

FLUCONAZOLE PROPHYLAXIS AGAINST FUNGAL COLONIZATION AND INFECTION IN PRETERM INFANTS

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ABSTRACT

Background Invasive fungal infection is associated with substantial morbidity and mortality in preterm infants. We evaluated the efficacy of prophylactic fluconazole in preventing fungal colonization and invasive infection in extremely-low-birth-weight infants.

Methods We conducted a prospective, randomized, double-blind clinical trial over a 30-month period in 100 preterm infants with birth weights of less than 1000 g. The infants were randomly assigned during the first five days of life to receive either intravenous fluconazole or placebo for six weeks. We obtained weekly surveillance cultures from all patients.

Results The 50 infants randomly assigned to fluconazole and the 50 control infants were similar in terms of birth weight, gestational age at birth, and base-line risk factors for fungal infection. During the six-week treatment period, fungal colonization was documented in 30 infants in the placebo group (60 percent) and 11 infants in the fluconazole group (22 percent; difference in risk, 0.38; 95 percent confidence interval, 0.18 to 0.56; $P=0.002$). Invasive fungal infection with positive growth of fungal isolates from the blood, urine, or cerebrospinal fluid developed in 10 infants in the placebo group (20 percent) and none of the infants in the fluconazole group (difference in risk, 0.20; 95 percent confidence interval, 0.04 to 0.36; $P=0.008$). The sensitivities of the fungal isolates to fluconazole did not change during the study, and no adverse effects of the fluconazole therapy were documented.

Conclusions Prophylactic administration of fluconazole during the first six weeks of life is effective in preventing fungal colonization and invasive fungal infection in infants with birth weights of less than 1000 g. (N Engl J Med 2001;345:1660-6.)

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DESPITE aggressive antifungal treatment of invasive candida infection, systemic fungal disease is increasing in prevalence and leads to high rates of illness and death among preterm infants.^{1,2} Candida species rapidly colonize the skin and mucous membranes of about 60 percent of critically ill neonates and can progress to invasive infection.^{2,3} Fungal infection accounts for 9 percent of cases of late-onset sepsis in infants who weigh less than 1500 g and is associated with a mortality rate of 28 percent, as compared with 7 percent among infants in whom no infection develops.¹ Critically ill neonates are at an increased risk for infection because of their developmentally immature immune systems, the in-

creased permeability of their skin and mucosal barriers, and the long-term need for central vascular access, parenteral nutrition, broad-spectrum antibiotics, postnatal steroids, and mechanical ventilation.^{2,4,5} This high-risk population could benefit greatly from effective prophylactic measures.

Reducing fungal colonization may prevent the development of invasive fungal infection in preterm infants.⁶ Antifungal prophylaxis with fluconazole has been successful in reducing fungal colonization and infection in patients who are undergoing bone marrow or solid-organ transplantation, those with leukemia or human immunodeficiency virus infection, and those who are receiving radiation and chemotherapy.⁷⁻¹² The efficacy of prophylaxis in these immunocompromised groups suggests that fluconazole may also be effective in preventing invasive fungal disease in preterm infants. However, a major concern regarding the prophylactic use of antifungal agents is the emergence of resistance. In the United States, national surveillance of bloodstream infections attributable to candida species demonstrated a relatively constant level of susceptibility of all candida species to fluconazole during a seven-year period from 1992 through 1998,¹³ but several other studies have demonstrated some resistance to fluconazole.¹⁴⁻¹⁸ Our goal in this study was to evaluate the safety and effectiveness of fluconazole in preventing fungal colonization and invasive infection in extremely-low-birth-weight infants, while maintaining surveillance for fluconazole resistance.

METHODS

Study Design

We conducted a prospective, randomized, double-blind clinical trial to evaluate the efficacy of fluconazole prophylaxis in 100 preterm infants. All infants admitted to our neonatal intensive care unit were eligible for the study, whether they had been born at our institution or transferred from another facility, if they had a birth weight of less than 1000 g and were less than five days old. The presence of liver failure was the only criterion for exclusion. Before enrollment, written informed consent was obtained from each infant's parent or guardian. The protocol was approved by the human investigations committee of the University of Virginia. Pfizer, which supported the study with a grant, was not involved in the study design; the collection, analysis, or interpretation of the data; or the preparation of the manuscript. The company had no control over the decision to publish this report.

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Using a computer-generated randomization table, the pharmacy randomly assigned infants, in a 1:1 ratio, to receive either fluconazole or placebo for six weeks. Six weeks was chosen as the duration of therapy on the basis of unpublished data from our neonatal intensive care unit demonstrating that approximately 85 percent of invasive fungal infections in preterm infants weighing less than 1000 g occurred during the first six weeks of life.

