J. Pharm. Pharmacol. 1984, 36: 283–284
Communicated August 22, 1983

In-vitro evaluation of the anticandidiasis activity of honey distillate (HY-1) compared with that of some antimycotic agents

E. E. OBASEIKI-EBOR*, T. C. A. AFONYA, *Department of Pharmaceutical Microbiology and Department of Pharmaceutical Chemistry, University of Benin, Benin City, Nigeria*

The susceptibility of 72 isolates of *Candida albicans* to the antimicrobial honey distillate fraction (HY-1) and several antimycotic agents is presented. All the isolates were sensitive to HY-1, H-115 and Jadit, while about 10% of the isolates were variably resistant to nystatin, miconazole nitrate and clotrimazole. The nystatin, miconazole nitrate and clotrimazole resistant isolates were inhibited by HY-1.

Candidiasis caused by a yeast-like fungus called *Candida albicans* manifesting vaginal discharge and irritation or burning sensation is generally considered more common than gonorrhoea. Several antifungal preparations commercially available such as miconazole nitrate (Gyno-Daktarin), clotrimazole (Canesten), nystatin (Mycostatin), etc. have been successfully used for the treatment of this infection (Willcox 1977). In this area of study, treatment failures have been reported (personal communication) when using the cream or pessary formulation of some of these antimycotic agents resulting in the use of several antifungal preparations before complete cure is obtained. This treatment failure indicates that there could be varying susceptibility profiles of *Candida albicans* isolates to the varying anticandidiasis agents. Preliminary study of the antimicrobial activity of honey distillate showed that a fraction (HY-1), had a broad spectrum antibacterial and antifungal activity, and excellent activity against a test *Candida albicans* compared with nystatin (Obaseiki-Ebor et al 1983).

We have examined the in-vitro activity of this fraction against 72 vaginal clinical isolates of *Candida albicans* compared with some commercial antimycotic preparations.

* Correspondence.

Table 1. Zones of inhibition (mm) of HY-1 and antimycotic agents against 72 vaginal *Candida albicans* isolates.

Drug	Dilution (% v/v)	Zones of inhibition (mm) 10–15	15–20	20–28	28–36
		Number of isolates			
HY-1	—	—	2	28	42
HY-1	50	1	40	31	—
HY-1	25	1	43	28	—
Mycostatin	—	3	10	43	16
Mycostatin	50	8	22	38	4
Canesten	—	4	—	36	32
Canesten	50	6	11	37	18
Daktarin	—	2	—	15	55
Daktarin	50	2	—	48	22
Jadit	—	—	—	38	34
H-115	—	—	8	60	4
H-115	50	—	38	33	1

Materials and methods

Organism. 72 clinical isolates of *Candida albicans* were obtained as vaginal swabs from the University of Benin Teaching Hospital, Benin City, Nigeria.

Antimycotic agents. Clotrimazole (canesten solution) Bayer; Miconazole nitrate (Daktarin powder) Janssen; Nystatin (Mycostatin suspension) Squibb; H-115 (chlormidazole) tincture (Grunnenthal); Jadit solution (Hoechst AG); HY-1 (antimicrobial distillate fraction). Daktarin powder was suspended in methanol at 100 mg ml^{-1} (10% w/v).

Media. Sabouraud liquid medium (Oxoid); Sabouraud dextrose agar (Oxoid).

Antimycotic assay. A modified method of Holder et al (1979) was used. Petri dishes containing 40 ml seeded sabouraud dextrose agar with *Candida albicans* had 9 mm holes punched around the periphery to which approximately 0·5 ml of the standard test antimycotic agent (and varying dilutions) as supplied by the manufacturer or honey distillate fraction (and its dilutions) was added. The plates were incubated at 35 °C for 36 h and the zones of inhibition measured. Each determination was carried out in at least three occasions.

Minimum inhibitory concentration (MIC). 10^{-2} dilution in sterile 0·9% NaCl (saline) of the two-day cultures of *Candida albicans* pure isolates were spotted respectively onto a series of overdried sabouraud agar plates containing progressively increasing concentration of the antimycotic agents. The plates were incubated at 35 °C

Table 2. Comparative in-vitro activity of HY-1 and four other antimycotic drugs against 72 vaginal *Candida albicans* isolates.

Organism (No. of isolates)	Drug	MIC (v/v %) which inhibited % of isolates 50	75	90	97
Candida albicans (72)	HY-1	0·5	1	2	4
	Mycostatin	0·1	0·2	0·5	1·0
	Canesten	0·1	0·5	0·5	2·0
	Daktarin	0·5 (w/v)	1·0 (w/v)	2·0 (w/v)	4·0 (w/v)
	Jadit	0·02	0·04	0·08	0·08

for 48 h. The lowest concentration of the agent inhibiting visible growth was regarded as the MIC.

