5-Fluorocytosine in the Treatment of Experimental Candidiasis in Immunosuppressed Mice

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A model infection with Candida albicans was established in mice. The animals were pretreated for 1 week with azathioprine, given dexamethasone, and then infected intravenously with Candida. Mortality for the group given Candida infection alone was 20%, 38% when azathioprine was added, and 50% when dexamethasone and azathioprine were given with infection. The titers of Candida in most mice were 10⁶ per gram of kidney. The rest of the mice were killed at 30 days. At this time, 16% had no evidence of Candida in the kidneys, but 40% of the mice had titers of 10³ or more. Mice treated with 5-fluorocytosine had a mortality of less than 4% in 30 days. Five percent of the treated mice killed at 30 days had titers of Candida of 10⁴. Therefore, 5-fluorocytosine increases survival in the presence of continued therapy with azathioprine with or without pretreatment with dexamethasone. The survival is associated with decreased titers of Candida in the kidneys.

5-Fluorocytosine (5-FC) is a new antifungal agent effective against Candida albicans and Cryptococcus neoformans under experimental conditions (6, 17, 23) and in a few reported clinical situations (3, 26, 27). It is relatively nontoxic and therefore may be an important advance over amphotericin B if its therapeutic effect can be more extensively confirmed. Systemic Candida infections occur frequently in the compromised host either concomitant with diseases known to affect host defenses, such as lymphoma and leukemia (7), or secondary to prolonged use of antibiotics or to immunosuppressive agents (20).

Published series from several transplantation centers indicate that the major cause of late deaths following transplantation is caused by or associated with infection, and that in 33 to 74% the causative organisms are Candida and Aspergillus (10, 16, 18, 25). After renal transplantation, most patients receive azathioprine (or similar immunosuppressive agents), and if signs of rejection occur, they are then treated with high doses of glucocorticoids (8). The data suggest that fungai infections are rare in posttransplant patients receiving azathioprine alone but increase after the addition of high doses of glucocorticoids (4, 12, 24). When fungal infection occurs, it is often unrecognized and even if the diagnosis is apparent, the therapeutic choice is frequently the nephrotoxic amphotericin B.

Experimental Candida infections in mice have been extensively studied by many investigators (1, 9, 11, 15, 28). Various agents which lower host resistance, such as antibiotics (21, 22), X ray (5), endotoxin (13), and cortisone (14, 19) have also been employed to increase the susceptibility of mice to a Candida challenge. We have extended the model by adding azathioprine in order to study 5-FC.

MATERIALS AND METHODS

Mice. Male white Swiss Webster mice (20 g), obtained from Arthur Sutter, Springfield, Mo., were allocated randomly to different groups. All were given standard laboratory food and water ad libitum. Mice receiving azathioprine and/or 5-FC were weighed every third day, and the dosage, given on a weight basis (mg/kg), was adjusted for average weight gain. After 7 days, azathioprine-treated mice weight averaged 30 g. Thirty days after Candida infection, mice were killed by chloroform. The kidneys were removed, weighed, and homogenized in normal saline in a Teflon grinder at a ratio of 1 g of kidney tissue per 2.0 ml of saline. The homogenate was diluted 1:10 and then serially diluted by the 10-fold dilution method to 10⁻⁴. Pour plates of appropriate dilutions were made using Sabouraud agar. The plates were
incubated at 37 C for 48 h, and the Candida titer per gram of kidney tissue was recorded. The fungus, when observed in cross sections of the kidney, had the hyphal form appearance under all experimental conditions used.

**Treatment groups.** Half of the eight groups received 5-FC (treated), half did not (untreated). One subgroup in each received Candida, and one received dexamethasone and Candida (Fig. 1).

**Candida inoculum.** Candida albicans recovered from the blood of a patient with fungicemia was maintained by serial monthly passage on Sabouraud agar. The minimum inhibitory concentration (MIC) of 5-FC was 6.25 μg/ml. Fresh Sabouraud slants were inoculated from the stock culture and incubated at 37 C for 24 h. The yeast was harvested, washed, and resuspended in saline. It was then counted directly in a Levy-Neubauer counting chamber, and the inoculum was adjusted to contain 2.0 × 10⁵ yeast cells per 0.1 ml for injection into the lateral tail vein of the mice. Four plates of the Candida inoculum were made and incubated at 37 C for 24 h, and the titer was compared to the hemocytometer count.

