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Renal candidiasis in the rat: effects of ureteral obstruction and diabetes

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Summary. The effect of ureteral obstruction on the course of renal candidiasis in a rat model was studied, using both normal and diabetic Sprague-Dawley rats, and a clinical isolate of *Candida albicans.* Diabetes was induced by streptozotocin injection 1 week prior to inoculation and transabdominal ligation of the left ureter. On day 9 post inoculation, mean titers of *Candida* were similar in right and left kidneys of obstructed rats. Mean left renal titers for obstructed and control rats were similar ($\log_{10}$ 2.68 CFU/g $\pm$ 0.73 (SE) vs. $\log_{10}$ 2.21 $\pm$ 0.09, $P > 0.01$). Diabetes produced higher renal titers of *Candida,* regardless of the presence of ureteral obstruction ($\log_{10}$ 5.74 CFU/g $\pm$ 0.57 (SE) vs. $\log_{10}$ 2.21 $\pm$ 0.09, $P < 0.01$). Animals treated for one week with amphotericin B showed a marked difference in *Candida* titers between obstructed and control animals ($\log_{10}$ 4.14 CFU/g $\pm$ 0.45 (SE) vs. 1.57 $\pm$ 0.38) for both kidneys, and between obstructed and nonobstructed kidneys in the same animals.

Key words: Candidiasis – Ureteral obstruction – Diabetes

Introduction

The incidence of infections due to *Candida albicans* and other fungi has increased markedly in recent decades [3, 5, 8]. Factors that predispose to systemic fungal infection include broad spectrum antibiotic therapy, immunosuppressive drugs, neutropenia, and the acquired immunodeficiency syndrome (AIDS). *C. albicans* is the most prevalent of the fungi affecting the genitourinary system [9]. Renal involvement occurs in up to 90% of patients with candidal septicemia [5]. Candidiasis may obstruct the urinary tract with fungus balls, or may occur as a complication of pre-existing obstruction to urine flow.

In the rat some type of damage to a kidney, such as hydronephrosis secondary to ureteral ligation, is necessary to establish bacterial renal parenchymal infections by either the hematogenous or ascending route [2]. Diabetic hyperglycemia also enhances hematogenous renal infection with both bacteria and *Candida* [4]. Levison has shown that renal infections as part of a systemic candidal infection can be produced by intravenous inoculation of the normal rat, and that diabetes induced with alloxan increases the severity of such infection [6]. The purpose of this study was to determine whether unilateral ureteral obstruction alone or in association with diabetes mellitus increased the severity of renal or systemic infection when *C. albicans* was injected intravenously in the rat, and whether unilateral ureteral obstruction decreased the response to standard therapy with amphotericin B.

Materials and methods

Outbred male Sprague-Dawley rats, 225–250 g, were housed in groups of 5–7 per cage with free access to water and rodent chow (Wayne). After 7 days acclimatization the rats were randomly assigned to experimental groups: diabetic obstructed (15 rats) and non-obstructed (13), non-diabetic obstructed (9) and non-obstructed (8). On day 0, after an overnight fast, all groups were injected intraperitoneally with either streptozotocin diluted in citrate-saline 65 milligrams per kilogram (mg/kg) or citrate-saline suspension alone. Daily urine glucose and ketone determinations were made with Keto-Diastix (Ames Co., Elkhart, IN) and serum glucose levels were determined after sacrifice using a spectrophotometric assay. During the experiment animals with weight loss > 15 g/24 h or ketones in the urine received 0.4 units protamine-zinc insulin (Eli, Lilly, Inc., Indianapolis, IN) subcutaneously per day.

A clinical isolate of *C. albicans* stored at −70° centigrade (C) in 1 ml aliquots was subcultured on Sabouraud-dextrose agar plates (Difco) 48 h prior to inoculation and incubated at 37°C. The original unpassaged isolate had been stored at −70°C several months at the time of the experiment and was subcultured fresh for this inoculation. This isolate and others similarly stored have been used for many experiments, and compared to passaged organisms under the same

Table 1. Comparison of mean kidney and liver *Candida* titers ($\log_{10}$ CFU/g tissue ± SE) four groups of rats. RK vs. LK within each groups NS differences between groups are similar whether comparing RK or LK both horizontally, vertically, and diagonally

nondiabetic nonobstructed	($N=8$)		nondiabetic obstructed	($N=6$)		
RK	LK	L	RK	LK	L	
2.33 ± 0.45	2.21 ± 0.09	1.63	2.78 ± 0.25	2.68 ± 0.73	2.71	NS
diabetic nonobstructed	($N=9$)		diabetic obstructed	($N=12$)		
RK	LK	L	RK	LK	L	
5.70 ± 0.51	5.74 ± 0.57	4.24	4.95 ± 0.68	4.90 ± 0.64	4.13	NS
$P<0.01$			$P<0.05$			

