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ORIGINAL ARTICLE

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Fungal colonization in neutropenic patients: a randomized study comparing itraconazole solution and amphotericin B solution

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Abstract We assessed the impact of prophylaxis with the oral itraconazole solution and amphotericin B solution on fungal colonization and infection in a randomized study among patients with hematological malignancies and neutropenia. Infecting and colonizing *Candida* strains of patients suffering from candidiasis were genotyped by random amplification of polymorphic DNA (RAPD) analysis. A total of 106 patients were evaluated in this study: 52 patients in the itraconazole and 54 in the amphotericin B arm. During neutropenia fungal colonization in the oropharynx occurred in 11 (19.6%) and 24 (40.6%) and in the rectum in 11 (19.6%) and 23 (38.9%) courses in the itraconazole and amphotericin B groups ($P<0.05$), respectively. *Candida albicans* was the most prevalent species in both study groups. Mixed fungal colonization with *Candida krusei* and *Candida glabrata* was increased in the amphotericin B group, yet without clinical importance since infections were due to *C. albicans*. The occurrence of invasive candidiasis was significantly increased in multicolonized compared to monocolonized patients. In the amphotericin B group 20 and in the itraconazole group 2 neutropenic patients showed multicolonization with *Candida* spp. ($P<0.05$). Overall fungal infections were 3.8% in the itraconazole and 14.8% in the amphotericin B group ($P<0.05$). RAPD typing showed oropharynx strains involved in superficial infections in four of five patients. In all four patients with deep fungal infections, it appears that the colonizing rectum strains were identical to infecting strains of *Candida* spp. Itraconazole solution significantly reduced *Candida* colonization and infection compared to amphotericin B solution. Most patients remained infected with the colonized strains for the entire study period, irrespective of antifungal prophylaxis.

Keywords Itraconazole · *Candida* · Prevention · Fungal colonization

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Introduction

Fungal infections remain a major cause of morbidity and mortality in neutropenic patients [1, 2]. Around 25% of patients with leukemia have signs of fungal infection at autopsy [3] and the medically most important opportunistic mycoses in Europe are caused by *Aspergillus* and *Candida* species [4]. Infections due to *Aspergillus* exceed the incidence of *Candida* infections in many centers as shown by Pfaffenbach and collegues [5]. *Candida* species account for 75% of fungal infections and result in 25–60% mortality [6]. Translocation of *Candida* of the endogenous gut flora across the intestinal mucosa and disruption of the biliary tract have been implicated in the pathogenesis of this infection [7]. Thus, major efforts have been made to reduce the incidence of fungal infections by use of prophylactic antifungal agents [8]. Yet, no general consensus has been reached on what regimen should be used for prophylaxis in neutropenic patients.

Early studies of the prophylactic use of oral drugs such as nystatin, clotrimazole, and miconazole yielded unsatisfactory results because of poor absorption, a narrow spectrum, and a poor compliance [9].

Prophylactic treatment with fluconazole, an azole antifungal agent, is widely used nowadays [10]. However, fluconazole, either oral or intravenous, offers no protection against *Aspergillus*, and several pathogenic *Candida* are either resistant or less sensitive to this compound [11]. Itraconazole, a broad-spectrum triazole has a wide spectrum of activity against *Candida* and *Aspergillus*. Nonrandomized studies suggest it is effective for treating and preventing systemic fungal infections in neutropenic patients [1, 12].

In this randomized study, we assessed the impact of prophylaxis with the oral itraconazole solution and amphotericin B solution on fungal colonization and infection among patients with hematological malignancies and neutropenia. To study the epidemiology of infecting and colonizing *Candida* strains of patients with fungal infections, genotyping by random amplification of polymorphic DNA (RAPD) analysis was performed.

Methods

Patients

Adult patients with hematological malignancies receiving treatment for chemotherapy were eligible for the study if their neutrophil count was expected to fall in the course of remission induction or consolidation chemotherapy or peripheral stem cell transplantation. Patients were excluded from the study if they had received fluconazole or i.v. amphotericin B previously or had previously proven systemic fungal infection. All patients were treated in units equipped with high-efficiency particulate air filters.

Prophylaxis regimes

Patients (n=158) were randomized to receive either itraconazole 5 mg/kg of body weight twice a day as a 10 mg/ml hydroxypropyl-ß-cyclodextrin solution (Itraconazole, Janssen-Cilag, Vienna, Austria) or amphotericin B solution 1000 mg three times a day as a 100 mg/ml suspension (Bristol Meyer Squibb, Vienna, Austria). Antifungal prophylaxis was started at the beginning of the cytotoxic chemotherapy or transplant conditioning regimen. Prophylaxis was continued until neutrophil levels had recovered to at least 1×10^9/l. Patients who did not develop a proven systemic fungal infection were permitted to reenter the study if they were receiving multiple courses of cytotoxic chemotherapy likely to induce neutropenia.