Data on the demographic and clinical characteristics of the infants were prospectively collected during their hospitalization and through review of their medical records. Microbiologic-culture results were collected during all episodes of fungal and bacterial infection of blood, urine, and cerebrospinal fluid during and after the six-week treatment period.

Drug Administration

Fluconazole was administered intravenously for six weeks at a dose of 3 mg per kilogram of body weight every third day for the first two weeks, every other day during the third and fourth weeks, and daily during the fifth and sixth weeks. Infants in the placebo group were given an equal volume of normal saline on the same schedule. This schedule of fluconazole dosing was based on data from pharmacokinetic studies involving preterm infants.^{19,20} The administration of the study drug (fluconazole or placebo) and the obtaining of surveillance cultures were discontinued before the end of the six-week treatment period if intravenous access was discontinued, if antifungal therapy was initiated for the treatment of a documented or presumed systemic fungal infection, if the infant was discharged from the hospital or transferred to another facility, or if the infant died.

Fungal Isolation and Identification

Fungal surveillance cultures of the nasopharynx (tracheal secretions if the infant was intubated and nasopharyngeal sample if the infant was not intubated), skin (groin), and stool or rectum were obtained, by two clinical nurses associated with the study, at base line and then weekly during the six-week study period on the day of the week of the patient's enrollment. Cultures of the umbilicus were also performed at base line and at one week.

Cultures were also obtained when they were considered by neonatal care physicians to be clinically indicated. Invasive fungal infection was defined by a positive culture of blood, urine, or cerebrospinal fluid. Stool and tracheal secretions were submitted in sterile containers, and skin specimens were obtained on swabs (Culturette, Becton Dickinson Microbiology Systems, Sparks, Md.). Each specimen was inoculated onto Inhibitory Mold Agar (Remel, Lenexa, Kans.), and the plates were incubated and examined for four weeks at 30°C unless yeast growth occurred within two weeks.

Speciation of fungal isolates was performed according to standard methods, including an initial screening for germ-tube formation and exoenzyme production (*Candida albicans* screen, Carr-Scarborough Microbiologicals, Decatur, Ga.) and a commercial auxanographic method (API 20 C AUX, BioMérieux, Hazelwood, Mo.).^{21,22}

Fluconazole-Susceptibility Testing

Each yeast isolate was tested for susceptibility to fluconazole by means of a standardized microbroth dilution assay, as described by the National Committee of Clinical Laboratory Standards.²³ We report the minimal inhibitory concentration (MIC) — the lowest concentration of drug resulting in a prominent decrease in growth at 48 hours as compared with the control.

Statistical Analysis

The primary efficacy end point was the incidence of invasive fungal infection during the administration of the study drug. The secondary outcomes were fungal colonization; bacterial infection of blood, urine, or cerebrospinal fluid; isolated intestinal perforation in the absence of pneumatosis; necrotizing enterocolitis; ligation of patent ductus arteriosus; threshold retinopathy of pre-

maturity requiring laser ablation; and abnormal findings on cranial ultrasonography, defined as grade 3 or 4 intraventricular hemorrhage or periventricular leukomalacia during hospitalization. Liver-function tests measuring aspartate aminotransferase, alanine aminotransferase, direct bilirubin, and alkaline phosphatase were performed at base line and at the end of the administration of the study drug.

The sample size of 100 infants was based on the assumption that the study would have a two-sided type I error rate of 0.05 or less and at least 90 percent power to detect an absolute difference of 75 percent in the cumulative incidence of invasive fungal infection between the placebo group and the fluconazole group after six weeks of treatment, given a pretrial incidence of invasive fungal infection of 30 percent.²⁴

The contingency data pertaining to the cumulative incidence of the primary and secondary outcomes during the administration of the study drug in the placebo group and the fluconazole group were analyzed by exact tests for nonparametric variables. The hypothesis tests were based on the nonparametric unconditional exact test described by Mehta and Patel,²⁵ and all tests were two-sided. The corresponding two-sided 95 percent confidence intervals for the differences in risk were constructed according to the Berger-Boos²⁶ modification of the exact method described by Santner and Snell.²⁷

The weekly incidence of infection in the placebo group was analyzed by the generalized-estimating-equation approach.²⁸ The covariates used in the model included the presence or absence of fungal colonization each week, the week of the screening, the number of sites that were colonized, and the presence or absence of fungal colonization at base line.

The presence or absence of invasive infection each week was modeled by means of a generalized linear model with a logit-link function. The parameters of the model were estimated on the basis of maximum-likelihood criteria, and an exchangeable correlation structure was used to model the correlations between the findings for the same infant at different times. Hypothesis tests and 95 percent confidence intervals were all two-sided and were based on the Wald normal approximation.

