Mice with Persistent Gastrointestinal *Candida albicans* as a Model for Antifungal Therapy

CALEB HERRERA AND M. NEAL GUENTZEL*

*Division of Allied Health and Life Sciences, The University of Texas, San Antonio, Texas 78285*

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Persistent infection of the gastrointestinal tract of CFW mice with *Candida albicans* was produced by the oral-intragastric inoculation of 6-day-old infants. Other intraabdominal organs (liver, kidneys, and spleen) were usually free of the organism in survivors at 20 days of age. However, all survivors retained high levels of the organism in the stomach and intestinal tract at 30 days of age. The possible utility of these persisting *C. albicans* infections of the gastrointestinal tract for the study of the efficacy of short-term antifungal therapy was studied. Drug treatment was initiated for a 2-week period when the survivors were 15 to 19 days old. Some representative antifungal agents in current use (i.e., amphotericin B, 5-fluorocytosine, and miconazole) effected significant reductions in the numbers of *C. albicans* in homogenates of gastrointestinal organs.

The number of antifungal agents for candidosis and other mycoses is small; most are toxic, and the indications for their use are varied (7). The lack of suitable animal models for the study of candidosis as it occurs in humans has hampered understanding of the host-pathogen interrelationships in the disease and impeded the development of improved antifungal therapy. Oral-intragastric inoculation of infant mice leads to systemic spread, lethality, or long-term colonization of the gastrointestinal tract (1, 5). These characteristics make mice a useful model for the study of gastrointestinal and systemic candidosis. In a separate report, we describe the use of mice with persistent gastrointestinal infections, initiated when the animals were infants, as a model for studying the role of compromising agents and procedures in exacerbating candidosis (3). This report describes the use of persistently infected mice as a model for studying the efficacy of anticanid agents.

**MATERIALS AND METHODS**

**Animals.** CFW mice for a breeding colony were purchased from Charles River Farms, Wilmington, Mass. Offspring of the animals, raised in the University of Texas Small Animals Facility, were used in all experiments. Food (Wayne Lab Blox, Allied Mills, Chicago, Ill.) and water for the adult mice were available ad libitum.

**Organism.** *C. albicans* CA 30 was obtained originally from D. G. Ahearn, Department of Biology, Georgia State University, Atlanta. Before use, the organism was passed four times through infant mice by repeated isolation from the livers of animals challenged by the oral-intragastric route. Stock cultures of the passed organism were preserved by lyophilization or freezing at -70°C.

**Challenge procedure.** Six-day-old mice were pooled to assure random distribution and then fasted for 6 to 8 h. *C. albicans* was grown on Sabouraud dextrose agar (Difco Laboratories, Detroit, Mich.) slants for 24 h at 37°C. Organisms were harvested with sterile nonpyrogenic saline (Travenol Laboratories, Deerfield, Ill.). The animals were challenged by the oral-intragastric route as described by Guentzel and Berry (2) with approximately 10^6 colony-forming units contained in 0.05 ml. The actual inoculum was determined by dilution plating on Sabouraud dextrose agar. The mice were returned to the mothers without regard for parentage. Approximately 50% of the animals survived the challenge dose. The surviving animals, who retained high levels of *C. albicans* in the gastrointestinal tract, were used in the experiments on persistence and antifungal therapy.

**Enumeration of organisms.** Mice were sacrificed by decapitation and dissected under aseptic conditions. Kidneys, spleen, liver, stomach, and the whole intestine were removed in that order and homogenized in volumes of sterile saline ranging from 2 to 5 ml depending on organ size. Dilutions of the homogenates were plated to Sabouraud dextrose agar containing 50 µg of chloramphenicol per ml. The numbers of *C. albicans* were counted after incubation for 48 h at 37°C. Indigenous yeasts were often present in homogenates of the gastrointestinal tract of older animals. However, they did not complicate enumeration of *C. albicans* because they had a smaller colony size and a colony morphology that was distinctly different from *C. albicans* CA 30. The plates were generally free of bacterial growth.

**Antifungal therapy.** *C. albicans* readily persists in the gastrointestinal tract of mice that survive the oral-intragastric challenge as infants (1, 5). The use of persistently infected mice as a model for the study of antifungal drugs was investigated. The efficacy of single therapy with amphotericin B (AMB), 5-fluorocytosine (5-FC), and miconazole, or a combination of AMB and 5-FC, was determined. The different drug
activities were assessed ad seriatim. In all cases, infected animals were mixed to assure random distri-
bution before separation into groups for controls and
for drug treatment. A 2-week drug treatment, as
described, was initiated when the mice were 15 to 19
days old (i.e., 9 to 13 days post-inoculation). The
following drugs were used: AMB (Fungizone, intrave-
nous, E. R. Squibb & Sons, Inc., Princeton, N.J.), 5-
FC (Ancobon, Roche Diagnostics, Div. Hoffmann-La
Roche, Inc., Nutley, N.J.), and miconazole (Monistat,
intravenous, Ortho Pharmaceutical Corp., Raritan,
N.J.).

Statistical analysis. The Mann-Whitney test was
used to determine whether the levels of C. albicans in
the stomach and intestinal tract were significantly
different in control and drug-treated animals. Data
were analyzed on an IBM model 370 computer, using a
Minitab package. Results were considered significant
at the 95% confidence level.

RESULTS

Persistence of C. albicans in mice. Infant mice
are most susceptible to candidosis, after oral-
intragastric inoculation, when approximately 6
days old (3). Heavy colonization of long dura-
tion was desirable for studies of antifungal drugs
in mice to permit treatment periods of at least 2
weeks. We determined the duration of coloniza-
tion by C. albicans CA 30 in mice. This permitted
selection of a time period for treatment and
for sacrificing the drug-treated animals when the
majority of control littermates were heavily col-
onized.

Mice, challenged when 6 days old, were sacri-
ficed at different ages, and the numbers of C.
albicans in the body organs were determined.
The levels of the organism in mice at three
different ages are shown in Table 1. All of the
animals retained C. albicans in the stomach and
intestinal tract at 30 days of age (i.e., 24 days
post-inoculation). Approximately 50% of the
littermates remained colonized 30 days later (54
days post-inoculation). However, organisms were
present at lower levels in the stomach and
intestines in these animals compared with the
30-day-old mice. Therefore, the persistently in-
fected mice were treated when they were 15 to
19 days old for a 2-week period. C. albicans was
absent from the extraintestinal sites (Table 1)
except in a few animals with low levels of the
organism in the liver or kidneys.

Effects of antifungal drugs. We examined the
possible utility of this mouse model, in which C.
albicans persists in the gastrointestinal tract, for
studies of antifungal drugs (Table 2). With the
exception of miconazole, the treatments studied
reduced the numbers of animals positive for C.
albicans in the stomach and intestine. Statistical
analyses were run on data for individual levels of
C. albicans in all control and drug-treated ani-
mal within each test group to determine whether
the effects of the treatments were significant.
However, the data for the different treatments
were not compared since they represented sepa-
rate experiments and single-drug dosages. The
effects of separate treatments with AMB and
miconazole were significant: AMB stomach (P
< 0.000075) and intestine (P = 0.0001); micona-
zeole stomach (P = 0.01) and intestine (P
< 0.025). Levels of significance comparing control
animals with those treated separately with 5-FC
or a combination of 5-FC and AMB were respec-
tively: 5-FC stomach (P < 0.05) and intestine (P
= 0.055); 5-FC and AMB stomach (P < 0.005)
and intestine (P = 0.0025).

DISCUSSION

The results of this investigation suggest that the
persistent gastrointestinal infection that oc-
curs in infant mouse survivors of oral-intra-
gastric inoculation with C. albicans provides a
potentially useful model for studies of the effica-
cy of antifungal agents. Harsh compromising
treatment of the animals before or after inocula-
tion is not required to establish the persistent
infection (Table 1). This contrasts with previous
failures to establish C. albicans in the gut of
adult conventional mice without compromising

| Table 1. Persistence of C. albicans in mice with infections initiated in infancya |
|---------------------------------|---------------------------------|---------------------------------|
| **Organ**                      | **20 Days**                     | **30 Days**                     | **60 Days**                     |
|                                | **Mean log10 CFU**              | **Mean log10 CFU**              | **Mean log10 CFU**              |
|                                | **positive/ total**             | **positives (±SD)**             | **positive/ total**             | **positives (±SD)**             | **positive/ total**             | **positives (±SD)**             |
| Liver                          | 2/6                            | 0.48 (±0.0)                     | 0/17                           | 0.0                           | 2/14                           | 1.04 (±0.37)                    |
| Spleen                         | 0/6                            | 0.0                             | 0/17                           | 0.0                           | 0/14                           | 0.0                             |
| Kidneys                        | 1/6                            | 0.48 (±0.0)                     | 1/17                           | 0.78 (±0.0)                   | 2/14                           | 0.78 (±0.0)                     |
| Stomach                        | 6/6                            | 5.23 (±0.38)                    | 17/17                          | 4.54 (±1.07)                  | 7/14                           | 2.04 (±0.99)                    |
| Intestines                     | 6/6                            | 4.82 (±0.92)                    | 17/17                          | 3.96 (±1.29)                  | 8/14                           | 2.25 (±1.37)                    |