RK = right kidney; LK = left kidney; L = liver; SE = standard error of the mean; NS = not significant; N = number in group

conditions, with no change in its virulence in the rat. A stock solution of 10^6 colony forming units (CFU)/ml was prepared the day of inoculation by suspending in 10 ml of non-bacteriostatic normal saline sufficient subcultured organisms to produce a turbid suspension when vortexed. The suspension was then adjusted to correspond to a McFarland standard of 0.5 which consistently produced a concentration of 10^6 CFU/ml. The stock concentration was verified by culturing serial tenfold dilutions of stock on Sabouraud-dextrose pour plates, counted after incubating at 37°C for 48 h. Inocula were prepared by serial dilutions of the stock suspension in sterile normal saline. On day 5 of this experiment all rats received 0.2 ml of a suspension containing 10^4 CFU/ml (1.68×10^4, 1.58×10^4, and 3.59×10^4 in replicate experiments) via lateral tail vein injection. On day 7 the animals in the two obstruction groups underwent transabdominal left ureteral ligation with 6-0 silk suture loosely tied under pentothal anesthesia. On day 14 all animals were sacrificed by pentothal injection. Serum was collected by decapitation from all animals for glucose determination. Both kidneys were excised under sterile conditions, decapsulated, and divided into halves. A slice from one half was preserved in formalin for subsequent histologic study. The remaining half of each kidney was weighed and homogenized in 1 ml of sterile saline. A segment of liver from each rat was similarly treated. Aliquots of 0.1 ml of undiluted homogenate and of two hundredfold dilutions of the homogenate were then cultured in duplicate on Sabouraud-dextrose agar pour plates inverted and incubated for 48 h at 37°C.

Histologic sections were stained with periodic acid Schiff stain and examined microscopically. Observations of acute and chronic inflammation, microabcess formation, fungus within the parenchyma or collecting system, and assessment of the degree of hydronephrosis were made by a blinded observer.

The CFU/g of tissue for each kidney and the liver from each rat was converted to $\log_{10}$ for statistical manipulation. This culture technique does not detect less than 10 CFU/g reliably, so all samples with 0 CFU/g were assigned a $\log_{10}=0$ corresponding to 1 CFU/g. The two-tailed Student's t test was applied to pairs of mean values (e.g. left vs right kidneys within a group, left kidneys or mean of both kidneys between two groups) to assess statistical significance of differences observed. A "p" value of <0.05 was considered to be statistically significant.

In another experiment rats were divided into two groups, one of which underwent left ureteral ligation at the time of inoculation. Both groups were inoculated with 5.8×10^5 CFU/ml *Candida,* treated with intraperitoneal amphotericin B 1 mg/kg/day (Fungizone intravenous; E. R. Squibb & Sons, Inc., Princeton, NJ, at a concentration of 0.2 mg/ml prepared fresh daily in a 5% dextrose solution) for seven days, and sacrificed. Differences between left and right kidneys of obstructed animals, and among both left and right kidneys of obstructed and non-obstructed groups were assessed.

Results

No differences were observed between the left and right kidney *Candida* titers in any group in the first experiment (Table 1). The differences in liver titers paralleled those seen among the kidneys; almost all liver cultures were positive indicating that animals in all groups had systemic infection of comparable severity. Comparisons were made among all the groups using the mean titer of the left and right kidneys combined, and using the left kidney only since the left ureter was ligated in the obstructed groups. The renal *Candida* titers in diabetic, nonobstructed rats were higher than those of nondiabetic obstructed or nonobstructed rats ($P<0.01$) and the titers of diabetic obstructed rats were higher than those of nondiabetic obstructed rats ($P<0.05$). No difference apeared between obstructed and nonobstructed animals in either the diabetic or control groups. Glucose levels performed on serum obtained at sacrifice confirmed the diabetic state where appropriate, although during the course of the experiment the presence of diabetes was established by weight loss and glycosuria. Normal rats have glucose levels below 150 mg/dl, while streptozotocin consistently produced glucose levels above 250 mg/dl. The variation in numbers of rats per group occured due to loss of animals from anesthetic and streptozotocin toxicity, untreated diabetes, and possibly candidiasis; animals dying prior to planned sacrifice were excluded from the analysis of results.

Table 2. Mean kidney colony counts ($\log_{10}$ CFU/g tissue) after 1 week of amphotericin B therapy (left kidney obstructed). Obstructed LK vs. control LK different at $P<0.01$; RK from two groups different at $P<0.05$

control	($N=10$)	obstructed	($N=12$)
LK	RK	LK	RK
1.57 ± 0.38	1.26 ± 0.30	4.14 ± 0.45	2.79 ± 0.39
NS		$P<0.05$	

In the second experiment (Table 2), after one week of amphotericin B the difference in *Candida* titers between the obstructed and non-obstructed kidneys within the obstructed group was 4.14 ± 0.45 (SE) CFU/g vs. 2.79 ± 0.39 CFU/g ($P<0.05$). The difference between left (obstructed) kidneys in the obstructed vs. control group was 4.14 ± 0.45 CFU/g vs. 1.57 ± 0.38 ($P<0.01$); that between right kidneys 2.79 ± 0.39 vs. 1.26 ± 0.30 ($P<0.05$). No difference existed between right and left kidneys in the non-obstructed group.