All the statistical computations were performed with the use of SAS software (version 8.1, SAS Institute, Cary, N.C.). Proc-StatXact for SAS users, a version of the StatXact-4 software package (Cytel, Cambridge, Mass.), was used to compute the nonparametric exact tests and the nonparametric exact 95 percent confidence intervals, and the SAS Proc Genmod procedure was used to perform the generalized estimating-equation analysis.

RESULTS

Study Participants

Fifty infants were randomly assigned to the placebo group and 50 infants to the fluconazole group during the 30-month period between May 5, 1998, and October 10, 2000. A total of 114 infants met the criteria for enrollment during the study period. Seven infants died before consent had been obtained; for four infants, consent was not obtained during the first five days of life; for one infant, consent was obtained but randomization did not occur; for one, an interpreter was not available to obtain informed consent; and one infant did not have an intravenous catheter or an endotracheal tube.

There were no significant differences between the placebo and fluconazole groups in the base-line demographic characteristics or risk factors for fungal infection (Table 1). At base line, eight infants in the placebo group and two in the fluconazole group were colonized (difference in risk, 0.12; 95 percent confidence interval, -0.05 to 0.29; $P=0.18$). There was

TABLE 1. DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF THE STUDY GROUP.*

VARIABLE	FLUCONAZOLE GROUP (N=50)	PLACEBO GROUP (N=50)	P VALUE
Base-line characteristics			
Birth weight — g	717±150	744±157	0.39
Gestational age — wk	25.5±1.6	25.7±2.0	0.61
Male sex — no. (%)	25 (50)	25 (50)	1.00
Nonwhite race — no. (%)	15 (30)	16 (32)	0.92
Antenatal steroids — no. (%)	40 (80)	37 (74)	0.62
Antenatal antibiotics — no. (%)	29 (58)	29 (58)	1.00
Rupture of membranes ≥24 hr before delivery — no. (%)	14 (28)	12 (24)	0.77
Born at study hospital — no. (%)	41 (82)	38 (76)	0.61
Vaginal delivery — no. (%)	21 (42)	22 (44)	0.92
Apgar score			
1 min	4±3	4±2	0.84
5 min	6±2	6±2	0.84
Age at enrollment — days	1.5±1.1	1.9±1.2	0.19
Risk factors during 6-wk treatment period			
Treatment received — no. (%)			
Steroids†	37 (74)	36 (72)	0.92
Parenteral nutrition	49 (98)	50 (100)	0.92
Histamine-2 receptor antagonists	14 (28)	11 (22)	0.62
Granulocyte colony-stimulating factor‡	6 (12)	4 (8)	0.74
Ampicillin and gentamicin	49 (98)	50 (100)	0.88
Vancomycin	31 (62)	36 (72)	0.37
Cephalosporins	37 (74)	34 (68)	0.62
Duration of antibiotic therapy — days	13±7	14±8	0.95
Vascular access — no. (%)			
Umbilical catheters	49 (98)	50 (100)	0.88
Central venous catheter	49 (98)	48 (96)	0.88
Peripheral arterial catheter	25 (50)	30 (60)	0.37
Endotracheal intubation — no. (%)	50 (100)	50 (100)	1.00
Major surgery — no. (%)§	5 (10)	10 (20)	0.33

*Plus-minus values are means ±SD. All maternal tests for human immunodeficiency virus were negative in both groups.

†One or more doses of hydrocortisone or dexamethasone were given.

‡Granulocyte colony-stimulating factor was given for neutropenia.

§Types of major surgery included ligation of patent ductus arteriosus, abdominal surgery, and placement of a ventricular peritoneal shunt.

no significant difference in the age at enrollment between the infants with base-line fungal colonization and those with no colonization at base line (mean [±SD] age, 2.1±1.5 vs. 1.7±1.1 days; $P=0.39$). All the infants were placed in a humidified incubator and treated with Aquaphor ointment (Beiersdorf, Norwalk, Conn.) for skin care during the first 7 to 14 days of life. One infant in the placebo group without a positive fungal culture was treated with amphotericin B and a systemic fungal infection later developed, and one patient in the fluconazole group without a positive fungal culture was treated with amphotericin B and later died from respiratory failure. Both patients were included in the analysis.

The mean (±SD) duration of the administration

of the study drug and the obtaining of the surveillance cultures for all study participants was 5.2 ± 1.4 weeks, and patients were followed for a mean of 13.7 ± 8.0 weeks. On three occasions, a dose of placebo was incorrectly administered in place of fluconazole to infants in the fluconazole group, and protocol surveillance cultures were not obtained from an infant three times in the placebo group and six times in the fluconazole group.