Results and discussion

Tables 1 and 2 show the antimycotic activity of HY-1 compared with the other agents. Several isolates resistant to mycostatin, canesten and daktarin were obtained. About 5% of the isolates cross-resistant to mycostatin, canesten and daktarin were inhibited by 4% HY-1. H-115 and Jadit did not select any resistant strains.

The results indicated that all the test agents were effective against *Candida albicans*. HY-1 from this study seemed promising because about 90% of the *Candida albicans* isolates tested were inhibited by 2% v/v HY-1. All strains resistant to antimycotic agents were inhibited by 4% v/v HY-1.

The authors are grateful to Miss Nony Erukwu of the Department of Medical Microbiology, University of Benin Teaching Hospital, Benin City, Nigeria, for isolating most of the *Candida albicans* strains; and to Miss Felicia Ikpim for technical assistance.

The receipt of the University of Benin, Nigeria, staff research grant is acknowledged.

REFERENCES

Holder, I. A., Schwab, M., Jackson, L. (1979) J. Antimicrob. Chemother. 5: 455–463

Obaseiki-Ebor, E. E., Afonya, T. C. A., Onyekweli, A. O. (1983) J. Pharm. Pharmacol. 35: 748–749

Willcox, R. R. (1977) Br. J. Venereal Dis. 53: 314–423

J. Pharm. Pharmacol. 1984, 36: 284–286
Communicated October 27, 1983

Parallel bioassay of physalaemin and hylambatin on smooth muscle preparations and blood pressure

G. FALCONIERI ERSPAMER*, G. MAZZANTI, T. YASUHARA, T. NAKAJIMA, *Institute of Pharmacology and Pharmacognosy, University of Rome I, Città universitaria, 00100 Roma, Italy, and Institute for Medical and Dental Engineering, Tokyo Medical and Dental University, Kandasurugadai, Chiyodaku, Tokyo 101, Japan*

Hylambatin, a novel tachykinin endecapeptide isolated from the skin of the African frog *Hylambates maculatus*, must be ascribed to the physalaemin subfamily. It differs structurally from all other known tachykinins mainly in having a methionine residue replacing the usual leucine residue at position 2 from the C-terminus. In parallel bioassay on a number of in-vitro and in-vivo test objects, hylambatin and physalaemin were nearly indistinguishable from each other, with few moderate quantitative differences.

Methanol extracts of the skin of the South African rhacophorid frog *Hylambates maculatus* contain, in addition to a kassinin-like peptide, Glu^2,Pro^5-kassinin, a physalaemin-like dodecapeptide, hylambatin (Yasuhara et al 1981). This differs from physalaemin by substitution of several amino acids in the *N*-moiety of the molecule and the presence, at position 2 from the *C*-terminus, of a Met residue, replacing the usual Leu residue, common to all other known tachykinins, including substance P and neuromedin K (Kangawa et al 1983).

However, considering the primary structures of physalaemin and hylambatin it may be seen that, leaving apart the *N*-terminal asparagyl residue, six of the amino acids (in italic) constituting the molecule of hylambatin are found in an equivalent region of physalaemin, while the remaining five amino acids may represent simple base exchanges

Asp-Pro-Pro-*Asp-Pro*-Asp-Arg-*Phe-Tyr-Gly*-Met-*Met-NH$_2$* Hylambatin

Pyr-Ala-*Asp-Pro*-Asn-Lys-*Phe-Tyr-Gly*-Leu-*Met-NH$_2$* Physalaemin

We describe the result of parallel bioassay of hylambatin and physalaemin on a number of in-vitro and in-vivo test objects. It will be seen that the two peptides were nearly indistinguishable from each other by parallel bioassay, with few moderate quantitative differences.

Materials and methods

Hylambatin and physalaemin were assayed in parallel on the following test preparations.

(a) Isolated smooth muscle preparations: rat uterus and colon (Tyrode solution at 32 °C); guinea-pig ileum (Krebs solution at 32 °C); rat urinary bladder, guinea-pig large intestine and urinary bladder, rabbit large intestine (Tyrode solution + 0·1% glucose at 37 °C).

(b) Rat urinary bladder in-situ (urethane anaesthesia, 1·2–1·5 g kg^{-1}, intraperitoneally).

(c) Blood pressure of the rabbit and the rat (urethane, 1·5 g kg^{-1}, intraperitoneally).

(d) Salivary secretion of the rat anaesthetized with urethane (1·2 g kg^{-1}, intraperitoneally) as described Lembeck & Starke (1968).

* Correspondence.