Antibacterial activity of Candida albicans aspartyl proteinase

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J Fac Med Baghdad
2005; Vol. 47, No.3
Received Dec.2003
Accepted Feb.2004

Summary:

Background: *Candida species are present as normal body flora on the skin, buccal cavity, vagina and intestinal tract of many individuals. Low molecular weight protein produced by many intestinal microflora, inhibits the growth of Candida species, while* broad-*spectrum antibiotics kill most of these microflora, in turn Candida becomes activated and overgrow.*

Aim: *to study the effect of Candida proteinase on some Gram-negative & Gram – positive bacteria.*

Material & methods: *Pour plate method used to incorporate the tested bacteria in nutrient agar medium. Wells were punched with sterile cork porer. 40 of different concentrations of the enzyme was placed in each well. Zones of inhibition were measured after 24 hrs of incubation at 37°C.*

Results: *Concentrations of 40:1 and 20:20 were inhibitory for Klebsiella, E.coli and Enterobacter; While dilutions of iess than 50% of the enzyme were not effective against these bacteria, with the exception to lactobacilli.*

Key words: *Antibacteria, C.albicans Proteinase.*

Introduction

Candida species are present as normal body flora on the skin, buccal cavity, vagina and intestinal tract of many individuals (Rippon, 1988)[1]. Iraqi populations carry Candida species in intestinal tract in a high percentage reachs up to 54% (Dabbagh, 1979)[2]. One of the problems we have encountered in individuals receiving broad-spectrum antibiotics as ampicillin, is overgrowth of Candida species in the intestine. Such persons may develop abdominal cramps, gases and other symptoms, which may lead to diarrhea. Low molecular weight protein produced by many intestinal microflora, inhibits the growth of Candida species (Mahdi et al. 1993)(3). Such broad-spectrum antibiotics kill most of these microflora, in turn Candida becomes activated and overgrow.

Aspartyl proteinase is considered to be one of the important factors produced by Candida albicans to play a major role in it's virulence (De Bernard's et al. 1995)(4). The ecological relationship between Candida and intestinal microflora is one of the deciding factors for allowing Candida to be present in sufficient number, so that under such conditions Candida albicans may arise (Mahdi et al. 1993)(5) In this work we noticed great effect of C.albicans proteinase on some Gram-negative and Gram-positive bacteria.

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MATERIALS AND METEHODS:

Candida albicans used in this work was isolated from a leukaemic patient. The organism showed typical characteristics of C.albicans by conventional methods (Emmons et al. 1970)[6].

Aspartyl proteinase was prepared from Candida albicans according to Odds (1988)[7]. Antibacterial activity of C.albicans proteinase enzyme was determined against each of E.coli ,Klebsielia spp., Enterobacter spp., Ps. aeruginosa and lactobacilli isolated from different clinical specimens of patients admitted to the teaching hospital of Kadhimya. These bacteria were identified by conventional methods according to Cruickshank (1975)[8]

Petri dishes were poured with two layers of nutrient agar, the upper layer of the agar medium was inoculated with one of the above mentiond bacteria from actively growing 24 hrs cultures of a concentration of 1% (bacteria/medium) (Cruickshank et al. 1975)(9). The inoculated medium was left to dry, then punched with sterile cork porer. Different concentrations of proteinase were used. A 40 [i\ of the enzyme was placed in each well. Inoculated media were incubated at 37°C for 18-24 hrs. Zones of inhibition were measured for each concentration used.

RESULTS:

Table 1 shows the activity of C.albicans proteinase enzyme on a group of Gram-negative bacteria namely E.coli, Klebsielia spp and Enterobacter spp. Concentrations of the enzyme 40:1 and 20:20 were active and gave zones of inhibition ranging from 8-15

mm in diameter; While dilution less than 50% didn't have any effect on the bacteria.

On the other hand the enzyme has no activity on Pseudomonas aeruginosa. Among the Gram-positive bacteria used in this contest was Lactobacilli. Table 2 shows activity of the enzyme against this bacteria. It is of interest to notice that the enzyme was active against Lactobacilli even in concentration less 50%.

Table 1- Antibacterial activity of C.albicans proteinase against different Gram-negative bacrteria. .

