STUDY OF THE EFFECT OF AMPHOTERICIN B, NYSTATIN AND MICONAZOLE ON THE POLAR LIPIDS OF *CANDIDA ALBICANS* AND C. *DUBLINIENSIS*

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Abstract

The aim of study was to determine whether nystatin, amphotericin B and miconazole have similar effects upon phospholipids of Candida albicans and C. dubliniensis. A serial dilution of antifungals was prepared in flasks, then, 50 µl of standardised suspension was inoculated into each flask and incubated in shaking water bath at 37°C for 48 h. The Candida growth in last flask was centrifuged and yeast cells harvested, washed and freeze-dried. Polar lipids were extracted from freeze-dried cells and then analysed by Fast Atom Bombardment Mass Spectrometry (FAB MS) in negative-ion mode. Nystatin, amphotericin B and miconazole different effects on phospholipids and fatty acids of two strains of C. albicans and a single strain of C. dubliniensis. The content of phosphatidylethanolamine (PE) in C. dubliniensis decreased in the presence of nystatin and amphotericin B, whereas this phospholipid was absent in cultures exposed to miconazole. PE in both examined strains of C. albicans decreased in the presence of amphotericin B and nystatin, whereas PE in one strain of C. albicans increased and in the other decreased when cultures were exposed to nystatin. It is concluded that biosynthesis of fatty acids and phospholipids of C. albicans and C. dubliniensis were affected by nystatin, amphotericin B and miconazole, in addition to effects on ergosterol previously described. Also, antifungals have various effects on different strains of C. albicans.

Keywords:

Amphotericin B, Miconazole, Nystatin, Polar lipids, Candida albicans, Candida dubliniensis

Introduction

Candida albicans is a saprophytic yeast that causes candidiasis. Superficial candidiasis is usually is treated with a topical preparation such as nystatin or miconazole but disseminated infections require systemic therapy e.g. amphotericin B. The most important topical preparations for candidiasis are miconazole and nystatin. The most important systemic preparation for systemic candidiasis is amphotericin B (1). Amphotericin B, nystatin and miconazole inhibit the growth of many fungi *in vitro*, for example, those are active against *C. albicans*. Studies show that the antifungal activity of amphotericin B, nystatin and miconazole is due to an interaction of the drug with sterol in the cell membrane. Therefore, the specific target is sterole, specially, ergosterol (2). The polyene resistant mutants of *C. albicans* have a different phospholipid fatty acid composition (3). Koh *et al*., have suggested that the variation in polyene sensitivity in *C. albicans* to be due to the differing fatty acid compositions (4). Azoles interact

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directly with membrane lipids without necessary binding to them (1, 5). For example, interaction with cell membrane phospholipids and fatty acids cause leakage of proteins and amino acids (6, 7). Some of the antifungal agents can inhibit fatty acid synthesis in yeasts (8, 9, 10). For example, cerulenin is a specific inhibitor of fatty acid (11). Iannitelli and Ikawa have shown that fatty acids are capable of protecting *Saccharomyces cerevisiae* against the action of polyenes (12). It is demonstrated that palmitoleic acid ($C_{16:1}$), oleic acid ($C_{18:1}$), α-linoleic acid ($C_{18:2}$), γ-linolenic acid ($C_{18:3}$) and arachidonic acid ($C_{20:4}$) have antagonistic effect against clotrimazole and miconazole (13). Also, Lomb *et al.*, have shown that the contents of fatty acids in the sensitive cultures of *C. glabrata* to polyenes differ from those of polyene resistant cultures (14). The imidazole antifungal agents, such as miconazole, econazole, clotrimazole and ketoconazole are decreased in the ratio of unsaturated:saturated fatty acids *in vivo* (15). The content of linoleate in *C. albicans* was increased from 15% in the phospholipid fraction of control cells to 22.5% and 25% in the phospholipid fraction of cells that were incubated in the presence of ketoconazole or miconazole (8). The aim of this study was to determine whether amphotericin B, nystatin and miconazole have similar effects upon fatty acids and phospholipid analogues of *C. albicans* and *C. dubliniensis*.

Materials and methods

Organisms

C. albicans C4, C70 and *C. dubliniensis* C1 obtained from the patients of Dental Hospital, at the University of Manchester. Isolates were maintained on Sabouraud's Dextrose Agar (SDA) (BBL, Becton Dickinson, USA). Isolates were confirmed as *C. albicans* and *C. dubliniensis* by production of yellow-green to blue-green colonies on CHROMagar Candida, germ tube tests on horse serum, production of chlamydoconidia on cornmeal agar, growth at 45°C and ID 32 C kits (BioMerieux SA, Marcy-L'Etoile, France).

Preparation of the inoculum

Psynchronous cultures were prepared according to the method of Johnson (16). For preparing fresh psynchronous cultures, test organisms were sub-cultured on SDA and incubated at 37°C for 24 h. Ten parts of plates were chosen and yeast cells were collected in 2 ml of sterile PBS by sterile cotton wool swabs to prepare a suspension of colonies (blastospores). The suspension was washed twice in PBS (2,000×*g* for 20 min) and re-suspended in 1 ml of sterile distilled water and adjusted to 70% Transmittance (T) by a spectrophotometer (Pye Unicam) set at 530nm. This should result in a suspension containing about 1×10^6 colony forming units (cfu)/ml.

Preparation of drug solution

Antifungal agents were obtained from Sigma (Sigma, Poole, Dorset, UK). 64 mg of amphotericin B, nystatin and miconazole were dissolved in 50 ml of dimethyl sulphoxide (DMSO) (Sigma, Poole, Dorset, UK) separately at 1280 mg/l. The solutions were left at room temperature for 30 minutes for self-sterilisation. The stock solutions were stored at –70°C for up to 1 month. The stock solutions of amphotericin B and nystatin were diluted to 640 mg/l with sterile distilled water.

Test method

5 ml of Sabouraud liquid medium (SLM) (Oxoid, Hampshire, England) was added to each of 10 sterile Erlenmeyer flasks (100 ml) and 5 ml of the drug solution (640 mg/l of amphotericin B and nystatin (2 fold dilution of the 1280 mg/ml stock) or 1280 mg/l of miconazole) was added to flask 1. The contents were mixed and 5 ml of solution was transferred into flask 2. These serial dilutions were repeated through to

flask 9. 5 ml of flask 9 was discarded. Flask 10 contained 5 ml SLM as control. 45 ml of sterile SLM was added to each flask. Then 50 µl of standardised suspension was inoculated into each flask and incubated in water bath shaker (37^{o}C) at 150 rpm for 48 h. The last flask that had growth was centrifuged at 3,000×*g* for 20 min.

Lipid extraction and analysis

All yeast cells were harvested, washed with sterile PBS and freeze-dried. Polar lipids were extracted from freeze-dried cells with chloroform-methanol (1:2, v/v). Lipid extracts were washed with sterile PBS for Fast Atom Bombardment Mass Spectrometry (FAB MS). Polar lipids were analysed by FAB MS in negative-ion mode with a Concept IS mass spectrometer (Kratos, Manchester, UK). All experiments were repeated three times.

Results

C. albicans

Nystatin

The minimal inhibitory concentration (MIC) of nystatin for both isolates of *C. albicans* was 0.25 mg/l and polar lipid data were obtained from cells growing on the next serial dilution of nystatin (0.125 mg/l). Carboxylate anion profiles of *C. albicans* (C70, C4) grown on nystatin showed that $C_{18:1}$ (41.4%, 29.8%), respectively was the major fatty acid in spectra, compared to control (31%, 28.1%) respectively (Table I). Phospholipid analogues of *C. albicans* (C70, C4), grown in the presence of nystatin, are shown in table II. Some phospholipid analogues, such as *m/z* 559(PA 26:1), 714(PE 34:2) and 715(PG 32:3) were detected in *C. albicans* (C70) and *m/z* 619(PG 25:2) and 761(PG 31:5) were found in *C. albicans* (C4). Overall, the content of PA peaks of *C. albicans* (C70) proportionately decreased compared to control from 36.7% to 24.7%. Other phospholipids, such as PE and PG, considerably increased compared to controls.

Amphotericin B

Polar lipids of *C. albicans* (C4, C70) were obtained from cultures grown on the dilution 0.125 mg/l. Amphotericin B decreased the content of *m/z* 253($C_{16:1}$), 255($C_{16:0}$), 279($C_{18:2}$), 281($C_{18:1}$) from a combined of 80.90% of total acid anions in control to only 41.3%. Amphotericin B inhibited the biosynthesis of *m/z* 239($C_{15:1}$), 241($C_{15:0}$), 267($C_{17:1}$) and 297($C_{19:0}$) in *C. albicans* (C70) and *m/z* 297($C_{19:0}$) and 267($C_{17:1}$) in *C. albicans* (C4) (Table I). The content of *m/z* 515(PA 23:2) considerably increased from 8.9% to 18.8% in *C. albicans* (C70) compared to controls, whereas this anion decreased in *C. albicans* (C4) from 11.4% to 3.0%. 501(PA 22:2) in *C. albicans* (C70) decreased from 10.5% to 3.1%. PA and PE decreased in both *C. albicans* isolates tested, whereas PG decreased from 20.6% in control to 9.4% in *C. albicans* (C70) and increased in *C. albicans* (C4) (Table II).

Miconazole

The MIC of miconazole for both *C. albicans* (C4, C70) was 2 mg/l and polar lipids were obtained from growing on the next serial concentration (1 mg/l). Miconazole was inhibited the biosynthesis of *m/z* 267($C_{17:1}$) in *C. albicans* (C70) and decreased the amount of *m/z* 253($C_{16:1}$) and 279($C_{18:2}$). The content of *m/z* 255($C_{16:0}$), 241($C_{15:0}$) and 283($C_{18:0}$) proportionately increased (Table I). Miconazole inhibited phospholipids with medium chain length fatty acid substituents, such as *m/z* 557(PA 26:2) and 659(PG 28:3) in *C. albicans* (C70). Overall, the content of PA, PG and PE decreased compared with unidentified peaks in higher mass which increased from 22.3% to 39.2%, in *C. albicans* (C70) (Table II).

C. dubliniensis

Nystatin

obtained from growing on the next serial dilution (0.5 mg/l). Carboxylate anion of *m/z* 295($C_{19:1}$) was seen in cultures when grown with nystatin compared to control. Nystatin caused the proportion of *m/z* 253($C_{16:1}$), 255($C_{16:0}$), 279($C_{18:2}$) and 281($C_{18:1}$) to decrease, while the proportions of *m/z* 283($C_{18:0}$) and 241($C_{15:0}$) increased (Table I). The most obvious changes were observed with *m/z* 515(PA 23:2) and 501(PA 22:2). The content of those phospholipid analogue anions in cultures grown with nystatin were respectively. 18.1% and 10.5% (Table II). The contents of PE and PG generally decreased in the presence of nystatin whereas PA increased.

Amphotericin B

In this study, the MIC of amphotericin B for *C. dubliniensis* (C1) was 1.0 mg/l and polar The MIC of nystatin for *C. dubliniensis* (C1) was 1 mg/l and polar lipids were lipid data were obtained from growth on the next serial dilution of amphotericin B (0.5 mg/l). Table I shows that *m/z* 255($C_{16:0}$) (25.4%) was the major carboxylate anion in culture growth with amphotericin B compared to *m/z* 281($C_{18:1}$) (28.1%) in control. When *C. dubliniensis* grew on amphotericin B the greatest variation in carboxylate anion was observed in the content of *m/z* 295($C_{19:1}$), 281($C_{18:1}$) and 279($C_{18:2}$). For example, *m/z* 279($C_{18:2}$) and 281($C_{18:1}$) decreased from 18.6% to 3.5% and 28.1% to 13.3%, respectively. Phospholipid analogues of *C. dubliniensis* grown on amphotericin B are shown in table II. In the presence of amphotericin B, phospholipid analogues of *m/z* 761 (PG 35:1), 762(PS 34:0) were detected and *m/z* 673(PA 34:1) and 715(PG 32:3), were inhibited.