Patients were withdrawn from the study if they received additional systemic antifungal treatment for a suspected or proven fungal infection. If a systemic fungal infection was suspected during the prophylaxis phase, study medication was stopped and treatment was given according to the hospital's normal practice.

Surveillance cultures

Samples for fungal cultures from the oropharynx and rectum were obtained at study entry and twice a week until patients' discharge from the hospital. Fungal colonization was considered to be present if surveillance cultures yielded a fungus isolated from the rectum, throat, or both in the absence of clinical signs of fungal infection. Patients colonized simultaneously in the oropharynx and rectum were considered multicolonized; patients colonized either in the oropharynx or rectum were considered monocolonized.

Fungal infection

Suspected cases of fungal infections were defined as clinical signs and symptoms (with or without radiological lesions) with fever of unknown origin unresponsive to broad-spectrum antibacterials and highly suggestive radiological lesions for deep fungal infection without mycological evidence by culture or histology. Superficial fungal infection was defined as clinically apparent infection of the oropharynx with positive cultures. Proven deep fungal infections were defined as the histopathological evidence of tissue invasion by fungi in specimens obtained by biopsy or autopsy, or a positive culture from a normally sterile body site, and clinical or radiological symptoms consistent with infection.

Fungal strains

Samples were cultured on Sabouraud agar (Merck, Vienna, Austria) for 5 days at 37°C, species identification was performed with api 20 C AUX (bioMerieux, Vienna, Austria), and examination of morphology on cornmeal agar (Merck, Vienna, Austria) at 20°C. *Candida* isolates cultured in case of infections were tested for in vitro susceptibility against amphotericin B and itraconazole using the microbroth dilution method according to the National Committee for Clinical Laboratory Standards Document M 27-A Guidelines [13]. RAPD typing of infecting and colonizing *Candida* strains of patients suffering from candidiasis were performed according to the method of Metzgar et al. [14].

Statistics

The study protocol of this randomized clinical trial was approved by the local Ethics Committee. All patients gave their written, confirmed consent before enrollment into the trial. Random codes were provided by the study statistician and study drugs were delivered to the study site in the hospital based on the random codes by the hospital pharmacy. Fungal colonizations or infections were compared between the two prophylactic groups itraconazole and amphotericin B using contingency table analysis and Fisher's exact test. Clinical features of the patients at baseline were compared by Fisher's exact test for dichotomous variables; the Mann-Whitney test was used to evaluate differences in continuous variables. The sample size of 80 patients in each treatment group based on the rate of colonization (20% in the itraconazole and 45% in the amphotericin B group) was prespecified. The power was set at 90% and the two-sided significance level at 0.05.

Results

Between May 1999 and October 2001, a total of 149 patients were randomized, and 43 patients (n=20 itraconazole, n=23 amphotericin B) became ineligible during the course of the study for reasons of receiving antifungal treatment before the study, not undergoing neutropenia, chemotherapy or transplantation, and allocation to another department; 106 patients were evaluated in this study: 52 patients in the itraconazole and 54 amphotericin B arm. Patients treated with amphotericin B experienced 59 neutropenic episodes compared with 56 in the itraconazole group. Both groups were similar in sex (P=0.16), age (P=0.13), and severity of underlying diseases (P=0.26). The mean age was 44.5 years and 38% of the patients were women. For detailed information see Table 1.

Table 1 Clinical features of patients. *AML* acute myeloid leukemia, *ALL* acute lymphoid leukemia, *NHL* non-Hodgkin's lymphoma, *Burkitt's* Burkitt's lymphoma, *AA* aplastic anemia, *CML* chronic myeloid leukemia, *MM* multiple myeloma, *HL* Hodgkin's lymphoma

	Itraconazole	Amphotericin B
No. of patients	52	54
Mean age	44.4	43.5
Male/female	26/33	27/29
No. of neutropenic episodes	56	59
No. of days with neutropenia (range)	19.4 (3–55)	14.5 (2–44)
Diagnosis		
AML	25	27
ALL	16	15
NHL	7	5
Burkitt's	3	2
AA	1	2
CML	1	1
MM	4	2
HL	1	2
Seminoma	1	–

Surveillance cultures

Fungal colonization before drug prophylaxis was not significantly different in the amphotericin B and itraconazole groups, regardless of the site analyzed (Table 2). At the time of admission *Candida* colonization in the oropharynx and rectum occurred in 53 (46%) and 46 (40%) of 115 neutropenic episodes. During neutropenia fungal colonization in the oropharynx occurred in 11 (19.6%) and 24 (40.6%) episodes, and in the rectum in 11 (19.6%) and 23 (38.9%) episodes in the itraconazole and amphotericin B groups ($P<0.01$), respectively.

