

## Evaluation of Miconazole Therapy in Experimental Disseminated Candidiasis in Laboratory Rats

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Miconazole, a broad-spectrum antimycotic agent with some antibacterial activity, has recently become available for experimental parenteral use in the United States. Its efficacy as an anticandidal drug was tested in adult Wistar rats. A previously established infectious dose of  $5 \times 10^6$  *Candida albicans* was intravenously injected into 250- to 300-g animals. This dose was fatal to 95% (20/21) of placebo-treated control animals within the 2-week postinfection observation period. Only 4% (2/53) of rats receiving intramuscular miconazole treatment died. Miconazole therapy in *Candida*-infected rats at a dosage of 50 mg/kg per day resulted in 85% survival, and, although 100 mg/kg per day was 100% efficacious, it was a relatively large volume to give intramuscularly to a rat. Therefore, 75 mg/kg per day was used as a therapeutic dose, and it gave favorable results in this study. Histological examination of all placebo-treated animals revealed *C. albicans* and a marked inflammatory response in the kidney, brain, and heart. *C. albicans* organisms were observed to be very prominent in these tissues by using the Gomori methenamine silver stain, and were cultured from these organs. Miconazole-treated rats that were killed after surviving the 2-week observation period had minimal histopathological changes, and the organisms present did not exhibit the same staining characteristics, nor were they isolated like those in the placebo-treated group. Miconazole appears to be an efficacious drug for parenteral therapy, as demonstrated in this reproducible model of disseminated candidiasis in laboratory rats, and more extensive experimental studies are indicated.

Systemic candidiasis is a life-threatening disease most commonly seen in people lacking a normal immune response, such as neonates and patients receiving X-ray therapy, corticosteroids, or other immunosuppressive agents. Commonly used drugs to treat systemic candidiasis are amphotericin B and 5-fluorocytosine. Amphotericin B, however, has been associated with severe nephrotoxicity, and *Candida albicans* is frequently resistant to 5-fluorocytosine (7). Therefore, there is a need for new antifungal agents for the treatment of systemic candidiasis. Miconazole (MON) is a  $\beta$ -substituted 1-phenethyl-imidazole derivative with a molecular weight of 479.16 that has been shown to have broad-spectrum antifungal and antibacterial activity (8). MON nitrate has been available for some time as a topical dermatological agent, but the recently developed form for parenteral use is the MON base, not MON nitrate.

Because rats offer definite advantages over other laboratory animals, this animal was chosen to develop a reproducible animal model of disseminated candidiasis to study the anticandidal efficacy of MON.

### MATERIALS AND METHODS

**Animals.** Adult Wistar rats (250 to 300 g) were obtained from our noninbred production colony. Animals were housed in groups of five in stainless steel suspension cages. The photo period was automatically controlled, providing 12 h of light and 12 h of dark. Standard laboratory chow and water were provided ad libitum. The environmental temperature ranged from 20 to 25°C.

**Organisms.** The strain of *C. albicans* used in this study was a clinical isolate recovered before initiation of antibiotic therapy. Organisms for animal inoculation were cultured for 18 h in Sabouraud dextrose broth at 37°C, harvested by centrifugation, and suspended in 0.01 M phosphate-buffered saline to pH 7.2. The cells were washed three times and resuspended to a concentration of  $10^7$  *C. albicans* per ml in phosphate-buffered saline. Organisms were injected within 30 min of preparation.

**Injection procedure.** All rats were injected with 0.5 ml of the above suspension via the tail vein. Because preliminary studies indicated that rats injected with this dose would die of disseminated candidiasis within 10 days postinjection, the observation period for development of fatal systemic disease was set at 2 weeks. It was important that each animal received the full dose of *C. albicans*, since those receiving less

showed only slight clinical signs of infection with a low fatality rate.

**MON administration.** MON was obtained from Janssen Research and Development, Inc., New Brunswick, N.J. Various doses (50, 75, and 100 mg/kg per day) of MON were examined, and preliminary studies indicated that 75 mg/kg per day, given twice daily in equal injections for 5 days, was the most efficacious dose for the intramuscular route. This dose was used throughout these studies. The onset of therapy was varied between pretreatment (4 h before *Candida* injection, 19 rats), 4-h delay treatment (24 rats), and 24-h delay treatment (10 rats). Twenty-one animals were injected twice daily with normal saline as placebo for 5 days beginning 4 h postinfection.