Table1: Mean[a] normalised carboxylate peaks for *C. albicans* (C4, C70) and *C. dubliniensis* C1 grown on SLM with nystatin, amphotericin B, miconazole and SLM as controls

m/z[b]	ID[c]	*Candida albicans* C4				*C. albicans* C70				*C. dubliniensis* C1			
		Amp	Mic	Nys	Con	Amp	Mic	Nys	Con	Amp	Mic	Nys	Con
200	Un[d]	0.0	0.0	0.0	0.0	9.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
209	($C_{13:2}$)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.7	0.0	0.0
210	Un	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6	0.0	0.0
211	($C_{13:1}$)	0.0	0.0	0.0	2.2	0.0	0.0	0.0	0.0	2.8	6.4	0.0	0.0
221	($C_{14:3}$)	0.0	0.0	5.0	2.7	7.5	6.2	0.0	0.0	0.0	1.9	0.0	0.0
222	Un	0.0	0.0	5.9	3.1	11.3	4.4	0.0	0.0	0.0	0.0	0.0	0.0
225	($C_{14:1}$)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2	1.2	0.0	0.0
227	($C_{14:0}$)	2.6	0.0	0.0	0.9	0.0	0.0	0.0	0.0	8.5	7.3	0.0	0.0
237	($C_{15:2}$)	0.0	0.0	0.0	0.0	2.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
239	($C_{15:1}$)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.0	0.0	0.0	0.0	0.0
241	($C_{15:0}$)	0.0	0.0	0.0	0.0	0.0	12.2	0.0	2.4	2.6	0.0	9.8	1.0
251	($C_{16:2}$)	0.0	0.0	0.0	0.0	4.7	0.0	0.0	1.8	0.0	0.0	0.0	0.0
253	($C_{16:1}$)	13.0	16.7	13.2	15.4	12.2	13.2	20.5	20.5	12.8	13.8	18.0	19.4
255	($C_{16:0}$)	22.4	19.0	19.6	20.5	9.9	14.8	18.1	13.2	25.4	28.6	21.3	24.9
257	OH-$C_{15:0}$	0.0	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
265	($C_{17:2}$)	0.0	0.0	0.0	0.0	3.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
267	($C_{17:1}$)	0.0	0.0	0.0	2.2	0.0	0.0	1.1	4.3	6.0	4.4	0.0	0.0
269	($C_{17:0}$)	0.0	0.0	0.0	0.0	0.0	1.5	0.0	0.0	0.0	0.0	0.0	0.0
277	($C_{18:3}$)	2.5	0.0	1.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9
279	($C_{18:2}$)	21.2	24.2	17.0	16.9	8.2	16.0	16.6	16.2	3.5	0.0	6.7	18.6
281	($C_{18:1}$)	29.7	25.5	29.8	28.1	11.0	15.9	41.4	31.0	13.3	12.2	27.6	28.1
283	($C_{18:0}$)	4.6	12.1	6.4	6.4	19.9	13.5	2.3	1.4	3.1	1.6	10.6	7.1
295	($C_{19:1}$)	3.9	0.0	1.5	0.0	0.0	0.0	0.0	0.0	21.0	18.1	6.1	0.0
297	($C_{19:0}$)	0.0	0.0	0.0	1.6	0.0	2.3	0.0	4.3	0.0	0.0	0.0	0.0

[a]Mean of three repeat analyses, [b]Mass-to-charge, [c]Identity which is tentative, [d]Unidentified, Amp: Amphotericin B; Mic: Miconazole: Nys: Nystatin; Con: Control

