Establishing an Ascending Urinary Tract Candidiasis Infection in Swiss Webster Mice

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Introduction:

Clinical studies have shown that Candida spp., especially Candida albicans, cause up to 13.7% of urinary tract infections in patients having a urinary catheter. These infections are more common among women, elderly patients, and the critically ill (Puri et al., 2002) as well as those receiving broad spectrum antibiotics (Guglielmo et al., 1994). As reported by Hsu et al. (1990), in a clinical setting, one of the most effective treatments for a bladder yeast infection is amphotericin B irrigation of the bladder using a catheter. One clinical study showed clearance of yeast in 72.3% of patients after just two daily amphotericin B deoxycholate treatments (Hsu et al. 1969). Intravenous amphotericin B deoxycholate and oral fluconazole treatments have also been shown to be successful in treating C. albicans bladder infections. In comparison, intravenous echinocandins do not get eliminated through the bladder in high enough concentrations to be a very effective treatment (Malani and Kauffman, 2007).

Given that AmBisome® (liposomal amphotericin B) is as effective against systemic fungal infections as amphotericin B deoxycholate but is significantly less toxic (Proffitt et al., 1991), this liposomal antifungal drug may be another option for treating bladder infections. To evaluate the role of AmBisome in this infection, additional preclinical studies are needed since the only published work in this area was done in rats with urinary candidiasis. This rat study was done to examine the pathogenesis of Candida albicans in ascending urinary tract infections and was not used to evaluate drug treatments (Tokunaga et al., 1993).
The focus of our study was to develop a mouse model of ascending urinary tract candidiasis and to begin to evaluate AmBisome's efficacy in this model. We did a series of experiments to establish a model for ascending urinary tract candidiasis in immunosuppressed mice that could be used to evaluate AmBisome using intravenous and/or irrigation treatment or treatment with other antifungal drugs. The pathogen, *C. albicans*, was selected for these experiments since it is such a common cause of bladder infections.

**Methods:**

Ketamine (80mg/kg)/Xylazine (16mg/kg) sedated Swiss Webster female mice were challenged with *C. albicans* via intraurinary catheterization. In study one all mice were immunosuppressed (IS) with 75mg/kg of cyclophosphamide intraperitoneally 3 days prior to challenge and then challenged with 50µL 1.8X10e5 cells of *C. albicans*. Tissues were collected from subgroups of mice each day (n=5/day) up to day +5 to follow the course of the infection by monitoring fungal burden in the tissues. In study two, eight groups of mice (n=10/group) were IS day -3. The mice were challenged with 50µL 7.5X10e5 cells of *C. albicans* at Day 0. Mice in groups 1-7 were treated once per day from day+1 to day +3. Mice in groups 1 and 2 received AmBisome intravenously (IV) at 3mg/kg and 5mg/kg, respectively. Group 3 received Fungizone 0.75mg/kg IV (highest tolerated dose of Fungizone given IV). Groups 4 and 5 received a catheter lavage with 50µL of a 20mg/mL solution where group 4 received AmBisome (4mg) and group 5 received Fungizone (5mg). Groups 6, 7, and 8 were 5% dextrose in water (D5W) IV, D5W lavage and untreated controls, respectively. All of the mice were sacrificed day +4. In both studies, bladder, liver, spleen, and kidneys were collected, homogenized and plated on Sabouraud's Dextrose agar to determine fungal burden (Colony Forming Units, CFU/g tissue).

**Results:**

In study one, which was done to characterize the ascending urinary infection model, on day 1, and the highest fungal burden was in the kidneys (2.6 X 10e5 CFU/gram). The average infection in the bladder and spleen (2.5 X 10e2 CFU/g for both organs) and liver (2.9 X 10e2 CFU/g) was lower than in the kidneys. The average fungal burden from day +2 to day +4 in the bladder and kidneys was higher (average=2.9 X 10e3 CFU/g and 5.4 X 10e4 CFU/g, respectively) than in the liver and spleen (average=1.9 X 10e1 CFU/g, and 3.8 X 10e1 CFU/g, respectively). By day +5, there was no detectable yeast in the liver and spleen, however, the yeast infection was still present in the bladder and kidneys (average= 1.1 X 10e3 CFU/g and 5.1 X 10e4 CFU/g, respectively). Thus, we had determined that we could
establish an ascending urinary tract infection with *C. albicans* in the Swiss Webster female mice that could be used for subsequent testing of antifungal drugs.

In study 2, tissues were collected at day 4 since the results from study 1 indicated that at this time, the infection would be present in both the bladder and the kidneys but would not be present in any of the other organs demonstrating that it was a localized infection. On day 4 in study 2 there was no detectable fungi in the bladder of any of the treated groups that received AmBisome or Fungizone. The control groups which included the D5W lavage, D5W IV and untreated control all had significant amounts of fungi in the bladder (average= 2.3X10ex4 CFU/g, 3.3X10ex4CFU/g, and 3.2X10ex4 CFU/g respectively). In the kidneys, in the control groups, the average CFU/g (average = D5W LAV 3.9X 10ex5 CFU/g, D5W IV 1.8X10ex5 CFU/g, and Untreated Control 2.5X10ex5 CFU/g) were markedly higher (10 to 100-fold) than in the treatment groups (average= AmBisome 3mg/kg IV 2.3X10e3 CFU/g, AmBisome 5mg/kg IV 9.5X10ex3 CFU/g, Fungizone 0.75mg/kg IV 9.8X 10ex3 CFU/g, AmBisome 4mg LAV 3.2X10ex4 CFU/g, and Fungizone 4mg LAV 2.0X10ex3 CFU/g).

**Conclusions:**

We demonstrated for the first time the establishment of a *C. albicans* ascending urinary tract infection in immunosuppressed Swiss-Webster female mice with localization primarily in the kidneys and bladder on day 4 post-challenge. Using this model, we also showed that treatment with AmBisome or Fungizone IV or via LAV resulted in clearance of the infection from the bladder. Residual infection in the kidneys was still present even with these treatments although the fungal load was reduced 10 to 100-fold compared to the control groups of mice.
Study 1, Developing the model

Day 1 post challenge with 1.8 $\times$ 10$^{5}$ C. albicans CP620 in Swiss Webster cyclophosphamide suppressed mice. 5 mice sacrificed each day.

Day 2 post challenge with 1.8 $\times$ 10$^{5}$ C. albicans CP620 in Swiss Webster cyclophosphamide suppressed mice.

Day 3 post challenge with 1.8 $\times$ 10$^{5}$ C. albicans CP620 in Swiss Webster cyclophosphamide suppressed mice.

Day 4 post challenge with 1.8 $\times$ 10$^{5}$ C. albicans CP620 in Swiss Webster cyclophosphamide suppressed mice.

Day 5 post challenge with 1.8 $\times$ 10$^{5}$ C. albicans CP620 in Swiss Webster cyclophosphamide suppressed mice. 5 mice sacrificed each day.
Study 2: AmBisome versus Amphotericin B deoxycholate administered Intravenously (IV) or by Catheter lavage (LAV)

Bladder
Cyclophosphamide immunosuppressed Swiss Webster female mice challenged with 7.5E5 cells via intrauterine catheterization, treated every day for 3 days, and sacrificed day +4 for tissue collection

Kidney
Cyclophosphamide immunosuppressed Swiss Webster female mice challenged with 7.5E5 cells via intrauterine catheterization, treated every day for 3 days, and sacrificed day +4 for tissue collection
Works cited:


