Experimental Urinary Tract Infection in Rats Caused by Candida albicans

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The increased clinical use of broad-spectrum antibiotics, immunosuppressives, and anti-inflammatory agents is thought to have contributed to the increased incidence of renal infections due to Candida albicans. To aid in the search for improved antifungal agents, we developed an experimental model in which C. albicans is injected into the medulla of the surgically exposed kidney of the rat. The ensuing infection is restricted to the urinary tract and can be treated successfully with amphotericin B. This model promises to be of merit in the evaluation of agents potentially useful in treating candidiasis of the urinary tract.

Although some 80% of urinary tract infections in man are caused by Escherichia coli and most of the remainder are accounted for by Proteus sp. and enterococci (2), Candida albicans has been isolated from urine with increasing frequency (1). The increasing incidence of urinary tract candidiasis (3, 4, 9, 10, 13) is often a consequence of the alteration of host resistance, especially after therapy with immunosuppressive agents, anti-inflammatory drugs, or broad-spectrum antibiotics (10).

Few experimental models of a urinary tract infection due to C. albicans have been reported (5, 6, 8), and for none of these has there been an effective control drug. This paper describes a model urinary tract infection in rats, caused by C. albicans and controlled by the oral administration of amphotericin B, which can be useful in evaluating new antifungal agents.

MATERIALS AND METHODS

Animals. Male, Holtzman rats (Holtzman Co., Madison, Wis.) weighing 200 to 220 g were housed individually. Water and antibiotic-free food (Rockland mouse/rat diet; TEKLAB Inc., Monmouth, Ill.) were provided ad libitum.

Inoculum. C. albicans SC (Squibb culture) 5314 was grown for 18 h at 37 C in Squibb F-4 broth medium (tryptone, 5 g; malt extract, 3 g; yeast extract, 3 g; glucose, 10 g; and distilled water to 1 liter). The challenge inoculum was 0.05 ml of this culture, which contained about 2 x 10^6 cells.

Infection. The animals were anesthetized with ether and the operative site was clipped of hair. The right kidney was exposed via the retroperitoneal approach, with the use of aseptic surgical technique. The inoculum was delivered into the medulla through a 27-gauge needle, and the wound was closed by means of stainless-steel clips (Clay Adams 9-mm surgical clips).

Therapy. A suitable therapeutic regimen for amphotericin B was determined by administering different oral doses of the compound daily to untreated normal rats for as long as 21 days. The sera and urine of these animals were collected at specified intervals and tested for amphotericin B activity by means of an agar diffusion assay, with Paecilomyces variotii as the test organism. Blood urea nitrogen (BUN) was determined at 3, 7, 14, and 21 days.

When used as the control drug in the experimental infection, amphotericin B was administered by gavage in a dose of 25 mg/kg, beginning 4 h after infection and continuing daily for three cycles of 6 consecutive days each. A 1-day rest period intervened before the cycle was repeated, for a total of 18 days of treatment.

Microbiology and pathology. Animals were divided into three groups: infected, infected-treated, and sham-infected. Animals in the first two groups were infected with 2 x 10^6 cells of C. albicans in the right kidney, and the infected-treated animals were given amphotericin B, 25 mg/kg, once daily by the oral route. Sham-infected animals were given an injection of sterile culture medium in the right kidney. At least three rats from each group were necropsied on days 3, 7, 14, and 21 after infection. All major organs except the brain were examined for gross signs of pathological changes. The blood, peritoneum, urine, and both kidneys were examined microbiologically. Immediately preceding necropsy, blood was obtained aseptically from anesthetized animals by percutaneous cardiac puncture. Samples from the peritoneal cavity were obtained for culture by rubbing a sterile swab moistened with saline over the peritoneal surfaces; urine was collected by direct aspiration of the surgically exposed bladder; both...
kidneys were hemisected sagitally and the cut surfaces were cultured by the imprint method. Where cell counts are reported, the organs were homogenized in 0.85% sodium chloride solution and plate counts were made of appropriate dilutions. All samples, except blood, were placed on Mycosel agar (BBL) and incubated at 37°C for 48 h. Blood samples were cultivated overnight in F-4 broth medium prior to being streaked on Mycosel agar.