* Inoculated at 6 days of age.
* Age of mice at examination.
* CFU, Colony-forming units.
treatments (4, 6). Short-term therapy with three antifungal drugs in current use significantly reduced colony counts in gastrointestinal organ homogenates of the mice (Table 2). The concentrations of antifungals in milligrams per kilogram of body weight used for this study were approximately the same as used for humans over a similar period of treatment. However, the frequencies and route (intraperitoneal) of administration differed from that normally used in humans (intravenous for AMB and miconazole and oral for 5-FC). Intraperitoneal administration was the most practical for all of the drugs in this study due to the small size of the animals. Smaller, more frequent dosages of the drugs, particularly of 5-FC, may have improved the efficacy of the treatments.

*C. albicans* was essentially cleared from extraintestinal intraabdominal organs (liver, kidneys, and spleen) in survivors of oral-intragastric challenge examined at 20 days of age or later (Table 1). Therefore, the animals examined in this study do not provide a model for antifungal therapy in disseminated infection. However, treatment of persistently infected animals with certain compromising agents leads to pronounced tissue invasion and dissemination of the organisms from the gastrointestinal tract (3). The use of such animals as models for antifungal therapy in disseminated candidosis is being investigated.

### TABLE 2. Effect of antifungal therapy on gastrointestinal counts of *C. albicans*

<table>
<thead>
<tr>
<th>Expt</th>
<th>Therapy</th>
<th>No. positive/total</th>
<th>Mean log$_{10}$ CFU$^a$ for positives (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Stomach Intestine</td>
<td>Stomach Intestine</td>
</tr>
<tr>
<td>A</td>
<td>Control</td>
<td>12/14</td>
<td>4.18 (±0.89)</td>
</tr>
<tr>
<td></td>
<td>AMB$^b$</td>
<td>6/12</td>
<td>2.32 (±0.58)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13/14</td>
<td>3.34 (±0.83)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2/12</td>
<td>1.68 (±0.60)</td>
</tr>
<tr>
<td>B</td>
<td>Control</td>
<td>5/6</td>
<td>4.25 (±0.92)</td>
</tr>
<tr>
<td></td>
<td>5-FC$^c$</td>
<td>5/6</td>
<td>3.31 (±0.64)</td>
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<tr>
<td></td>
<td></td>
<td>2/6</td>
<td>3.52 (±0.36)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2/6</td>
<td>3.03 (±0.15)</td>
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<tr>
<td>C</td>
<td>Control</td>
<td>6/6</td>
<td>4.54 (±0.74)</td>
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<tr>
<td></td>
<td>Miconazole$^d$</td>
<td>6/6</td>
<td>3.97 (±0.75)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6/6</td>
<td>3.38 (±0.42)</td>
</tr>
<tr>
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<td></td>
<td>6/6</td>
<td>3.00 (±0.49)</td>
</tr>
<tr>
<td>D</td>
<td>Control</td>
<td>6/6</td>
<td>4.89 (±1.06)</td>
</tr>
<tr>
<td></td>
<td>AMB plus 5-FC$^e$</td>
<td>3/6</td>
<td>4.49 (±0.84)</td>
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<tr>
<td></td>
<td></td>
<td>6/6</td>
<td>1.76 (±0.99)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2/6</td>
<td>1.53 (±0.08)</td>
</tr>
</tbody>
</table>

$^a$ CFU, Colony-forming units.
$^b$ Three times weekly, 3 mg/kg, intraperitoneally.
$^c$ Five times weekly, 100 mg/kg, intraperitoneally.
$^d$ Three times weekly, 100 mg/kg, intraperitoneally.
$^e$ Three times weekly, 3 mg of AMB per kg and 100 mg of 5-FC per kg, intraperitoneally.

### ACKNOWLEDGMENTS

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### LITERATURE CITED