In the amphotericin B group serial surveillance cultures were persistently negative for *Candida* spp. in 29 neutropenic patients. Five neutropenic patients showed monocolonization, 20 multicolonization with *Candida* spp. In the itraconazole group serial surveillance cultures were persistently negative for *Candida* spp. in 32 neutropenic patients. Eighteen neutropenic patients showed monocolonization, two multicolonization with *Candida* spp. as shown in Table 3. Table 4 shows the spectrum of *Candida* spp. isolated from surveillance cultures. Overall, *C. albicans* was the most frequent species in both study groups. Mixed fungal colonization with *C. krusei* and *C. glabrata* was increased in the amphotericin B group. The development of invasive candidiasis was significantly increased in multicolonized compared to monocolonized patients (Table 3). In all cases of deep infections *C. albicans* was cultured from blood and tissue specimens.

Fungal strains

None of the isolates of *Candida* spp. tested showed in vitro resistance. The minimal inhibitory concentration (MIC) for amphotericin B ranged between 0.125 μg/ml and 1 μg/ml and for itraconazole $\leq$0.125 μg/ml. RAPD typing showed that each patient was infected by his or her own distinct DNA type of *Candida*. The 14 isolates from 5 patients with superficial infections felt into 8 different RAPD genotypes. The colonizing strains of the oropharynx were identical to superficial infecting strains in four of five patients. One patient displayed a clear switch between colonizing and infecting strain in the amphotericin B group. The 11 isolates from 4 patients with deep infections felt into 6 different RAPD genotypes. The colonizing rectum strains were identical to the infecting strains obtained from blood cultures and tissue specimens in both treatment groups.

Table 2 Fungal colonization of 115 episodes in the oropharynx and rectum before, during, and after chemotherapy

	Throat			Rectum		
	Itraconazole	Amphotericin B		Itraconazole	Amphotericin B	
Before neutropenia	25 (44.6%)	28 (47.4%)	$P=0.45$	19 (33.9%)	27 (45.7%)	$P=0.32$
During neutropenia	11 (19.6%)	24 (40.6%)	$P<0.05$	11 (19.6%)	23 (38.9%)	$P<0.05$
After neutropenia	6 (10.7%)	26 (44.0%)	$P<0.05$	6 (10.7%)	21 (35.5%)	$P<0.05$

Table 3 Colonization and infection due to *Candida* spp. among patients with hematologic malignancies receiving prophylaxis with itraconazole and amphotericin B

Drug	Surveillance cultures of neutropenic patients	No. of patients		
			Superficial infections	Deep infections
Amphotericin B	No colonization	29	0	0
	Monocolonization	5	1	0
	Multicolonization	20	3	3
Itraconazole	No colonization	32	0	0
	Monocolonization	18	0	0
	Multicolonization	2	1	1

Table 4 Fungal colonization species in neutropenic patients during antifungal prophylaxis

Fungal organisms	No. of patients colonized			
	Monocolonized		Multicolonized	
	Amphotericin B	Itraconazole	Amphotericin B	Itraconazole
Candida albicans	2	8	9	2
Candida glabrata	1	2	2	0
Candida parapsilosis	1	0	0	0
Saccharomyces cerevisiae	0	2	1	0
Candida krusei	0	0	1	0
Candida rhodotorula	0	2	1	0
C. albicans and *C. glabrata*	1	1	1	0
C. glabrata and *C. krusei*	0	0	5	0
C. albicans and *S. cerevisiae*	0	1	0	0
Others	0	2	0	0
Total	5	18	20	2

Clinical outcome

The overall incidence of proven fungal infections in our patients was 9.4%. One patient (1.9%) in the itraconazole and four patients (7.4%) in the amphotericin B group had superficial fungal infections. Invasive candidiasis developed in one (1.9%) in the itraconazole and in three (5.5%) patients in the amphotericin B group by *C. albicans*. Invasive aspergillosis occurred in one patient in the amphotericin B group. The incidences of suspected fungal infection were not different between the groups; three patients (5.5%) of each group were identified. Overall mortality in the itraconazole group was 5.7% and in the amphotericin B group 5.5%. Deaths were attributed to fungal infection in two amphotericin B recipients. Compliance was good without showing differences in both treatment groups.

