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Ketoconazole in the Prevention of Experimental Candidal Vaginitis

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The prophylactic and therapeutic activities of ketoconazole were evaluated in rats inoculated intravaginally with *Candida albicans*. Daily doses of 2.5 mg/kg initiated 48 h before challenge and continued for 48 h thereafter protected 80% of the rats against infection. A similar result in rats with established infection was attained with daily doses of 5.0 mg/kg.

Ketoconazole (Janssen Pharmaceutica, Piscataway, N.J.), an imidazole derivative, at a dosage of 7.5 mg/kg for 5 days is highly effective in the treatment of symptomatic candidal vaginitis in both humans and experimental animals (1). In the present study, rats were treated prophylactically with ketoconazole commencing 48 h before vaginal inoculation of *C. albicans* in an attempt to prevent the establishment of vaginal colonization and infection.

Pseudoestrous was induced in 80 oophorectomized female Sprague-Dawley rats (175 to 200 g) by subcutaneous injection of estradiol valerate in sesame oil (0.5 mg/0.1 ml, E. R. Squibb & Sons, Princeton, N.J.) and was maintained by similar weekly injections. At 48 h after the initial estrogen injection, rats were inoculated vaginally with 10^4 *C. albicans* blastospores suspended in 0.1 ml of sterile phosphate-buffered saline, pH 7.2. *C. albicans* used for infection was obtained by inoculating 10 ml of sterile 1% phytone-peptone-glucose broth with 100 μl of a stock blastospore suspension and incubating the preparations for 22 h at 25°C in a shaking water bath. The resulting culture was centrifuged for 10 min at 2,000 rpm at 4°C and resuspended in 1 to 2 ml of sterile phosphate-buffered saline. The number of blastospores in this suspension was counted in a hemacytometer. Thereafter, the yeast suspension was administered, 0.1 ml per animal, via a short segment of butterfly tubing (1.5 in. [ca. 3.8 cm]) affixed to a 1-ml tuberculin syringe. Vaginal infection was confirmed by vaginal lavage performed 5 days after candida inoculation. Quantitation of the candida organisms present was made by serial dilution of the retrieved lavage fluid and plating on Sabouraud glucose agar. All plates were incubated at 30°C for 48 h for each series of dilutions; the plate, containing between 30 and 300 colonies, was used to calculate the log of the CFU per milliliter. The MIC of the clinical isolates of *C. albicans* used for ketoconazole was 0.098 μg/ml as determined by agar dilution with a Steers-Foltz replicator and Kimmig nutrient agar (E. Merck AG, Darmstadt, Federal Republic of Germany).

Prophylaxis with ketoconazole administered by daily gavage at a dosage of 1.25, 2.5, 5.0, and 7.5 mg/kg was commenced 48 h before vaginal inoculation of *C. albicans* and continued for an additional 48 h after infection for a total of 5 days, i.e., five doses of ketoconazole. For each dosage regimen, 10 oophorectomized rats were used, except for the group receiving 1.25 mg of ketoconazole per kg, in which only 5 rats were used. A control group of 10 untreated rats was similarly inoculated and lavaged, and quantitative cultures were performed. Successful prophylaxis or treatment was equated with sterile vaginal lavage fluid. All experimental studies at all drug dosages were performed at least twice. Statistical evaluation of the effect of ketoconazole prophylaxis was performed using the Student *t* test.

All 10 untreated control rats became infected, with a mean vaginal titer of log 5.4 ± 0.6 CFU/ml of vaginal fluid. Prophylaxis with ketoconazole commencing 48 h before vaginal inoculation of *C. albicans* at dosages of 7.5 and 5.0 mg/kg prevented the establishment of vaginal colonization and infection in 100% of rats, and prophylaxis with 2.5 mg of ketoconazole per kg prevented vaginal infection in 80% of inoculated animals (Table 1). Since prophylaxis was continued on the day of vaginal inoculation and for an additional 48 h thereafter, it may be argued that our observations at all dosages may reflect the effects of early treatment rather than true prophylaxis. Accordingly, additional groups of 10 rats each were treated with identical daily doses of ketoconazole commencing the day after inoculation of *C. albicans* for a total of 2 days. Therapy with 7.5 mg of ketoconazole per kg starting 48 h postinoculation sterilized the vaginal fluid in 10 out of 10 rats, whereas treatment for 2 days with 5.0 mg of ketoconazole per kg eradicated infection in only 7 of 10 rats. Ketoconazole at 2.5 mg/kg sterilized vaginal fluid in only 2 of 10 rats. Significant differences were thus detected between the early treatment group and the animals receiving additional preinoculation prophylaxis with 2.5 mg of ketoconazole per kg ($P < 0.01$).

These results suggest that pretreatment of susceptible hosts with low-dosage ketoconazole may be effective in reducing the risk of vaginal colonization and symptomatic infection. In this study, we used a relatively large inoculum of *C. albicans*, i.e., 10^4 blastospores, to induce infection. In the rat model we have been able to induce vaginal infection with an inoculum as small at 10^2 blastospores of *C. albicans*. The critical inoculum required in humans is unknown, although 10^4 microorganisms is probably a high concentration even in adults, providing a stern test for this prophylactic regimen.

TABLE 1. Comparison of efficacy of ketoconazole prophylaxis and early treatment regimen in experimental candidal vaginitis

Ketoconazole (mg/kg)	No. of infected rats cured/total	
	Prophylactic regimen	Early treatment
1.25	1/5	1/5
2.5	8/10[a]	2/10[a]
5.0	10/10	7/10
7.5	10/10	10/10

[a] $P < 0.01$.

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The mechanism of action of low-dosage ketoconazole in preventing vaginal infection is still obscure, although germination inhibition which prevents the development of the invasive form of *C. albicans* occurs at extremely low concentrations of ketoconazole (2). Human studies are necessary to verify these experimental observations; such studies in women with recurrent and chronic candidal vulvovaginitis are under way.

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