Table 2: Mean normalised phospholipid analogue peaks for *C. albicans* (C4, C70) and *C. dubliniensis* C1 grown on SLM with nystatin, amphotericin B, miconazole and SLM as controls

m/z	ID	*Candida albicans* C4				*C. albicans* C70				*C. dubliniensis* C1			
		Amp	Mic	Nys	Con	Amp	Mic	Nys	Con	Amp	Mic	Nys	Con
500	Un	0.0	0.0	2.8	2.5	2.0	9.2	0.0	3.3	0.0	3.1	2.5	0.0
501	PA 22:2	0.0	6.2	2.7	3.7	3.1	10.7	3.4	10.5	3.9	5.1	10.5	0.0
502	Un	0.0	0.0	0.0	0.0	5.0	0.0	1.9	0.0	0.0	2.4	0.0	0.0
503	Un	0.0	0.0	0.0	0.0	2.3	0.0	0.0	3.0	0.0	0.0	0.0	0.0
505	Un	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.1	4.5	0.0	0.0
513	Un	0.0	0.0	0.0	0.0	3.9	10.1	0.0	0.0	0.0	2.3	2.4	0.0
514	Un	0.0	6.5	0.0	3.3	14.5	13.9	1.9	4.1	2.4	3.8	10.4	0.0
515	PA 23:2	3.0	15.8	7.9	11.4	18.8	16.2	5.6	8.9	8.9	9.0	18.1	0.0
516	Un	0.0	2.5	0.0	0.0	23.9	6.0	0.0	3.3	0.0	2.7	3.0	0.0
517	Un	0.0	0.0	0.0	0.0	3.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
619	PG 25:2	0.0	0.0	4.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
525	Un	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.3	0.0	0.0
530	Un	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.0	0.0	0.0	0.0	0.0
531	Un	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.9	0.0	0.0	0.0	0.0
533	Un	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.6	0.0	0.0
545	Un	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.4	2.7	0.0	0.0
557	PA 26:2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.9	0.0	0.0	0.0	0.0
573	Un	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.2	0.0	0.0
559	PA 26:1	0.0	0.0	0.0	0.0	0.0	0.0	2.7	0.0	0.0	0.0	0.0	0.0
604	PE 26:1	0.0	0.0	0.0	0.0	2.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
613	Un	0.0	0.0	0.0	0.0	7.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
659	PG 28:3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.3	0.0	0.0	0.0	0.0
660	PE 30:1	0.0	0.0	0.0	0.0	0.0	0.0	2.2	2.7	0.0	0.0	0.0	0.0
671	PA 34:2	10.2	2.7	9.0	3.0	0.0	7.4	2.9	2.7	3.0	0.0	0.0	8.5
672	PE 31:2	0.0	0.0	0.0	0.0	10.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
673	PA 34:1	2.9	2.9	5.5	5.2	0.0	0.0	10.1	11.7	0.0	2.5	0.0	8.7
674	PE 31:1	0.0	0.0	0.0	0.0	0.0	0.0	3.8	3.9	0.0	0.0	0.0	0.0
676	PE 31:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.6	0.0	0.0	0.0	0.0
687	PG 30:3	0.0	0.0	0.0	0.0	0.0	0.0	4.2	5.0	4.1	2.9	2.7	0.0
688	PE 32:1	0.0	2.5	0.0	0.0	0.0	0.0	3.2	3.4	0.0	0.0	0.0	2.4
689	PE 32:1*	0.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
697	PG 31:5	6.2	0.0	3.2	0.0	0.0	0.0	0.0	2.8	0.0	0.0	0.0	0.0
698	PE 33:3	0.0	2.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
699	PG 31:4	8.1	0.0	8.5	3.2	0.0	0.0	5.6	3.6	3.3	0.0	0.0	7.2
700	PE 33:2	0.0	0.0	0.0	0.0	0.0	0.0	3.3	2.7	0.0	0.0	0.0	0.0
701	PG 31:3	0.0	6.3	0.0	0.0	0.0	0.0	5.3	2.7	0.0	2.1	2.6	0.0
702	PE 33:1	0.0	5.7	0.0	0.0	0.0	0.0	5.5	0.0	0.0	0.0	2.5	0.0
713	PG 32:4	0.0	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
714	PE 34:2	11.0	3.9	11.1	12.3	0.0	7.3	4.0	0.0	3.6	0.0	2.9	13.1
715	PG 32:3	3.5	7.3	0.0	6.4	0.0	4.7	7.5	0.0	0.0	0.0	6.0	8.5
716	PE 34:1	7.3	3.1	10.8	11.2	0.0	4.6	7.2	0.0	2.9	0.0	3.0	14.4
717	PG 32:2	0.0	3.1	0.0	3.1	4.9	0.0	3.4	0.0	0.0	0.0	0.0	5.1
733	PG 33:1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.7	7.1	0.0	0.0
734	PS 32:0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.8	2.5	0.0	0.0
740	PE 34:3	6.3	0.0	5.1	3.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.3
741	PG 34:2	0.0	5.6	0.0	0.0	0.0	2.5	0.0	0.0	0.0	0.0	0.0	0.0
742	PE 36:2	10.8	5.7	13.6	11.2	0.0	5.2	3.2	4.6	4.6	0.0	8.1	11.8
743	PG 34:3	5.3	9.4	5.2	8.9	3.7	2.2	7.5	3.2	2.6	0.0	8.3	6.1
744	PE 36:1	9.0	0.0	10.6	9.0	0.0	0.0	2.9	3.2	3.2	0.0	2.9	9.2
745	PG 34:2	0.0	2.6	0.0	0.0	0.0	0.0	2.6	0.0	0.0	0.0	0.0	2.8
759	Un	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.0	8.2	0.0	0.0
760	PS 34:1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.8	5.8	0.0	0.0
761	PG 35:1	7.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	19.8	15.5	6.5	0.0
762	PS 34:0	0.0	0.0	0.0	0.0	2.0	9.2	0.0	3.3	8.0	10.6	7.5	0.0
768	PS 35:4	3.4	0.0	0.0	0.0	3.1	10.7	3.4	10.5	0.0	0.0	0.0	0.0
770	PS 35:3	5.9	0.0	0.0	0.0	5.0	0.0	1.9	0.0	0.0	0.0	0.0	0.0
835	PI 34:1	0.0	0.0	0.0	2.4	2.3	0.0	0.0	3.0	0.0	0.0	0.0	0.0