Discussion

In our trial involving 106 patients, prophylactic itraconazole decreased fungal colonization and infection in neutropenic patients undergoing chemotherapy. Colonizing and infecting strains showed identical RAPD patterns in almost all neutropenic patients.

Since colonization is a risk factor for systemic fungal infection, major efforts have been made in the reduction of *Candida* colonization within the endogenous flora [8, 15]. This study was designed to test the efficacy of two prophylactic regimens in neutropenic patients. The oral nonabsorbable agent amphotericin B widely used in our hospital was selected in comparison to itraconazole solution. There were statistically significant differences between amphotericin B solution and itraconazole solution as prophylaxis for *Candida*. Itraconazole had a higher success rate than amphotericin B in reduction of fungi in the oropharynx and rectum ($P<0.05$). Fungal colonization during neutropenia occurred in 37% and in 83%, infections occurred in 3.8% in the itraconazole and in 14.8% in the amphotericin B recipients ($P<0.05$), respectively. *C. albicans* was the most frequent species in both study groups followed by *C. glabrata.* Mixed fungal colonization with *C. krusei* and *C. glabrata* was increased in the amphotericin B group, yet without showing clinical importance since infections were due to *C. albicans*, with a lack of non-*C. albicans* infections; similar data were found by Vreugdenhil et al. [16] using itraconazole capsules as antifungal prophylaxis. However, the number of proven cases of invasive candidiasis was too low in our study to attempt any exhaustive evaluation of the relative incidence of species variation in deep infections.

The occurrence of invasive candidiasis was significantly increased in multicolonized compared to monocolonized patients. In the amphotericin B group, 6 of 20 and in the itraconazole group 2 of 2 multicolonized neutropenic patients developed infection due to *C. albicans* ($P<0.05$). No case of invasive infection was documented in the non-colonized patients. Therefore, the use of a compound selectively effective against *Candida* seems to be justified in this particular subgroup of patients. Also, Glasmacher reported in a fungal surveillance study that the use of itraconazole solution led to a reduced growth rate of *Candida* spp. ($P<0.05$) [15].

Candida species now rank among the four pathogens most frequently isolated in blood cultures [17]. The source of infection has been the subject of considerable debate [18], with some suggesting the gastrointestinal tract [19] and others favoring the skin [20]. Our examinations showed that the colonizing rectum strains were identical to infecting strains in four patients suffering from deep fungal infections. The colonizing oropharynx strains were identical to superficial infecting strains in four of five patients. No colonizing oropharynx strain was involved in deep infections. This is in contrast to a study of Marr et al. [21] showing that the majority of patients who developed bloodstream infection with either *C. glabrata* or *C. krusei* had previous oral colonization with this species. It is unclear whether this observation is a direct result of fluconazole administration or of the fungal species involved. However, our data show that the gut represents an important endogenous source of deep infection in neutropenic patients. Overall, reducing multicolonization may have a favorable impact in decreasing the incidence of *Candida* infections.

In the present study patients were treated with itraconazole, 10 mg/kg per day because it was anticipated that higher doses of itraconazole may also have antifungal effects on hyphae of *Aspergillus* [22]. So far, the low incidence of proven *Aspergillus* infections in this study precludes any conclusions about the effectiveness of prophylactic itraconazole for prevention of aspergillosis. Yet, in the current study cases of suspected invasive aspergillosis were similar in the amphotericin B and itraconazole groups. The administration of intravenous amphotericin B was not lower in the itraconazole arm. These results are comparable to those observed in a multicenter trial comparing itraconazole and placebo [23].

The overall incidence of fungal infections in our patients was about 9.4%, and the reasons for this are unknown since none of the isolates tested showed in vitro resistance against amphotericin B or itraconazole. The results of itraconazole may have been related to inadequate serum levels of the drug, which has been observed during neutropenia [24]. Glasmacher and colleagues have shown that the incidence of fungal infection was higher if no appropriate plasma levels were obtained. So far, using itraconazole in a dose of 10 mg/kg per day, we observed the same rate of fungal infection which occurred in patients described by Harrousseau and colleagues [10]. However, several mechanisms could be responsible for these differences in prevention of fungal infections.

In conclusion, itraconazole oral solution significantly reduced *Candida* colonization and infection compared to amphotericin B solution. Most patients remained infected with the colonized strains for the entire study period, irrespective of antifungal prophylaxis.

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