Fungal Colonization

During the six-week treatment period, fungal colonization was present at one or more sites in 30 of the infants in the placebo group (60 percent) and 11 of those in the fluconazole group (22 percent, $P=0.002$) (Table 2). Fungal colonization at two or more sites occurred in 26 of the infants in the placebo group (52 percent), as compared with 9 of those in the fluconazole group (18 percent, $P=0.003$). Fluconazole prophylaxis led to a significantly lower incidence of colonization at three of the culture sites (skin, stool, and nasopharynx) than that found in the placebo group during the six-week treatment period (Table 2).

The distribution of fungal species and the sites of colonization are shown in Table 3. There were 177 fungal isolates documented in 769 surveillance cultures (23.0 percent) obtained from infants in the placebo group and 42 isolates among 861 surveillance cultures (4.9 percent) obtained from infants in the fluconazole group during the treatment period (difference in risk, 0.18; 95 percent confidence interval, 0.15 to 0.22; $P<0.001$). The skin and stools were the most common sites of evidence of fungal colonization in both groups, whereas fungal colonization of the nasopharynx occurred only once in one infant in the fluconazole group, as compared with 37 times in 17 infants in the placebo group ($P=0.002$).

Fluconazole prophylaxis significantly reduced the incidence of *C. albicans* colonization overall, as well as in the skin, stools, and nasopharynx (Table 2). After the second week, no infants in the fluconazole group had colonization with *C. albicans*, as compared with 10 infants in the placebo group ($P=0.008$). There was no significant difference between the groups in the number of patients colonized with *C. parapsilosis* (Table 2), but overall, there were fewer isolates of *C. parapsilosis* in the fluconazole group (35 of 861 cultures) than in the placebo group (79 of 769 cultures) (Table 3). The total number of infants colonized with *C. parapsilosis* increased from 2 at base line to 16 by week 2 of the study.

Invasive Fungal Infection

Fungal infection, as documented by growth of fungi in cultures of blood, urine, or cerebrospinal fluid, developed in 10 of the infants in the placebo group (20 percent) and none of those in the fluconazole

TABLE 2. EFFECTS OF FLUCONAZOLE PROPHYLAXIS ON FUNGAL COLONIZATION.*

EVIDENCE OF COLONIZATION	FLUCONAZOLE GROUP (N=50)	PLACEBO GROUP (N=50)	DIFFERENCE IN RISK (95% CI)	P VALUE
no. of infants (%)				
≥1 site	11 (22)	30 (60)	0.38 (0.18 to 0.56)	0.002
≥2 sites	9 (18)	26 (52)	0.34 (0.14 to 0.52)	0.003
Any fungal species				
Skin	10 (20)	24 (48)	0.28 (0.07 to 0.47)	0.008
Stool	9 (18)	27 (54)	0.36 (0.16 to 0.54)	0.003
Nasopharynx	1 (2)	21 (42)	0.40 (0.21 to 0.57)	0.002
Umbilicus	2 (4)	6 (12)	0.08 (−0.08 to 0.24)	0.40
<i>Candida albicans</i>				
Any site	3 (6)	14 (28)	0.22 (0.03 to 0.40)	0.02
Skin	1 (2)	10 (20)	0.16 (−0.002 to 0.33)	0.05
Stool	3 (6)	13 (26)	0.20 (0.02 to 0.38)	0.03
Nasopharynx	0	11 (22)	0.22 (0.06 to 0.39)	0.005
Umbilicus	0	4 (8)	0.08 (−0.05 to 0.23)	0.31
<i>C. parapsilosis</i>				
Any site	8 (16)	14 (28)	0.14 (−0.08 to 0.31)	0.26
Skin	8 (16)	12 (24)	0.08 (−0.11 to 0.27)	0.47
Stool	5 (10)	10 (20)	0.10 (−0.08 to 0.28)	0.33
Nasopharynx	1 (2)	8 (16)	0.14 (−0.02 to 0.30)	0.09
Umbilicus	2 (4)	1 (2)	0.02 (−0.12 to 0.16)	0.89

*Data are for the infants with surveillance cultures that tested positive for fungal growth one or more times during the treatment period (mean [±SD], 5.2±1.4 weeks). CI denotes confidence interval.