Miconazole

Polar lipids were obtained from cells grown on the dilution of 1.0 mg/l. Small amount of some carboxylate anion, such as *m/z* 209($C_{13:2}$), 211($C_{13:1}$), 227($C_{14:0}$), and a greater amount of *m/z* 295($C_{19:1}$) (18.0%) were detected in *C. dubliniensis* when exposed to miconazole. Miconazole decreased the proportions of *m/z* 253($C_{16:1}$) and 281($C_{18:1}$) from 19.4% to 13.8% and 28.1% to 12.2%, respectively (Table I). Table II shows the content of phospholipid analogue anions of *C. dubliniensis* when it was grown on SLM with miconazole. PA and PG proportions were similar in cultures with miconazole or control, whereas PE and PS were absent.

Discussion

Many researchers have studied the mechanisms by which amphotericin B, miconazole and nystatin affect both the morphology of *C. albicans* cells and their mechanisms of drug resistance. However, the effect of antifungals on phospholipid analogues and fatty acids of yeasts have not been examined. In this study, the effects of three anti-*Candida* agents on polar lipids of *C. albicans* and *C. dubliniensis* were studied. Our results showed that the content of polar lipids (carboxylic acids and phospholipids) were indicated in *Candida* isolates when grown with nystatin, miconazole and amphotericin B. Koul *et al*., have suggested that lipids are targeted by some of the anti-*Candida* drugs (17). Van Den Bossche *et al*., have suggested that this shift to more unsaturated fatty acid results in increased membrane fluidity (8). According to Niimi *et al*., unsaturated fatty acids bind to azole (clotrimazole) (18). Hitchcock *et al*., have found that changes in phospholipid composition affect polyene sensitivity (3).