For microscopy examination, kidneys, cut as above, were imprinted on glass slides. These slides were fixed with methanol and stained with Gram stain. BUN. BUN was determined spectrophotometrically for sera separated from blood immediately after the cardiac puncture. The Harleco BUN kit was employed and the reaction was read in a Gilford model 300N spectrophotometer.

RESULTS

Experiments to determine a suitable therapeutic regimen for amphotericin B showed that 6 h after an initial oral dose of amphotericin B (25 mg/kg) the serum concentration reached 0.05 μg/ml (Fig. 1). On this dosage schedule, the serum concentration remained consistently above this level until the termination of the experiment. The mean concentration of amphotericin B in the 24-h urine sample was 0.5 μg/ml as determined by an agar diffusion assay. Thus, a dosage of 25 mg per kg per day was chosen in the experimental infection, since it resulted in serum and urine levels of amphotericin B consistently in excess of the concentration expected to be inhibitory for the organism used in the model infection. The BUN values obtained for normal animals receiving 25 mg of amphotericin B per kg per day were consistently within normal limits (23.4 ± 3.4 mg/100 ml), as compared with those for normal untreated controls (23.8 ± 3.0 mg/100 ml). This dosage had no apparent effect on renal function.

The results of studies in which the urine and kidneys from infected animals and treated controls were cultured at 3, 7, 14, and 21 days after infection are summarized in Table 1.

Amphotericin B reduced the incidence of infection of the right kidney from 93 to 69% by day 3, after only two treatments. The number of culturally positive right kidneys grew progressively lower as additional treatments were administered until, by day 21, Candida could no longer be recovered. The contralateral kidney showed an infection rate on day 3 only half that of the right kidney. In addition, it was more responsive to therapy, becoming culturally negative for Candida as early as day 7.

Although the data already referred to in Table 1 present qualitative evidence that amphotericin B decreased the infection, enumeration of the Candida cells in the infected kidney at various times after infection provided quantitative confirmation (Fig. 2). In the untreated groups, the cell population decreased from 2 × 10^4 to 1 × 10^4 cells/g (wet weight) of kidney between the time of infection and day 3. Growth of Candida in the kidneys of these untreated animals, however, continued from day 3 onward, until a population of about 10^7 cells had been reached in the right kidney by day 21. Similar measurements made on the right kidney of treated rats showed a marked decrease in the population of Candida from 10^4 on day 3 to fewer than 10^2 cells by day 21.

Pathological signs increased in severity, progressing from a few well-isolated necrotic foci extending from the medulla to the cortex early in the infection to large confluent necrotic areas 2 to 3 weeks later. The right kidney was more frequently affected than was the left. The urinary bladder remained essentially normal in gross appearance throughout the experimental period.

Examination of the affected kidney generally revealed masses of pseudohyphae, concentrated chiefly in the renal pelvis and calices, that frequently extended to the proximal portion of the ureter and caused obstruction. When this obstruction occurred, the renal pelvis was grossly distended by masses of intertwining mycelial elements. The presence
of necrosis and obstruction was in marked contrast to the condition of treated animals, which rarely exhibited pathological changes.