TABLE 3. DISTRIBUTION OF FUNGAL SPECIES AND SITES OF COLONIZATION.*

VARIABLE	FLUCONAZOLE GROUP (N=50)	PLACEBO GROUP (N=50)
No. of surveillance cultures obtained	861	769
Fungal isolates from surveillance cultures — no. (% of cultures)	42 (4.9)	177 (23.0)
Fungal species — no. (% of isolates)		
<i>Candida albicans</i>	3 (7.1)	77 (43.5)
<i>C. parapsilosis</i>	35 (83.3)	79 (44.6)
<i>C. guilliermondii</i>	0	5 (2.8)
<i>C. glabrata</i>	4 (9.5)	5 (2.8)
<i>C. lusitanae</i>	0	9 (5.1)
<i>Trichosporon mucoides</i>	0	2 (1.1)
Site — no. (% of isolates)		
Skin	21 (50.0)	54 (30.5)
Stool	18 (42.9)	81 (45.8)
Nasopharynx	1 (2.4)	37 (20.9)
Umbilical cord	2 (4.8)	5 (2.8)

*Data are for colonization found in cultures obtained during the period when the study drug was being administered (mean [±SD] duration, 5.2±1.4 weeks).

group (difference in risk, 0.20; 95 percent confidence interval, 0.04 to 0.36; $P=0.008$). Four of the infants with systemic fungal infection died. The 10 invasive infections were caused by *C. albicans* (5), *C. parapsilosis* (3), *C. glabrata* (1), and *C. dubliniensis* (1). The lowest concentration of fluconazole that inhibited the growth of 90 percent of these isolates (MIC_{90}) was not different from the MIC_{90} of the isolates from the surveillance cultures obtained from the infants in the placebo group in whom invasive infection did not develop. There were eight episodes of sepsis (one with meningitis, one with necrotizing enterocolitis, and one with a urinary tract infection) and two isolated episodes of urinary tract infection. In one infant with a systemic fungal infection, an antecubital skin abscess developed; in another infant, endocarditis developed; and an appendiceal abscess and peritonitis developed in a third infant. Systemic fungal infection was treated in all cases with intravenous amphotericin B, with the addition of fluconazole in two cases. After the six-week treatment period, invasive fungal infection occurred in three infants in the placebo group and one infant in the fluconazole group ($P=0.30$). No clusters of invasive fungal infections occurred at any time during the study period.

There was no association between fungal colonization, as defined by a positive surveillance culture at any time during the study period or at base line, and the incidence of invasive fungal infection. Systemic

fungal infection was preceded by colonization with the same fungal species at some time point in 8 of the 10 infants with invasive infections (80 percent). By comparison, 21 of the 40 control infants without systemic fungal infection (52 percent) also had colonization. All eight patients who had colonization before invasive fungal infection developed had positive surveillance cultures during the week before the infection appeared. In one case, *C. dubliniensis* fungemia with endocarditis developed in an infant who had had colonization with *C. albicans*. In another case, *C. glabrata* sepsis developed in an infant who had been colonized with *C. parapsilosis*. In the placebo group, colonization with a specific fungal species or at a specific site was not associated with an increased risk of invasive fungal infection.

There was an association between the occurrence of invasive fungal infection and the number of sites that had been colonized at the time of the last screening. For each additional site colonized, the odds of invasive fungal infection increased by a factor of three (odds ratio, 3.0; 95 percent confidence interval, 1.4 to 6.8).

Minimal Inhibitory Concentration

Sensitivity to fluconazole as measured by the MIC did not change significantly for infants in either group. One isolate of *C. parapsilosis* in one fluconazole-treated patient had an increase in MIC from 0.25 μg per milliliter at base line to 1.00 μg per milliliter at the end of the treatment period, but invasive fungal infection did not occur in this patient. In addition, patterns of sensitivity to fluconazole did not change over the 30-month study period (Table 4).

Secondary Outcomes

There was no significant difference in mortality between the two groups (4 infants in the fluconazole group died, as compared with 10 in the placebo group; $P=0.22$). The frequencies of the secondary outcomes were also similar in the two groups. The incidence of bacterial infections (73 percent of which were due to gram-positive organisms) was similar in the two groups during and after the treatment period. Bacterial infection developed in 17 infants in the placebo group (34 percent) and 19 infants in the fluconazole group (38 percent). Necrotizing enterocolitis occurred in six infants in the placebo group (12 percent) and two infants in the fluconazole group (4 percent). There were also no significant differences between the placebo and fluconazole groups in the incidence of isolated intestinal perforation (5 infants and 2 infants, respectively), ligation of patent ductus arteriosus (5 infants and 2 infants, respectively), threshold retinopathy of prematurity (11 infants and 15 infants, respectively), or abnormal findings on cranial ultrasonography (9 infants and 8 infants, respectively). No adverse effects of the fluconazole