Azathioprine. Azathioprine was kindly supplied as bulk Imuran for parenteral preparation by George H. Hitchings of Burroughs-Wellcome Co., Tuckahoe, N.Y. It was administered intraperitoneally (IP) to mice at 3 mg per kg per day, beginning 7 days before infection with Candida and continuing for 30 days.

**Dexamethasone.** Dexamethasone (0.015 mg in 0.2 ml) was given subcutaneously the day before the day of, and the day after, Candida infection.

5-FC. 5-FC was kindly supplied by W. E. Scott of Hoffman-LaRoche, Inc., Nutley, N.J. This agent was administered IP at 100 mg per kg per day, beginning within 1 h after infection with Candida and continuing for 30 days until the end of the study. The time interval between administration each day of the two IP medications was at least 6 h.

**Statistical methods.** Statistical analysis was performed by the chi square method.

**RESULTS**

In Fig. 1, comparison is made between each treated experimental group and its corresponding untreated control. The Candida titers in all of the untreated experimental groups show a peak distribution in the 10^5 to 10^7 range. In the treated groups, the Candida titers are more evenly distributed with absence of a peak titer, indicated by the flattening of the bar groups.

The effect of 5-FC both on the Candida titers and on the longevity of the mice over an arbitrary time period of 30 days is shown in Fig. 2. Forty-two of 118 mice without therapy died spontaneously, whereas in the treated group only 4 of 109 succumbed (P = 0.001). Of mice in the untreated groups, 92% died before 30 days, and they had titers of Candida between 10^5 to 10^7. The number of treated mice dying spontaneously was small (n = 4); however, three out of four mice in this group had titers of 10^10, which are considerably lower than titers in the comparable untreated group. At 30 days the number of mice (n = 105) with a titer of 10^4 was decreased by almost two-thirds compared with the untreated groups. Figure 3 shows the percentage of survivors of all groups, treated and untreated, over the 30-day time period.

There was an 80% survival of mice given only Candida (Fig. 3). In the untreated groups, survival rate decreased to 62% in those given azathioprine and Candida, and to 50% in those given dexamethasone and Candida, and dexamethasone, azathioprine, and Candida. This indicates that untreated groups receiving the immunosuppressives dexamethasone or azathioprine, or both, did poorly when presented with a Candida challenge, compared with groups...
receiving only Candida ($P = 0.01$). At 30 days, survival of all treated groups was significantly better than that of all untreated groups ($P = 0.001$). These data cannot be used to demonstrate a relationship between immunosuppression and mortality because of the minimal mortality in all groups receiving 5-FC. There was no statistically significant difference among the subgroups treated with 5-FC.

**DISCUSSION**

The immunosuppressed host has a higher mortality after *Candida* challenge than does the normal host. The experimental evidence for immunosuppression by glucocorticoids is well documented in the literature. Previous toxicity studies using azathioprine in mice showed minimal cumulative toxic effects with repeated doses. The maximum tolerated dose was 100 mg/kg administered IP for 5 days, and this was accompanied by anorexia weight loss (2). In this study, the dose of azathioprine was given on a milligram-per-kilogram basis in order to approximate the posttransplant doses usually given in man and thereby to keep the toxicity low enough to allow the antifungal effect of 5-FC to be demonstrated. With this dosage, immunosuppression occurred as shown by differences in mortality. It is not possible to differentiate statistically the effect of either dexamethasone alone or azathioprine alone on immunosuppression.

5-FC significantly prolonged survival and lowered the number of viable *Candida* cells in both survivors and nonsurvivors. The increased survival was apparent in the presence of continued therapy with azathioprine, with or without dexamethasone. The animals given azathioprine or dexamethasone, or both, demonstrated immunosuppression at least in regard to the ability of *Candida* to multiply in larger numbers than in the controls. No increased fatality was observed from 5-FC, which agrees with other studies of 5-FC pertaining to animal toxicology (17).

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**LITERATURE CITED**

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