

Effect of Prophylactic Ketoconazole and Nystatin on Fungal Flora

Wirkung von prophylaktischen Ketoconazol- und Nystatin-Gaben auf die Pilzflora

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Summary: The effect of prophylactic antifungal drugs on oropharyngeal and anterior nares fungal colonization was studied in 20 patients receiving nystatin and 19 patients receiving ketoconazole. Surveillance cultures were obtained weekly for a mean of 27.1 ± 4.8 days in the nystatin group and 44.0 ± 6.7 days in the ketoconazole group. Initially, 63.2 % of nystatin patients and 77.8 % of ketoconazole patients had yeasts in their oropharynx. Neither drug eliminated oropharyngeal yeast colonization; by the end of the first four weeks of surveillance, 66.7 % of the nystatin group and 63.6 % of the ketoconazole group still had yeasts in the oropharynx. However, both drugs caused a reduction in the quantity of yeasts grown on successive cultures. Filamentous fungi were isolated in baseline cultures in 42.1 % of the nystatin patients and 33.3 % of the ketoconazole patients. Prophylaxis did not appear to alter carriage of filamentous fungi in the upper airways. Pathogenic filamentous fungi were only rarely isolated, and this rate did not increase with prophylaxis. Resistance to polyene antifungals (nystatin, amphotericin B) or to ketoconazole did not occur as a result of prophylaxis.

Zusammenfassung: Untersuchung über die Pilzflora im Oropharynx und im vorderen Nasenraum unter dem Einfluß einer prophylaktischen antimykotischen Behandlung. 20 Patienten erhielten Nystatin und 19 Patienten Ketoconazol. Wöchentliche Kontrollkulturen wurden in der Nystatin-Gruppe über einen durchschnittlichen Zeitraum von $27,1 \pm 4,8$ Tagen und in der Ketoconazol-Gruppe über $44,0 \pm 6,7$ Tage durchgeführt. Zu Beginn der Untersuchung hatten 63,2 % der Nystatin-Patienten und 77,8 % der Ketoconazol-Patienten Hefen im Oropharynx. Keines

der beiden Antimykotika eliminierte die oropharyngeale Hefebesiedlung. Nach 4wöchiger Beobachtung wurden Hefen in der Nystatin-Gruppe bei 76,7 % und in der Ketoconazol-Gruppe bei 63,6 % im Oropharynx festgestellt. Durch beide Antimykotika wurde jedoch eine Verringerung der kulturell nachweisbaren Hefemengen hervorgerufen. Schimmelpilze fanden sich zu Beginn der Untersuchung bei 42,1 % der Nystatin-Patienten und bei 33,3 % der Ketoconazol-Patienten. Unter der prophylaktischen Behandlung war keine Verringerung des Vorkommens derartiger Pilze in den oberen Luftwegen feststellbar. Pathogene Schimmelpilze wurden nur sehr selten isoliert und das Vorkommen dieser Pilze stieg unter der prophylaktischen Behandlung nicht an. Eine Resistenz gegenüber Polyen-Antimykotika (Nystatin, Amphotericin B) oder Ketoconazol trat unter der prophylaktischen Behandlung nicht auf.

Introduction

Patients with prolonged neutropenia due to cancer chemotherapy are at high risk for developing infections with yeast-like fungi, such as *Candida* (4, 16). Overgrowth of yeasts in the gastrointestinal tract may cause locally severe infections, such as thrush and esophagitis, and may culminate in disseminated infection (20). In an attempt to decrease fungal colonization and to prevent serious local yeast infections, as well as disseminated candidiasis, many centers routinely use polyene or imidazole antifungal agents prophylactically (3, 5, 20).

Ketoconazole is an imidazole which has been shown to be effective in the treatment of chronic mucocutaneous candidiasis (17). It is attractive for use as a prophylactic agent in cancer patients because it is easily administered orally and has minimal toxicity.

This study was undertaken 1) to assess the efficacy of ketoconazole versus nystatin in the prevention of infection with yeast-like fungi in neutropenic patients and 2) to study the effect of ketoconazole and nystatin on the fungal flora of the oropharynx and anterior nares. We were interested in the possible emergence of potentially pathogenic filamentous fungi or the development in yeasts of resistance to antifungal drugs during prophylaxis. A prior report documented those aspects of the study dealing with the clinical efficacy of ketoconazole versus nystatin in preventing fungal infections (14); this report details the studies of fungal colonization and antifungal drug susceptibility.

Materials and Methods

Patients

Between March 1982 and March 1983, all patients over the age of 16 hospitalized at the University of Michigan Hospital with acute leukemia or lymphoma, which was to be treated with chemotherapy leading to severe neutropenia, were eligible for this study. None of the patients had received systemic antifungal therapy prior to entering the study. Patients were treated in single rooms while hospitalized. Special isolation precautions were not employed, and regular hospital food was served.