Fatty acid analysis of *C. albicans* and *C. dubliniensis* grown on nystatin revealed $C_{18:2}$, $C_{18:1}$, $C_{16:1}$ and $C_{16:0}$ as major fatty acids. In this study nystatin caused the ratio of unsaturated:saturated fatty acids to increase in both species compared with controls. Nystatin causes abnormal growth of *C. albicans* (19). Iannitelli and Ikawa (1980) have shown that fatty acids decreased the activity of nystatin against *S. cerevisiae* (12). Also, longer chain length fatty acids, and those with more unsaturating, are more effective. Nystatin had different effects on phospholipids of yeasts tested. For example, the content of PE and PG were increased in *C. albicans* C70 but proportionately decreased both phospholipids in *C. albicans* C4 and *C. dubliniensis* C1. Also, PS was biosynthesised in *C. dubliniensis*, whereas PS was not found in controls. Azoles have a direct effect on the fatty acids of cell membranes (6). For example, Georgopapadakou *et al*., have reported that miconazole increases the content of $C_{16:0}$ and $C_{18:0}$ whereas it decreases the proportion of $C_{18:2}$ and $C_{18:3}$ *in vitro* (15). In this study the proportion of $C_{18:0}$ increased in *C. albicans*, whereas $C_{18:2}$ increased proportionately in one strain but decreased in the other. Long-chain fatty acids, such as, palmitoleic acid ($C_{16:1}$), oleic acid ($C_{18:1}$), α-linoleic acid ($C_{18:2}$), γ-linolenic acid ($C_{18:3}$) and arachidonic acid ($C_{20:4}$) have antagonistic effects against miconazole (13). The content of linoleate ($C_{18:2}$) was found to be 15% of the total fatty acid in phospholipid fractions of *C. albicans*. While that increased up to 25% in the phospholipid fractions of *C. albican* grown in the presence of miconazole (8). In this study, $C_{18:2}$ and $C_{18:3}$ proportionately decreased in *C. dubliniensis* and small changes happened for $C_{18:2}$ in *C. albicans*. The presence of miconazole mainly affects on proportions of $C_{18:1}$ and $C_{16:1}$ in spectra. This possibly indicates that fatty acids involve in the binding site of the drug at the membrane. Georgopapadakou *et al*., have reported that miconazole has no effect on $C_{18:1}$ (15). However, in this study, the proportion of $C_{18:1}$ considerably decreased in *C. dubliniensis* and *C. albicans* (C70).

In this study, phospholipid analogues were affected by miconazole and proportions of unidentified phospholipids increased. PA, PG and PE proportions decreased in one isolate of *C. albicans* and increased in the other isolate. The proportions of phospholipid classes in *C. dubliniensis* exposed to miconazole were similar to data for controls (no miconazole). There are no published data on phospholipids and fatty acids of *C. albicans* and *C. dubliniensis* with which to compare the present data. Amphotericin B had the most effect on $C_{18:0}$ and increased the proportion of $C_{18:0}$ by 14.2 times in *C. albicans* (C70) compared to control. The major fatty acid in control was $C_{18:1}$ compared to $C_{18:0}$ in amphotericin B cultures. The ratio of unsaturated:saturated fatty acid was 1.7 and 2.4 in *C. albicans* C70 and C4, respectively. In *C. dubliniensis* the greatest changes were seen with proportion of $C_{18:2}$ and $C_{19:1}$. In this species the ratio of unsaturated to saturated fatty acids decreased. PG 32:2 is biosynthesised under the presence of amphotericin B and the content of PG 34:3 increased. Generally, amphotericin B caused PS synthesis in *C. dubliniensis* and one isolate of *C. albicans*. Also, PA and PE proportions decreased in both *C. albicans* strains, whereas PG increased in *C. albicans* C4 only. There are no previously published data on carboxylic acid and phospholipids analogues of *C. dubliniensis* grown on amphotericin B with which to compare the present findings. The proportion of unsaturated fatty acids in both species increased when exposed to nystatin, whereas amphotericin B and miconazole decreased the proportion of unsaturated fatty acids. It was found that antifungals have different effects on different strains of *C. albicans*.

It is concluded that the biosynthesis of fatty acids and phospholipids are affected by nystatin, amphotericin B and miconazole, In addition to effects on ergosterol previously described. Also antifungals have different effect on polar lipids of different strain of *C. albicans.*

References

1) Odds, F.C., *Candida and Candidosis.* 2nd ed., Bailliere Tindall, London, 1988; 279- 313.