Type of bacteria	*Dilution*	*Inhibition zone (mm)*	*Activity of enzyme (unit)*
E. coli	0 (Control: dist. Water)	0	0
	40: 1 30:10 20:20	 0	7.28 5.46 3.64
Klebsiella spp.	0 (control: Dist. Water)	0	0
	40: 1 20:20 15:25	17 12 0	7.28 3.64 2.73
Enterobacrter spp.	0 (Control: dist. Water)	0	0
	40: 1 20:20 15:25	15 8 0	7.28 3.64 2.73

Table-2: Antibacterial activity of C.albicans proteinase against Lactobacilli.

Type of bacteria	*Dilution*	*Inhibition zone Mm*	*Activity of enzynte(unit)*
Lactobacilli	0 (Control: dist.water)	0	0
	40: 1 20:20 15:25 10:30	15 9.0 6.0 0	7.28 3.64 2.73 1.82

DISCUSSION

One of the attracting phenomenon observed is antibacterial activity of aspartyl proteinase of Candida albicans. Some of the intestinal microflora were tested in this respect. Among the organisms tested were E.coli, Klebsiella spp., Enterobacter spp., and Pseudomonas spp. E.coli was inhibited by proteinase in concentration of 7.28 and 5.46 units. The inhibition zones were 10 and 8 mm in diameter. Enterobacter spp. was inhibited by proteinase enzyme in concentrations of 7.28 and 3.64 units showing zones of inhition of 15.0 and 8.0 mm in diameter. On the same token Klebsiella spp. and lactobacilli showed sensitivity toward aspartyl proteinase of C.albicans as shown in table 1 and 2.

Intestinal candidiasis often follows administration of broad spectrum antibiotics orally or even systemically. These antibiotics usually kill most of the intestinal flora. It is well documented that many of these bacteria produce low molecular weight protein (L-proien) which has anticandidal activity (Mahdi et al. 1993)[3]. Removal of most of these bacteria allows Candida to overgrow and flourish (Mahdi et al. 1993)[5]. and (Hummel et al 1973)[10]. Aspartyl proteinase of C.albicans activity against E.coli, Klebsiella and others to be reported for the first time to the best of our knowledge.

Such activity of this enzyme may contribute in continuation of candidal intestinal infection, in a sense it inhibits growth of these microflora and may prevent their colonization in the intestine or delay their reestablishment for some time. These in turn create a situation of absence of L-protein or its presence in enough concentration to inhibit flourish of Candida.

REFERENCES:

1. *Rippon JW. Medical Mycology, 3rd ed. Philadelphia: W.B. Saunders; 1988, pp: 534.*
2. *Dabbagh R. Candida species inhabiting the intestine of healthy individuals and patients with G.I tract disturbance. J Fac Med (Baghdad) 1979; 21: 74-78.*
3. *Mahdi LK and Dabbagh R. Candidal inhibitor produced by some intestinal flora. J Comm Med 1993; 6: 217-227.*
4. *De Bernard's F, Cassone A, Sturternt J and Calderone RA. Expression of C.albicans Sap 1 and Sap 2. In experimental vaginitis. Infect and Immunity 1995; 63: 1887-1891.*
5. *Mahdi LK and Dabbagh R. Candida species as intestinal flora, their overgrowth and absence. M.sc thesis. Baghdad University, 1993.*
6. *Emmons CW, Binford CH and Uptz JP. Medical Mycology. 3rd ed. New Yourk, London: Churchil Livingstone; 1970, pp: 18.*
7. *Odds FC. Candida and Candidiasis. 2nd ed. Bailliere Tindal; 1988, pp: 1-3.*
8. *Cruickshank R. Medical Microbiology. 12th ed. N.Y., London: Churchill Livingstone; 1975, vol. I, pp: 151 - 159.*
9. *Cruickshank R, Dugoid JP, Marmion BP and Swain RH. Medical Microbiology. 12th ed. Edinburgh: Churchil Livingstone; 1975, vol. II, pp: 306.*
10. *Hummel RP, Ostreicher EJ, Mefey MP, and Mcmilan BG. Inhibition of Candida by E.coli. J Surgical Research 5: 1973; 15: 53-58.*