TABLE 4. MINIMAL INHIBITORY CONCENTRATION OF FLUCONAZOLE FOR FUNGAL ISOLATES DURING THE 30-MONTH STUDY PERIOD.*

FUNGAL SPECIES	MIC ₅₀		MIC ₉₀	
	FIRST 6 MO	LAST 6 MO	FIRST 6 MO	LAST 6 MO
	$\mu\text{g/ml}$			
<i>C. albicans</i>	1.0	0.25	2.0	0.5
<i>C. parapsilosis</i>	2.0	1.0	4.0	8.0

*MIC₅₀ denotes the lowest concentration required to inhibit the growth of 50 percent of the isolates; MIC₉₀ denotes the lowest concentration required to inhibit the growth of 90 percent of the isolates. Data represent the highest value for each specimen site (skin, stool, nasopharynx, or umbilicus). There were sufficient data to allow separate analysis of only two species, *Candida albicans* and *C. parapsilosis*. Data are for the placebo and fluconazole groups combined. There were no significant differences between periods in the MIC₅₀ or MIC₉₀ for either species.

therapy were documented. Specifically, there was no appreciable adverse effect of therapy on levels of aspartate aminotransferase, alanine aminotransferase, total bilirubin, or alkaline phosphatase. No infant was withdrawn from the study because of changes in liver-function results, and the results returned to normal after treatment in all infants who survived to discharge. Aminotransferases were elevated (more than 2 SD above the mean) in three infants in each group (6 percent) during the treatment period.

DISCUSSION

The results of this study demonstrate that fluconazole is effective in preventing invasive fungal infection in extremely-low-birth-weight preterm infants. In the past 10 years, several studies have investigated the use of fluconazole prophylaxis in immunocompromised adults.^{7-12,29-32} In patients receiving bone marrow transplants, 75 days of prophylactic treatment with fluconazole reduced the incidence of fungal colonization, superficial infection, systemic infection, and candidiasis-related death, despite colonization with azole-resistant fungal species.^{7,9,30} Fluconazole resistance did not occur. The extremely-low-birth-weight infant is an unusual host in whom the risk of fungal infection decreases with the maturation of the immune system, extubation, the discontinuation of parenteral nutrition, and the removal of central vascular access as postnatal age increases. For these reasons, the preterm infants in this study required a shorter course of medication and a lower total dose of fluconazole than adults have required in previous studies. Since azole resistance has been reported to be dependent on the total dose received, this difference in exposure may partially explain why flucona-

zole resistance did not develop in this cohort.¹⁴ In addition, the use of amphotericin B for primary therapy for invasive fungal disease limits the total dose of fluconazole to which fungi are exposed and may help prevent the development of resistant fungi.^{15,33}

The elimination, as a result of fluconazole prophylaxis, of fungal colonization at multiple sites or, in some cases, of colonization altogether appeared to contribute to the prevention of invasive fungal infection. In addition, in patients who had fungal colonization, it is possible that fluconazole prophylaxis may have rendered the fungi more susceptible to the innate defenses of the host.³⁴⁻³⁹

Fluconazole prophylaxis appeared to have its most dramatic effects on *C. albicans*—a finding that is consistent with other data suggesting that *C. albicans* is more susceptible to fluconazole than other fungal species.⁴⁰ The marked effect of fluconazole on nasopharyngeal colonization suggests that effective tissue drug concentrations are achieved in the nasopharyngeal mucosa.⁴¹ By the third week of life in the infants in our study, fluconazole prophylaxis eliminated colonization by *C. albicans* that was most likely acquired by means of vertical transmission from the mother.⁴² The majority of colonizations with *C. parapsilosis* occurred during the second week of the study, implying that *C. parapsilosis* has a different mechanism of colonization, which most commonly occurs by means of horizontal transmission, through the nosocomial spread of fungi from health care workers or colonized infusates.⁴³

The fluconazole dosing used in this study was based on published data regarding the pharmacokinetics of fluconazole in preterm infants.^{19,20} Our study demonstrated no adverse effects of fluconazole, and the lower dose and the intermittent dosing may be responsible for this favorable side-effect profile. Because of concern about the development of azole resistance, we limited our study to preterm infants who required vascular access or endotracheal intubation during the first six weeks of life. Therefore, this study does not address the effectiveness of fluconazole in infants who continue to require vascular access or endotracheal intubation beyond six weeks of age. The prophylactic administration of fluconazole prevented fungal colonization and invasive fungal disease in extremely-low-birth-weight preterm infants without adverse effects or development of fluconazole resistance.