Patients were randomized to receive either ketoconazole, 200 mg daily, or nystatin, 500,000 units, taken as a swish and swallow preparation four times daily. Patients received prophylactic treatment throughout the entire period of expected neutropenia (PMN < 500/ μ l), including outpatient days.

Fungal surveillance cultures

Surveillance cultures for fungi were obtained from the oropharynx and anterior nares prior to starting prophylaxis and every week thereafter while on the study. The anterior nares were sampled with cotton swabs, which were then placed immediately into Stuart's transport medium; cultures of the oropharynx were obtained with a gargle of 5 ml of sterile 0.9% saline.

Within an hour of collection, all specimens were inoculated, using the four-quadrant method, onto plates containing Sabouraud dextrose agar and Sabouraud dextrose agar with chloramphenicol (GIBCO Inc., Grand Island, NY) (13). The plates were incubated at 25°C and examined weekly for 4 weeks. The growth of yeasts was graded as rare, few, moderate, or abundant by standard criteria (13). The growth of filamentous fungi could not be quantitated accurately in this manner and simply was noted as present or absent.

Identification of fungi

Yeast-like organisms were identified by using the API Clinical Yeast System (Analytab Products, Inc., Plainview, NY) and germ tube formation (7). Filamentous fungi were identified by hyphal and conidial morphology and color using tease-mount preparations, microslide cultures, and differential media for *Aspergillus* (8, 18).

Antifungal susceptibility studies

An agar dilution replicate plate method was used for testing the susceptibility of the isolates to ketoconazole, nystatin, and amphotericin B. Ketoconazole powder (Janssen Pharmaceutica, Inc., New Brunswick, NJ), nystatin powder (Squibb, Inc., Princeton, NJ), and amphotericin B powder (Squibb, Inc.) were each dissolved in 100 % dimethyl sulfoxide (DMSO) yielding stock solutions of 1000 µg/ml. The drugs were further diluted in sterile distilled water to give concentrations of 0.06 µg/ml to 500 µg/ml. The final dilutions of the drugs were made in molten agar to achieve concentrations of 0.006 µg/ml to 50 µg/ml in the assay plates. The agar used for ketoconazole assays was Kimmig agar (E. Merck, Inc., Darmstadt, Federal Republic of Germany), adjusted to pH 6.5 (19); the agar used for nystatin and amphotericin B studies was antibiotic medium No. 3 in purified agar (Difco, Inc., Detroit, MI).

Organisms were grown overnight in yeast nitrogen base with dextrose (Difco, Inc.) and diluted in 0.9 % NaCl to a concentration of 5×10^4 organisms/ml using a barium standard. Plates containing antifungal drugs of varying concentrations were inoculated with a Steers replicator which delivered 0.003 ml of each test organism. Therefore, the final inoculum was 1.5×10^2 organisms. The plates were incubated 25°C for 48 hours. The minimum inhibitory concentration (MIC) was defined as the lowest concentration of drug which prevented visible growth on the plates. The MIC⁵⁰ was the concentration of drug which inhibited 50 % of the isolates, and the MIC⁹⁰ was that concentration of drug which inhibited 90 % of the isolates.

Results

Patients

Forty-three patients were entered into the study. Twenty were randomized to receive ketoconazole; three of this group were excluded from analysis because of poor compliance (2 patients) or transfer to another facility within 7 days of beginning the study (1 patient). Twenty-three were randomized to receive nystatin; three were excluded from analysis because of poor compliance (1 patient) or death within 7 days of beginning the study (2 patients). In addition, 2 patients who failed on nystatin and who were placed on ketoconazole 2 months and 3 months later, respectively, were included in the ketoconazole group as well as in the nystatin group. Thus, there were 19 patients in the ketoconazole group and 20 patients in the nystatin group who were assessed for fungal colonization.

The mean time over which surveillance cultures were obtained was 27.1 ± 4.8 days for the group on nystatin and 44.0 ± 6.7 days for the group on ketoconazole. The total number of times cultures were obtained was 77 for those on nystatin and 115 for those on ketoconazole. The patients given nystatin were followed for a shorter period of time and had fewer cultures

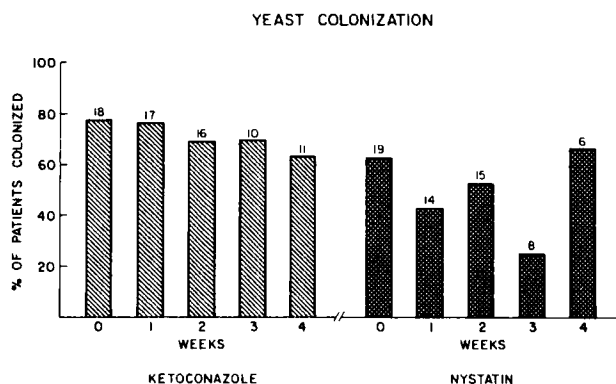


Fig. 1: The percent of patients with oropharyngeal colonization with yeasts before prophylaxis was begun and during the first four weeks of prophylaxis with ketoconazole or nystatin. Numbers above the bars refer to the number of patients from whom surveillance cultures were obtained that week.