DISCUSSION

Rats are known to be refractory to the induction of infections, and numerous efforts have been made by many investigators to compromise the animal by a variety of means prior to infection. Attempts to develop a urinary tract infection with Candida by injecting the organism directly into the surgically exposed bladder of rats, as reported by Hatala et al. (5), were unsuccessful. Placing the animals under a variety of stress conditions prior to challenge also failed to yield suitable infection rates. Extensive studies conducted in this laboratory have established that 10⁶ to 2 x 10⁶ viable units of Candida, injected directly into the renal medulla, are required to produce a high (85 to 100%) rate of infection. The infected process remains limited to the kidneys and the urinary tract of all animals throughout the experimental period. Although infection of the experimental tract by Candida was considered to be secondary to a generalized Candida sepsis, as reported by Hatala et al. (5), were unsuccessful. Placing the animals under a variety of stress conditions prior to challenge also failed to yield suitable infection rates. Extensive studies conducted in this laboratory have established that 10⁶ to 2 x 10⁶ viable units of Candida, injected directly into the renal medulla, are required to produce a high (85 to 100%) rate of infection. The infected process remains limited to the kidneys and the urinary tract of all animals throughout the experimental period. Although infection of the experimental tract by Candida was considered to be secondary to a generalized Candida sepsis, recent reports have stated that a candidal

Table 1. Cultures of urine and kidneys of rats positive for Candida at various periods after infection

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Percentage of cultures positive*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 days*</td>
</tr>
<tr>
<td></td>
<td>Urine</td>
</tr>
<tr>
<td>Sham-infected</td>
<td>0 (0/4)</td>
</tr>
<tr>
<td>Infected</td>
<td>86 (24/28)</td>
</tr>
<tr>
<td>Infected-treated</td>
<td>13 (2/15)</td>
</tr>
</tbody>
</table>

* Number positive/total number tested is given in parentheses.
* Days after infection.
* Right kidney.
* Left kidney.

Fig. 2. Number of viable units/g wet weight of kidney cultured at various periods after infection.
urinary tract infection can be present without demonstrable involvement of other organs (9).

In addition to the presence of Candida in the urine and kidneys of infected animals, the occurrence of masses of pseudomycelium and necrotic debris within the renal pelvis, and often in the ureter, causing obstruction, is similar to the conditions found in human renal candidiasis (9, 10, 13).

The mechanism by which urinary tract candidiasis occurs is not definitely known, "but it may be a retrograde extension from the urethra, proliferation of fungal organisms already present in the bladder or by hematogenous dissemination to the genito-urinary tract from another area" (11). In our studies in rats, the ascending route of infection is suggested, since attempts to isolate the organism from the blood, as well as the peritoneum, were consistently unsuccessful.

The contralateral kidney was presumably infected by reflux of urine, which is known to occur in rats (7). The presence of reflux is one of the factors that has been reported to perpetuate and aggravate an infection in the urinary system (12). It is not surprising that the left kidney, not having been traumatized by surgery nor infected by direct injection into the medulla, showed an infection rate only half that of the right kidney.

In an attempt to ascertain the extent of renal damage caused by the candidal infection and any ameliorating effects of therapy, BUN was determined for both infected and treated-control rats. The BUN was elevated (96.1 ± 16.3 mg/100 ml) only in infected animals that showed extensive damage to the kidney, evident also by gross examination at necropsy. Treated and sham-operated control rats had BUN values within normal limits throughout the experiment. However, since alteration of BUN in the rat is apparent only when both kidneys have been damaged severely, it is unlikely that this parameter will prove useful in following the course of the infection or the effect of therapy. In contrast, in man the BUN can be a meaningful indicator of renal damage.

Oral administration of amphotericin B was used, not only because it is infinitely easier than intravenous administration in a study such as this, but also because effective levels of the drug can be attained by this route in the rat.

We conclude that C. albicans infection of the urinary tract of the rat can be established and controlled with antibiotic therapy and that the therapeutic efficacy of test compounds can be accurately assessed by determining the percentage of kidneys positive for Candida in the infected versus the treated group. This technique qualifies as a primary screening test for new antifungal agents. A quantitative examination, i.e., a comparison of the numbers of organisms in the infected kidneys of the two groups of rats, in addition, may be useful in evaluating chemotherapeutic agents nearly equivalent in activity.

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LITERATURE CITED