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REFERENCES

1. Stoll BJ, Gordon T, Korones SB, et al. Late-onset sepsis in very low birth weight neonates: a report from the National Institute of Child Health and Human Development Neonatal Research Network. *J Pediatr* 1996; 129:63-71.
2. Baley JE. Neonatal candidiasis: the current challenge. *Clin Perinatol* 1991;18:263-80.
3. Baley JE, Kliegman RM, Boxerbaum B, Fanaroff AA. Fungal colonization in the very low birth weight infant. *Pediatrics* 1986;78:225-32.
4. Stoll BJ, Temprosa M, Tyson JE, et al. Dexamethasone therapy increases infection in very low birth weight infants. *Pediatrics* 1999;104(5):1121. abstract.
5. Kicklighter SD, Springer SC, Cox T, Hulsey TC, Turner RB. Fluconazole for prophylaxis against candidal rectal colonization in the very low birth weight infant. *Pediatrics* 2001;107:293-8.
6. Xanthou M, Valassi-Adam E, Kintsonidou E, Matsaniotis N. Phagocytosis and killing ability of *Candida albicans* by blood leukocytes of healthy term and preterm babies. *Arch Dis Child* 1975;50:72-5.
7. Goodman JL, Winston DJ, Greenfield RA, et al. A controlled trial of fluconazole to prevent fungal infections in patients undergoing bone marrow transplantation. *N Engl J Med* 1992;326:845-51.
8. Winston DJ, Chandrasekar PH, Lazarus HM, et al. Fluconazole prophylaxis of fungal infections in patients with acute leukemia: results of a randomized placebo-controlled, double-blind, multicenter trial. *Ann Intern Med* 1993;118:495-503.
9. Slavin MA, Osborne B, Adams R, et al. Efficacy and safety of fluconazole prophylaxis for fungal infections after marrow transplantation—a prospective, randomized, double-blind study. *J Infect Dis* 1995;171: 1545-52.
10. Powderly WG, Finkelstein D, Feinberg J, et al. A randomized trial comparing fluconazole with clotrimazole troches for the prevention of fungal infections in patients with advanced human immunodeficiency virus infection. *N Engl J Med* 1995;332:700-5.
11. Lumberras C, Cuervas-Mons V, Jara P, et al. Randomized trial of fluconazole versus nystatin for the prophylaxis of *Candida* infection following liver transplantation. *J Infect Dis* 1996;174:583-8.
12. Mucke R, Kaben U, Libera T, et al. Fluconazole prophylaxis in patients with head and neck tumours undergoing radiation and radiochemotherapy. *Mycoses* 1998;41:421-3.
13. Marr KA, Seidel K, White TC, Bowden RA. Candidemia in allogeneic blood and marrow transplant recipients: evolution of risk factors after the adoption of prophylactic fluconazole. *J Infect Dis* 2000;181:309-16.
14. Millon L, Manteaux A, Reboux G, et al. Fluconazole-resistant recurrent oral candidiasis in human immunodeficiency virus-positive patients: persistence of *Candida albicans* strains with the same genotype. *J Clin Microbiol* 1994;32:1115-8.
15. Lewis RE, Klepser ME. The changing face of nosocomial candidemia: epidemiology, resistance, and drug therapy. *Am J Health Syst Pharm* 1999; 56:525-33.
16. Pfaller MA, Messer SA, Hollis RJ, et al. Trends in species distribution and susceptibility to fluconazole among blood stream isolates of *Candida* species in the United States. *Diagn Microbiol Infect Dis* 1999;33:217-22.
17. Rex JH, Rinaldi MG, Pfaller MA. Resistance of *Candida* species to fluconazole. *Antimicrob Agents Chemother* 1995;39:1-8.
18. Nguyen MH, Peacock JE Jr, Morris AJ, et al. The changing face of candidemia: emergence of non-*Candida albicans* species and antifungal resistance. *Am J Med* 1996;100:617-23.
19. Saxen H, Hoppu K, Pohjavuori M. Pharmacokinetics of fluconazole in very low birth weight infants during the first two weeks of life. *Clin Pharmacol Ther* 1993;54:269-77.
20. Brammer KW, Coates PE. Pharmacokinetics of fluconazole in pediatric patients. *Eur J Clin Microbiol Infect Dis* 1994;13:325-9.
21. Spicer AD, Hazen KC. Rapid confirmation of *Candida albicans* identification by combination of two presumptive tests. *Med Microbiol Lett* 1992;1:284-9.
22. Murray PR, ed. Manual of clinical microbiology. 7th ed. Washington, D.C.: ASM Press, 1999:1191-7.
23. Reference method for broth dilution antifungal susceptibility testing of yeasts: approved standard. Wayne, Pa.: National Committee of Clinical Laboratory Standards, 1997. (NCCLS document no. M27-A.)
24. Hennekens CH, Buring JE. Epidemiology in medicine. 2nd ed. Boston: Little, Brown, 1994:87-8.
25. Mehta C, Patel N. Proc-StatXact 4 for SAS users. Cambridge, Mass.: Cytel Software, 1999:257-92.
26. Berger RL, Boos DD. P values maximized over a confidence set for the nuisance parameter. *J Am Stat Assoc* 1994;89:1012-6.
27. Santner TJ, Snell MK. Small-sample confidence intervals for $p_1 - p_2$ and p_1/p_2 in 2×2 contingency tables. *J Am Stat Assoc* 1980;75:386-94.
28. Diggle PJ, Liang K-Y, Zeger SL. Analysis of longitudinal data. Oxford, England: Clarendon Press, 1994:143-5.
29. Kern W, Behre G, Rudolf T, et al. Failure of fluconazole prophylaxis to reduce mortality or the requirement of systemic amphotericin B therapy during treatment for refractory acute myeloid leukemia: results of a prospective randomized phase III study. *Cancer* 1998;83:291-301.
30. Marr KA, Seidel K, Slavin MA, et al. Prolonged fluconazole prophylaxis is associated with persistent protection against candidiasis-related

