concern. Am. J. Med. 77 : 1, 1984.
5. Meunier F : Candidiasis : Eur. J. Clin. Microbiol. Infect. Dis. 8 : 438, 1989.
6. Beachey FH : Bacterial adherence : adhesin receptor interaction mediating the attachment of bacteria to surface. J. Infect. Dis 143 : 325, 1981.
7. Kennedy MJ : Adhesin and association mechanisms of *Candida albicans*. Curr. Top. Med. Mycol. 2 : 73, 1987.
8. King RD, Lee JC and Morris AL : Adrence of *Candida albicans* and other *Candida* species to mucosal epithelial cells. Infect. Immun. 27 : 667, 1980.

9. Kimura LJ and Pearsall NN : Adherence of *Candida albicans* to human buccal epithelial cell. Infect. Immun. 21 : 64, 1978.
10. Williamson MI, Samaranayake LP, MacFarlane TW : Biotypes of oral *Candida albicans* and *Candida tropicalis* isolates. J. Med. Vet. Mycol 24 : 81, 1986.
11. Williamson MI, Samaranayake LP, MacFarlane TW : A simple new method for biotyping *Candida albicans*. J. Dent. Res. 65 : 523, 1986.
12. Williamson MI, Samaranayake LP, MacFarlane TW : Biotype of *Candida albicans* using the API 20C system. FEMS Microbiol. Lett 37 : 27, 1986.
13. Rams TE, Slots J : *Candida* biotypes in human adult periodontitis. Oral Microbiol. Immunol 6 : 191, 1991.
14. 鄭在奎 : *Candida albicans*에 대한 miconazole nitrate, nystatin 및 rifampin의 單獨 및 併合 抗菌作用에 關하여, 대한화학요법학회지 1 : 68, 1983.
15. 곽창훈, 양인섭, 전장원, 김재원, 김중환 : 칸디다증에 대한 ketoconazole의 치료효과. 대한화학요법학회지 3 : 166, 1985.
16. 고춘명, 김수기 : *Candida albicans*의 ketconazole, miconazole 및 rifampin복합처리에 의한 항균력 검사. 대한미생물학회지 23 : 535, 1988.
17. Fromptling RA : Overview of medically important antifungal azole derivatives. Clin. Microbiol. Rev. 1 : 187, 1988.
18. Graybill JR : New antifungal agents. Eur. J. Clin. Microbiol. Infect. Dis. 8 : 402, 1989.
19. National Committee for Clinical Laboratory Standards. Reference Method for broth Dilution Susceptibility Testing of Yeast : Proposed Standard. NCCLS document M27-P (ISBN 1-56238-186-5). National Committee for Clinical Laboratory Standards, Villanova, Pa, 1992.
20. Budtz-Jorgensen, E : "Denture stomatitis : III. Histopathology of truma and *Candida*-induced inflammatory lesions of the palatal mucosa", Acta. Odontol. Scand 28 : 251, 1970.
21. Buchner A, Helf M : "Pathologic conditions of the oral mucosa associated with ill-fitting dentures : I. Denture stomatitis" Isr. J. Dent. Med. 27 : 5-9, 1978.
22. Budtz-Jorgensen, E : "Clinical aspects of *Candida* infection in denture wearers" J. Am. Dent. Assoc. 96 : 474, 1978.
23. Budtz-Jorgensen, E : "Oral mucosal lesions associated with the wearing of removable dentures" J. Oral Pathol, 10 : 65, 1981.
24. Budtz-Jorgensen E, Theilade E, Theilade J : "Quantitative relationship between yeast and bacteria in denture-induced stomatitis" Scand. J. Dent. Res 91 : 134, 1983.
25. Budtz-Jorgensen E, Theilade E, Theilade J : "Method for studying the development structure and microflora of denture plaque." J. Dent. Res. 89 : 149, 1981.
26. Makila E, Hopsu-Havu V : "Mycotic growth and soft denture lining material". Acta. Odontol. Scand. 35 : 197, 1977.
27. Bergendahl T, Holmberg K, Nord C : "Yeast colonisation in the oral cavity and feces in patients with denture stomatitis". Acta. Odontol. Scand. 37 : 257, 1979.
28. Arendorf TM, Walker DM : "Oral candidal population in health and disease". Br. Dent. J. 147 : 267, 1979.
29. Budtz-Jorgensen E : "The significance of *Candida albicans* in denture stomatitis". Scand. J. Dent. Res. 82 : 151, 1974.

30. Greenspan D, Greenspan J : "Oral manifestation of AIDS". *Dermatol, Clin*, 5 : 733, 1987.
31. Fromptling RA : Overview of medically important antifungal azole derivatives. *Clin. Microbiol. Rev* 1 : 187, 1988.
32. Graybill JR : New antifungal agents. *Eur. J. Clin. Microbiol. Infect. Dis* 8 : 402, 1989.
33. Isenberg HD : In vitro antifungal susceptibility testing of yeasts. p. 5.15.1–5.15.16. *Clinical microbiology procedures handbook vol. 1*. American Society for Microbiology/Washington, DC, 1992.
34. .Ruley JF, Wilson RG, Barrett–Bee : Azole resistance in *Candida albicans*. *Sabouraudia* 22 : 53, 1984.
35. Saag MS, Dismukes WE : Azole antifungal agents : Emphasis on new triazoles. *Antimicrobiol. Agent Chemother* 32 : 1, 1988.
36. Doern GV, Tubert TA, Chapin K, Rinald MG : Effect of medium composition on result of macrobroth dilution antifungal susceptibility testing of yeasts. *J. Clin. Microbiol* 24 : 507, 1986.