**Histopathological procedure.** Animals that died during the observation period and those that survived the 2-week observation period were killed and autopsied, and tissue taken for histopathological examination was fixed in 10% buffered Formalin. Paraffin sections were made from each of the tissues and stained with hematoxylin and eosin and Gomori methenamine silver for examination by light microscopy. Representative tissues from treated and untreated animals were also cultured for *C. albicans*. Tissues were minced, added to an equal volume of phosphate-buffered saline, and homogenized in a tissue grinder. Samples of the homogenate were plated on Sabouraud dextrose agar and blood agar, incubated at 37°C for 48 h, and observed. Isolation of *C. albicans* was confirmed using standard techniques.

## RESULTS

**Clinical disease.** Within 3 days of inoculation, placebo-treated rats exhibited lethargy, anorexia, hunched posture, and ruffled hair coat and had porphyrin-stained ocular and nasal secretions. Signs of neurological disease, such as

head tilt and/or circling, developed by 5 days postinjection in some of the animals. MON-treated animals appeared clinically ill for 3 to 5 days as noted by lethargy, ruffled hair coats, and mild anorexia. However, after at least 3 days of MON therapy these animals began to appear and act clinically normal, whereas most of the placebo animals died. Figure 1 shows that with pretreatment and 4-h delay treatment with 75 mg of MON per kg there was 100% survival. There was 80% survival when onset of therapy was delayed 24 h. All three of the treatment groups were statistically significantly different from controls ( $P < 0.01$ ), and there was no significant difference between treatment groups by the  $\chi^2$  method of analysis.

**Necropsy findings.** All rats that died had gross abscesses in the kidneys, and most animals had them also in the heart. These lesions consisted of small white foci on the surface and were also present throughout the parenchyma. MON-treated animals that survived the 2-week postinjection observation period were killed and also autopsied. The gross lesions found in the organs of the rats dying from disseminated candidiasis were not present in the survivors. Numerous candida were isolated from organs (brain, heart, kidney) of placebo-treated animals. Candida were not isolated from MON-treated animals that survived the 2-week observation period; organisms, however, were isolated in small numbers from the kidneys and brains of those few MON animals that died before 2 weeks postinjection.

**Histological findings.** The kidneys of all

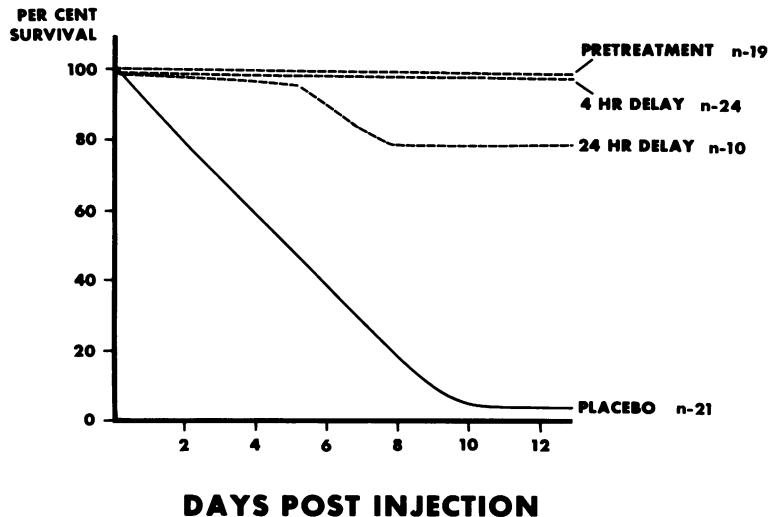


FIG. 1. Results of various treatment regimens of MON therapy (75 mg/kg) in rats with experimental disseminated candidiasis.

rats that died had histopathological changes present, ranging from localized foci of organisms and inflammatory cells to extensive abscesses filled with polymorphonuclear leukocytes in both the cortex and medulla. Many tubules were packed with polymorphonuclear leukocytes and cellular debris, and these purulent areas corresponded to the location of *C. albicans* as demonstrated with Gomori methenamine silver stain (Fig. 2). *Candida* were also frequently found within glomeruli, but the severe inflammatory changes present in the tubules were not seen in the glomeruli.

The brain was the second most common location of lesions due to *C. albicans*. Focal granulomatous lesions were found in the cerebral cortex, cerebellum, and brain stem. Giant cells of the Langhans type were present in many of these lesions; the brain was the only tissue in which these cells were found. The focal lesions corresponded well to the location of the *C. albicans* as seen with Gomori methenamine silver stain (Fig. 3). Occasionally, perivascular cuffing

with mononuclear cells was seen in the cerebral cortex, and some animals had inflammatory cells invading the meninges.