Histologic examination of the livers was unremarkable in all cases. The kidneys of the animals with high renal *Candida* titers ($>10^3$ CFU/g) showed parenchymal inflammation and occasional microabscesses, and fungus in the tubular lumen and collecting system, regardless of the presence of hydronephrosis. Only a few of the diabetic kidneys showed cortical lesions; most of the kidneys showed inflammation primarily in the medulla. Kidneys with low *Candida* titers were histologically normal.

Discussion

This study addressed the severity of infection and the response to antifungal therapy as measured by renal and hepatic *Candida* titers after a relatively short incubation, and the effect of diabetes and ureteral obstruction singly and in combination. We determined by intravenous pyelography that the kidney remained functional two weeks after ureteral ligation, although it became severely hydronephrotic. As indicated by the presence of positive cultures in all the controls, the inoculum used produced systemic infection in normal animals. Thus the model did not assess susceptibility, the risk of acquiring candidiasis, nor could the short course of the experiment evaluate spontaneous regression of infection. However, our experience with this model in experiments of longer duration had indicated that renal *Candida* titers after inocula of 10^4 CFU/ml increase with time. Whether the presence of ureteral obstruction would affect the risk of acquiring candidiasis from a small inoculum, or progressing from primary renal to systemic candidiasis, were not addressed in these studies.

Streptozotocin produced an extreme diabetic state that was lethal if untreated. All animals injected became diabetic which was not always the case with alloxan in our experience. In this short study animals were treated with insulin as required however, their diabetes remained uncontrolled. Any effect of diabetes on the course of the infection would thus be magnified and easily detected in a small group of animals. The effect was modified by insulin therapy by others [4], suggesting that it was in fact due to the diabetic state rather than to the drug itself. Our results in the diabetic group paralleled those reported using the alloxan diabetic mouse model [6].

The presence of unilateral ureteral obstruction produced no deleterious effect in the severity study in either diabetic or nondiabetic rats. Prior studies of ureteral obstruction showed increased susceptibility to gram negative bacterial infection with ureteral ligation [2, 7] and increased severity of *S. aureus* infection with ureteral ligation but not partial ureteral obstruction [7]. Like *S. aureus* [7], *C. albicans* was selectively pathogenic to the kidney [1, 6, 10], as both organisms infect the normal kidney hematogenously. This pathogenicity and the size of the inoculum might have masked an effect of obstruction. Although bloodborne gram negative bacilli will not infect a kidney whose ureter is ligated 48 h or more after inoculation [2], our ureteral ligation was done two days after inoculation because our preliminary work with this model showed that ligation more than 24 h prior to inoculation resulted in sterile cultures or very low colony counts in the obstructed kidney, presumably due to decreased blood flow and failure of the bloodborne organisms to reach that kidney. We also had no success infecting kidneys with pre-existing mild partial ureteral obstruction. In the absence of amphotericin therapy, no difference occurred between obstructed and control rats whether ligation was performed at the time of or 48 h after inoculation. Renal *Candida* counts increase between 0 und 7 weeks, and vary directly with inoculum size between 10^3 and 10^7 CFU/ml. Parenthetically, the use of different suture types (silk, nylon and prolene) did not affect counts since the suture remains outside the urinary tract.

Diabetes, however, did increase the severity of infection in both obstructed and nonobstructed groups, as reported previously using the alloxan diabetes model [6], providing another point of similarity between our model and previous ones. We had anticipated that the combination of two predisposing factors might unmask an effect of obstruction on severity which could not be observed in healthy animals. On the

contrary, the overwhelming infection seen in the uncontrolled diabetic rats would have obscured any effect of ureteral ligation. No synergy was found in this situation.

The treatment experiment suggests that ureteral obstruction made the infection more difficult to eradicate. A higher inoculum (10^5 CFU/ml) could be used in this experiment because no diabetic animals were included; the higher renal *Candida* titers achieved facilitated discrimination of differences between smaller groups of rats and obviated an untreated control group. The renal colony counts in the obstructed animals after one week of amphotericin (Table 2) were similar to those observed in untreated animals (data not shown), while in the control group the counts were much lower. Furthermore the colony counts in the unobstructed right kidneys were significantly higher in the animals with left ureteral ligation, suggesting that the presence of one obstructed kidney adversely affects antifungal therapy at the systemic level. Our previous experience had been that even after three weeks of therapy not all animals are free of *Candida,* and relapses occurred, as is the case with *Candida* infections in the human. Still, the one week treatment study provided preliminary evidence that the obstructed animals would require more intensive therapy, perhaps including removal of the obstructed organ.

This rat model would be useful for the study of other predisposing factors for *C. albicans* infection. As in humans, the kidney is preferentially involved, other organs are also affected (liver, spleen, gastrointestinal tract), and infection is reliably induced with convenient inocula. Unlike the mouse, which is suitable for acute studies because it succumbs rapidly to the disease [1], the rat develops a subacute pyelonephritis and systemic candidiasis. Its use would facilitate longer experiments to study ascending infection, other predisposing factors, efficacy of new therapeutic modalities, and the relationship between primary renal and systemic candidiasis which would further elucidate the pathogenesis of candidiasis and the role of the urinary tract as both a primary target of *Candida* infection and a route by which it may be acquired.

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