Table 1
Yeast-like organisms isolated in surveillance cultures from 19 patients on ketoconazole and 20 patients on nystatin

Organisms	Number (%) isolates	
	Ketoconazole	Nystatin
<i>Candida albicans</i>	73 (74.5)	34 (70.8)
<i>Saccharomyces cerevisiae</i>	11 (11.2)	0 -
<i>Torulopsis glabrata</i>	2 (2.0)	6 (12.5)
<i>Candida parapsilosis</i>	5 (5.1)	0 -
<i>Candida tropicalis</i>	3 (3.1)	2 (4.2)
<i>Candida krusei</i>	3 (3.1)	4 (8.3)
<i>Candida guilliermondii</i>	0 -	2 (4.2)
<i>Candida species</i>	1 (1.0)	0 -
Total	98	48

taken due to a higher rate of development of fungal infections (9 infections compared with 3 in the ketoconazole group), leading to withdrawal of these patients from the study (14).

Effect of prophylaxis on yeast-like flora

The percentage of patients whose baseline cultures yielded yeast-like organisms was similar in both groups (Fig. 1). Neither drug eliminated colonization during the first four weeks of surveillance (Fig. 1). By the end of four weeks, 66.7% of the nystatin group and 63.6% of the ketoconazole group had yeasts remaining in their oropharynx. However, of those patients who had yeast present in baseline cultures, 66.7% on nystatin and 57.1% on ketoconazole showed a fall in the quantity of yeasts grown on successive cultures. Two patients on nystatin and 3 on ketoconazole had abundant yeast growth throughout prophylactic therapy.

The yeast most commonly isolated was *Candida albicans*, with all other species occurring much less frequently (Tab. 1). Neither drug led to overgrowth by a predominant yeast, such as *Torulopsis glabrata* or *C. tropicalis*. On only four occasions did anterior nares cultures yield yeasts; in all four instances, the growth was very scanty and the organism was present in the oropharynx at the same time.

Effect of prophylaxis on filamentous fungal flora

The percentage of patients whose baseline cultures yielded filamentous fungi was similar in both groups (Fig. 2). Neither drug eliminated carriage of filamentous fungi nor enhanced

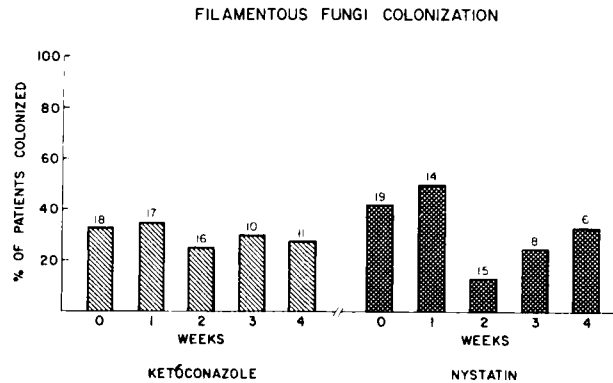


Fig. 2: The percent of patients with anterior nares or oropharyngeal colonization with filamentous fungi before prophylaxis was begun and during the first four weeks of prophylaxis with ketoconazole or nystatin. Numbers above the bars refer to the number of patients from whom surveillance cultures were obtained that week.

Table 2
Filamentous fungi isolated in surveillance cultures from 19 patients on ketoconazole and 20 patients on nystatin

Organisms	Number (%) isolates	
	Ketoconazole	Nystatin
Cladosporium	11 (22.0)	8 (18.6)
Penicillium	5 (10.0)	5 (11.6)
Alternaria	7 (14.0)	2 (4.7)
Basidiomycetes	1 (2.0)	7 (16.3)
Aspergillus versicolor	6 (12.0)	5 (11.6)
Aspergillus fumigatus	1 (2.0)	0
Aspergillus niger	1 (2.0)	1 (2.3)
Rhizopus	1 (2.0)	1 (2.3)
Other Aspergillus species	6 (12.0)	5 (11.6)
Other filamentous fungi	11 (22.0)	9 (21.0)
Total	50	43

their growth during the first four weeks of surveillance (Fig. 2). Seventy percent of the positive cultures were from the anterior nares in both patient groups. The organisms isolated were almost entirely commensal organisms; *Aspergillus niger* was isolated twice, *A. fumigatus* only once, and *A. flavus* was not found in any culture (Tab. 2).