2) Pierce, A.M., Pierce HD, Unrau. AM, Oehlschlager AC. Lipid composition and polyene antibiotic resistance of *Candida albicans* mutants. *Can J Biochem* 1978; **56**: 135-42.

3) Hitchcock CA, Barrett-Bee KJ, Russell NJ. Sterols in *Candida albicans* mutants resistant to polyene or azole antifungal, and of a double mutant *Candida albicans*. *CRC Crit Rev Microbiol* 1987; **15**: 111-15.

4) Koh TY, Marriott MS, Taylor J, Gale EF. Growth characteristics and polyene sensitivity of a fatty acid auxotroph of *Candida albicans. J Gen Microbiol* 1977; **102**: 105-10.

5) Hitchcock CA, Barrett-Bee KJ, Russell NJ. The lipid composition and permeability to azole of an azole- and polyene-resistant mutant of *Candida albicans. J Med Vet Mycol* 1987; **25**: 29-37.

6) Bodey GP. *Candidiasis: Pathogenesis, Diagnosis and Treatment*, 2nd ed., Raven Press, New York. 1993: 371-406.

7) Kuroda S, Uno J, Arai T. Target substance of some antifungal agents in the cell membrane. *Antimicrob Agents Chemother* 1978; **13**: 454-59.

8) Van den Bossche H, Willemsens G, Cools W, Marichal P, Lauwers W. Hypothesis on the molecular basis of the antifungal activity of N-substituted

imidazole and triazoles. *Biochem SocTrans* 1983; **11**: 665-67.

9) Awaya J, Ohno T, Ohno H, Omura S. Substitution of cellular fatty acids in yeast cells by the antibiotic cerulenin and exogenous fatty acids. *Biochim Biophys Acta* 1975; **409**: 267-73.

10) Haraguchi H, Taniguchi M, Yano Y, Tanaka T, Oi S, Hashimoto K. Mode of the antifungal action of chrysodin in *Candida albicans*. *Agric Biol Chem* 1990; **54:** 2417-22.

11) Mago N, Khuller GK. Influence of lipid composition on the sensitivity of *Candida albicans* to antifungal agents. *Indian J Biochem Biophys* 1989; **26**: 30-3.

12) Iannitelli RC, Ikawa M. Effects of fatty acids on action of polyene antibiotics. *Antimicrob Agents Chemother.* 1980; **17:** 61-4.

13) Yamaguchi H. Antagonistic action of lipid components of membranes from *Candida albicans* and various other lipids on two imidazole antimycotics, clotrimazole and miconazole. *Antimicrob Agents Chemother* 1977; **12**: 16-5.

14) Lomb M, Fryberg M, Oehlschlager AC, Unrau AM. Sterol and fatty acid composition of polyene macrolide antibiotic resistant *Torulopsis glabrata*. *Can J Biochem* 1975; **53**: 1309-15.

15) Georgopapadakou NH, Dix BA, Smith SA, Freudenberger J, Funke PT. Effect of antifungal agents on lipid biosynthesis and membrane integrity in *Candida albicans*. *Antimicrob Agents Chemother* 1987; **31**: 46-51.

16) Johnson EM, Richardson MD, Warnock DW. *In-vitro* resistance to imidazole antifungals in *Candida albicans*. *J Antimicrob Chemother* 1984; **13**: 547-8.

17) Koul A, Chandra J, Prasad R. Status of membrane lipids and amino acid transport in morphological mutants of *Candida albicans*. *Biochem Mol Biol Int* 1995; **35**: 1215-22.

18) Niimi M, Kamiyama A, Tokunaga M, Tokunaga J, Nakayama H. Germ-tube-forming cells of *Candida albicans* are more susceptible to clotrimazole-induced killing than yeast cells. *Sabouraudia* 1985; **23**: 63-8.

19) Pugh D, Cawson RA. The effect of nystatin on the morphology of *Candida albicans*. *Sabouraudia* 1978; **16**: 307-11.