death in allogeneic marrow transplant recipients: long-term follow-up of a randomized, placebo-controlled trial. *Blood* 2000;96:2055-61.

31. Gotzsche PC, Johansen HK. Meta-analysis of prophylactic or empirical antifungal treatment versus placebo or no treatment in patients with cancer complicated by neutropenia. *BMJ* 1997;314:1238-44.

32. Kanda Y, Yamamoto R, Chizuka A, et al. Prophylactic action of oral fluconazole against fungal infection in neutropenic patients: a meta-analysis of 16 randomized, controlled trials. *Cancer* 2000;89:1611-25.

33. Gleason TG, May AK, Caparelli D, Farr BM, Sawyer RG. Emerging evidence of selection of fluconazole-tolerant fungi in surgical intensive care units. *Arch Surg* 1997;132:1197-202.

34. Abu-el Teen K, Ghannoum M, Stretton RJ. Effects of sub-inhibitory concentrations of antifungal agents on adherence of *Candida* spp. to buccal epithelial cells in vitro. *Mycoses* 1989;32:551-62.

35. Hazen KC, Mandell G, Coleman E, Wu G. Influence of fluconazole at subinhibitory concentrations on cell surface hydrophobicity and phagocytosis of *Candida albicans*. *FEMS Microbiol Lett* 2000;183:89-94.

36. Ghannoum MA, Filler SG, Ibrahim AS, Fu Y, Edwards JE Jr. Modulation of interactions of *Candida albicans* and endothelial cells by fluconazole and amphotericin B. *Antimicrob Agents Chemother* 1992;36:2239-44.

37. Van 't Wout JW, Meynaar I, Linde I, Poell R, Mattie H, Van Furth R. Effect of amphotericin B, fluconazole and itraconazole on intracellular *Candida albicans* and germ tube development in macrophages. *J Antimicrob Chemother* 1990;25:803-11.

38. Garcha UK, Brummer E, Stevens DA. Synergy of fluconazole with human monocytes or monocyte-derived macrophages for killing of *Candida* species. *J Infect Dis* 1995;172:1620-3.

39. Minguez Minguez F, Lima JE, Garcia MT, Prieto J. Effects of antifungal pretreatment on the susceptibility of *Candida albicans* to human leukocytes. *Chemotherapy* 1997;43:346-51.

40. Pfaller MA, Messer SA, Hollis RJ, et al. In vitro susceptibilities of *Candida* bloodstream isolates to the new triazole antifungal agents BMS-207147, Sch 56592, and voriconazole. *Antimicrob Agents Chemother* 1998;42:3242-4.

41. Vaden SL, Heit MC, Hawkins EC, Manaugh C, Riviere JE. Fluconazole in cats: pharmacokinetics following intravenous and oral administration and penetration into cerebrospinal fluid, aqueous humour and pulmonary epithelial lining fluid. *J Vet Pharmacol Ther* 1997;20:181-6.

42. Waggoner-Fountain LA, Walker MW, Hollis RJ, et al. Vertical and horizontal transmission of unique *Candida* species to premature newborns. *Clin Infect Dis* 1996;22:803-8.

43. Huang YC, Lin TY, Leu HS, Peng HL, Wu JH, Chang HY. Outbreak of *Candida parapsilosis* fungemia in neonatal intensive care units: clinical implications and genotyping analysis. *Infection* 1999;27:97-102.

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