Antifungal susceptibility studies

Comparison of MIC⁵⁰ and the MIC⁹⁰ for each drug showed no differences between the yeasts isolated in baseline cultures and those isolated during the course of prophylaxis with either nystatin or ketoconazole (Tab. 3).

Discussion

This study was concerned with finding a prophylactic antifungal regimen which would be efficacious in eliminating yeast infections in neutropenic patients. It was of interest to determine if efficacy could be related to decreasing colonization by yeasts in the oropharynx. Also, we felt it important to document the consequences of long-term prophylaxis, with regard to overgrowth of filamentous fungi, such as *Aspergillus*, and development of resistance to ketoconazole or polyene antifungal agents.

Table 3
Susceptibility of yeasts to antifungal agents before and during prophylaxis with ketoconazole or nystatin

	MIC Values Before Prophylaxis				MIC Values During Prophylaxis										
	Keto		Nyst		Keto		Nyst		AmB						
	MIC ⁹⁰	MIC ⁵⁰	MIC ⁹⁰	MIC ⁵⁰	MIC ⁹⁰	MIC ⁵⁰	MIC ⁹⁰	MIC ⁵⁰	MIC ⁹⁰	MIC ⁵⁰					
C. albicans															
Keto group (11)	0.02	0.05	1.56	3.12	0.20	0.20	0.20	0.20	(63)	0.01	0.20	1.56	3.12	0.02	0.20
Nyst group (10)	0.01	6.25	1.56	3.12	0.20	0.20	0.20	0.20	(22)	0.01	0.02	1.56	3.12	0.20	0.20
T. glabrata															
Keto group (1)	0.20		1.56		0.20				(1)	0.20		1.56		0.10	
Nyst group (1)	0.40		1.56		0.20				(4)	0.10	0.40	0.78	1.56	0.20	0.20
S. cerevisiae															
Keto group (1)	0.10		1.56		0.20				(10)	0.20	0.40	1.56	3.12	0.10	0.20
Nyst group (0)	-		-		-				(0)	-	-	-	-	-	-
Other Candida species															
Keto group (6)	0.02	3.12	1.56	1.56	0.20	0.20	0.20	0.20	(5)	3.12	6.25	1.56	3.12	0.20	0.20
Nyst group (2)	0.10	12.50	1.56	1.56	0.20	0.20	0.20	0.20	(5)	0.02	0.02	1.56	3.12	0.20	0.20

() numbers in parantheses refer to the number of isolates tested.

The clinical aspects of this study, reported previously, showed that ketoconazole was slightly more efficacious than nystatin in preventing serious local yeast infections, such as thrush, esophagitis, and vaginitis (14). However, clinical efficacy of ketoconazole was not associated with eradication of fungal colonization in the oropharynx. Other studies with ketoconazole, as well as those with miconazole or amphotericin B, have shown this dissociation between clinical efficacy and effect on colonization (6, 10, 20). Our study, as well as prior ones using other antifungal agents, has shown that the quantity of yeasts in the oropharynx does decrease with prophylaxis even though eradication of yeasts does not occur (10, 20).

Overgrowth by strains of yeasts, such as *T. glabrata*, which may be more resistant to ketoconazole, was noted in one preliminary study of ketoconazole prophylaxis in neutropenic patients (9). However, our study failed to show emergence of *T. glabrata* or other resistant yeasts. The few patients who were colonized with *T. glabrata* carried this yeast before prophylaxis and demonstrated persistence of the organism in the oropharynx in successive cultures.

Likewise, another preliminary report noted the emergence of and infection with filamentous fungi, such as *Aspergillus*, in patients on ketoconazole prophylaxis (1). Our patients did not show an increase in colonization with filamentous fungi while on prophylaxis. Pathogenic *Aspergillus* species such as *A. fumigatus* and *A. flavus*, were rarely isolated in our patients. Colonization with pathogenic *Aspergillus* species is probably most dependent on environmental sources (2) and is uncommon in the absence of such a source (15). Previous surveillance cultures in neutropenic patients at our institution revealed a seasonal variation in colonization with filamentous fungi, with more isolates recovered in the summer months (15).

Prophylactic therapy with ketoconazole or nystatin did not lead to the development of ketoconazole-resistant or polyene-resistant yeasts. Polyene resistance has been reported in 7.4% of isolates from oncology patients at one center (11), but all isolates were from patients who had received systemic amphotericin B, as well as nystatin prophylaxis. Oral nystatin alone did not lead to emergence of resistance in our population. The development of resistance to ketoconazole has been reported only rarely (12). Our study confirms that prophylaxis in neutropenic cancer patients for as long as 4 months is not associated with the development of ketoconazole resistant organisms.

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