In the few MON-treated rats that died, gross changes on the surface of the kidney were also present, as was seen in the placebo-treated animals. Gross changes in body organs were not evident in MON-treated animals that survived and were subsequently killed and autopsied. Microscopic changes, however, were present in the kidneys and brains of all MON-treated rats. As in the control animals, there was a marked inflammatory response radiating from the cortex into the medulla with many polymorphonuclear leukocytes within the tubules. However, there were very few candida present within this area of polymorphonuclear leukocyte response. The brains of the MON-treated animals also evidenced histopathological changes, as seen by the granulomatous response within the cerebrum and some Langhans type giant cells. However, as found in the kidney by using Gomori methenamine silver stain, very few candida were

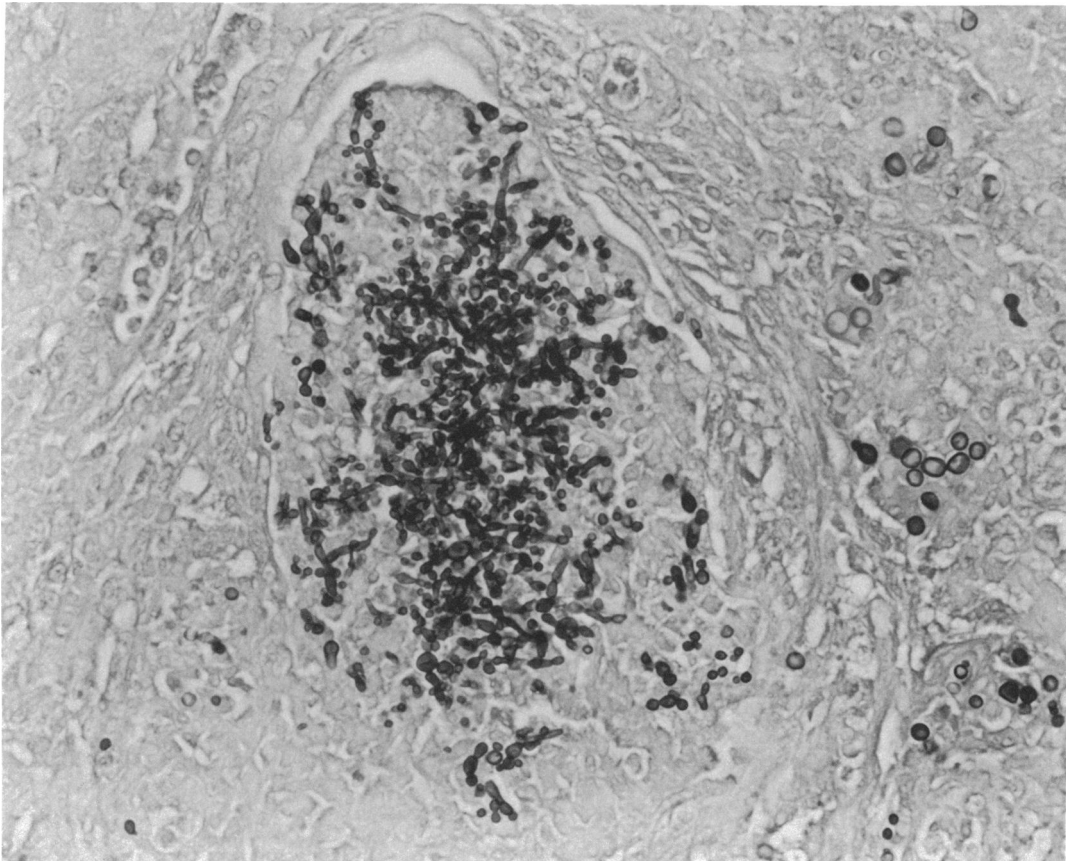


FIG. 2. Renal tubule filled with dark-staining *C. albicans* corresponding to the areas with marked polymorphonuclear leukocytes and cellular debris. Gomori methenamine silver stain. Magnification,  $\times 360$ .

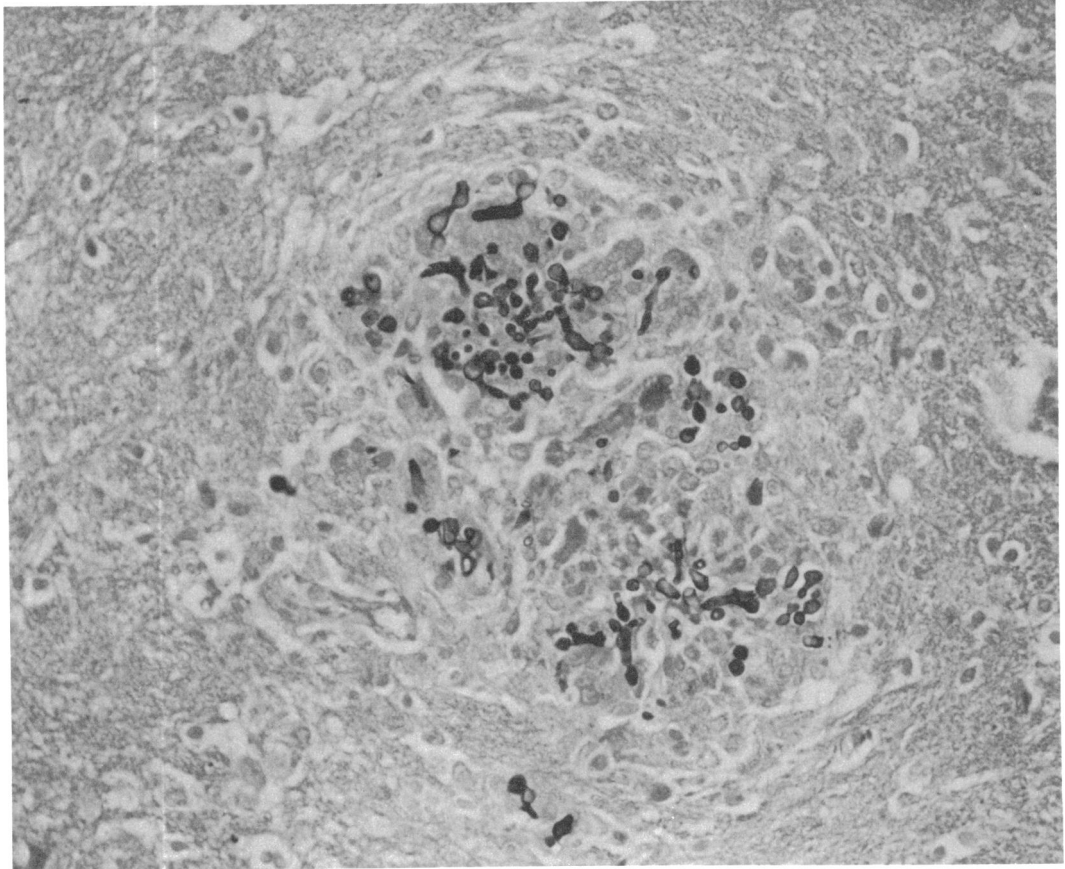


FIG. 3. Focal granulomatous lesion in cerebral cortex. Presence of *C. albicans* is generally confined within the inflammatory lesion. Gomori methenamine silver stain. Magnification,  $\times 360$ .

prevalent within the granulomas, and those present had a nonuniform staining pattern.

#### DISCUSSION

This model of disseminated candidiasis in laboratory rats gave reproducible results, with clinical and histopathological changes similar to those reported for other animals and humans dying of this disease (1, 2, 4, 5).

In human cases of fatal disseminated candidiasis, gross and microscopic histopathological changes are similar to those reported in this study. Multiple abscesses are found routinely in the kidney, heart, and brain, corresponding to the location of the organisms (2, 5, 6). These organs are also affected in rats with experimental disseminated candidiasis.

MON used for this study was base, not MON nitrate, which is commercially available for topical dermatological use. MON was efficacious in the treatment of disseminated candidiasis even with a delay of therapy up to 24 h postinjection

of intravenous *C. albicans*. The normal route of administration of MON to humans is intravenous, but, because of the small veins in rats and the multiple injections required, intramuscular injections were used; these still proved to be efficacious and statistically significantly better than placebo alone. Toxicity studies in 20 rats in our laboratory showed no adverse reactions to parentally administered MON at doses of 100 mg/kg per day divided in two equal doses for 10 days of treatment. When the rats were killed 3 weeks after MON therapy, some muscle degeneration was found at the site of injection, probably due to the pH of MON, which is  $4 \pm 0.5$ . No other significant histopathological lesions were present in these rats. Human studies have shown that 600 to 2,000 mg of MON given intravenously in three divided daily doses is safe and efficacious for systemic candidiasis (3, 9). MON appears to be a drug that may be useful in patients with impaired renal function, in whom amphotericin B cannot be used, and in patients

that have *C. albicans* resistant to 5-fluorocytosine.

As demonstrated in this reproducible animal model of fatal disseminated candidiasis, MON is an efficacious parenteral anticandidal agent, and more extensive study of the use of this drug should be encouraged.

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