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Urological Research

Ventral Bladder Hernia Facilitates Study of Urinary Tract Infections in Rats

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Summary. Previous studies have demonstrated that after intravesical inoculation of Escherichia coli, rats drinking 5% glucose-water remained bacteriuric for up to 21 days while rats drinking tap water became abacteriuric within a few days. To facilitate accurate monitoring of bacteriuria, we created a ventral bladder hernia for percutaneous aspiration of urine. After intravesical inoculation with 10^8 E. coli, rats with ventral bladder hernias demonstrated clearance of bacteria at a rate comparable to that observed in rats with intrapelvic bladders ($p < 0.01$). Of rats drinking tap water, 6 of 7 (85%) with intrapelvic bladders and 8 of 9 (89%) with ventral hernias had less than 10 colony forming units per ml of urine within 9 days of inoculation. Of rats drinking 5% glucose-water, 4 of 5 (80%) with intrapelvic bladders and 6 of 8 (75%) with ventral bladder hernias had $>10^5$ colony forming units per ml of urine 9 days after inoculation. The results suggest that this technique does not alter the antibacterial response of control or polyuric rats to E. coli inoculated intravesically.

Key words: Urinary tract infection, Rats, Escherichia coli.

INTRODUCTION

The study of experimental ascending urinary tract infection has been greatly facilitated by the well-known model of Freedman (5, 8). When healthy mice or rats were allowed unlimited access to 5% glucose water, they responded with a markedly increased fluid intake and subsequent non-glycosuric polyuria. Intravesical inoculation of pathogenic E. coli resulted in bacteriuria for up to 21 days if the animals were maintained on oral glucose water; prompt clearance of bacteriuria resulted when plain tap water was substituted.

Our initial experience with this model, however, suggested that accurate daily urine cultures were impossible. Urine specimens obtained by stimulating the animal to void or by urethral catheterization were invariably contaminated with urethral or perineal organisms. Percutaneous aspiration of urine was impossible because the bladder, even when distended, remained deep in the pelvis. Because frequent, reliable cultures are essential for bacteriological monitoring of individual animals on a longitudinal basis, we modified Freedman's model surgically by creating a ventral bladder hernia. This modification permits safe, percutaneous apsiration of bladder urine with minimal risk of contaminating culture specimens or of introducing organisms into the urinary tract. The results of our study demonstrated that this technique did not alter the antibacterial response of control or polyuric rats to E. coli inoculated intravesically.

MATERIAL AND METHODS

Bacteria

The E. coli strain used in all experiments was isolated from bladder urine and serotyped (O18) according to the method of Brent and Vosti (4). The E. coli were grown in brain heart infusion broth (BHI, Difco, Detroit, Michigan) for 72 h, harvested by centrifugation, and washed once in phosphate buffered saline (PBS, Difco; pH 7.2). To minimise bacterial variation between experiments, the E. coli were suspended in BHI plus dimethyl sulphoxide (Fisher Scientific) at a final concentration of 5% (v/v) and frozen immediately in an acetone-dry ice bath. The frozen aliquots

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of bacteria were stored at -20°C in screw-capped tubes. When preparing bacteria for an experiment, an aliquot was thawed at 37°C, washed once in PBS, inoculated into yeast nitrogen base (YNB, Difco; pH 7) and incubated overnight at 37°C. The bacteria were harvested, washed once in PBS, and inoculated to an optical density (OD) of 0.03 at 540 mm (Coleman Spectrophotometer Model 44) into YNB. Log phase bacteria (OD = 0.15) were harvested, washed once in PBS, resuspended, and serially diluted in sterile 0.85% sodium chloride to a concentration of 10^9 colony forming units (CFU)/ml as determined by quantitative cultures. An inoculum of 10^8 bacteria suspended in 0.1 ml of 0.85% NaCl was used for all experiments. Cultures were performed by plating 0.1 ml of urine on blood agar and McConkey agar plates (Difco). Plates were incubated at 37°C for 48 h, the colonies were counted and the results recorded as CFU/ml.

Experimental Animals

Male albino Sprague-Dawley rats (Harlan-Sprague Dawley, Madison, Wisconsin) weighing 200-300 g were allowed unlimited access to Purina Rat Chow. The animals were divided into 2 groups: those with the bladder in its normal intrapelvic position and those with a ventral bladder hernia. Through a suprapubic midline incision, the subcutaneous tissue was bluntly dissected free of the rectus fascia laterally until the femoral vessels were visualized. The rectus was then divided in the midline and the bladder exposed. A stay suture of 6-0 chromic catgut served to anchor the dome of the bladder to the lateral subcutaneous tissue. Traction on the bladder was not excessive; in all cases the base of the bladder remained in its normal position within the pelvis. The rectus fascia was then approximated with a continuous suture of 4-0 chromic catgut, leaving an opening which allowed the bladder to slide easily yet did not allow herniation of intraperitoneal contents. Three additional sutures of 6-0 chromic catgut were placed to anchor the bladder superiorly, caudally and to the subcutaneous tissue immediately overlying the bladder ventrally. Care was taken to avoid placing any sutures within the lumen of the bladder where they might serve as a nidus for infection or stone. The skin was closed with a running suture of 4-0 chromic catgut, and animals were allowed 2 weeks recuperation before inoculation with bacteria.

Both operated and non-operated groups were further subdivided into those drinking tap water or 5% glucose water. The daily tap water intake for each rat was always less than 50 ml. Animals allowed unlimited access to glucose water generally drank more than 100 ml/day; if oral intake was below this amount, an animal was removed from the study, since Freedman has determined that this is the average amount required for maintenance of infection in the polyuric rat (5). Animals were started on glucose water 6 days prior to bacterial inoculation.

Bacterial Inoculation and Culture

Intrapelvic Bladder. Intramuscular methohexital sodium (Brevital, Eli Lilly, Indianapolis, Indiana) was used in a concentration of 6 mg/100 g animal weight to assure adequate anaesthesia, and the bladder was exposed through a suprapubic midline incision. Urine was aspirated through a 27-gauge needle for culture and to reduce the residual volume to approximately 0.2 ml. With a second syringe, 10^8 *E. coli* in 0.1 ml of 0.85% sodium chloride were introduced into the bladder. This left a total volume of approximately 0.3 ml in a bladder normally holding up to 1.5 ml, thereby minimizing the possibility that the rat would void upon awakening from anaesthesia. After inoculation, the bladder was left in its normal intrapelvic position and the wound was closed with 4-0 chromic catgut. At the end of an experiment, the animals were killed by decapitation, the bladder was exposed and urine was aspirated for culture.

Ventral Bladder Hernia. The skin overlying the easily palpable bladder was prepared with Betadine and alcohol. Urine was aspirated for culture and bacteria were introduced percutaneously through a 27-gauge needle using the technique outlined above for the intrapelvic bladder group.

Subsequent daily urine cultures were obtained by percutaneous aspiration. *E. coli* isolates were periodically serotyped to confirm that they were the same as the original inoculum.

Statistical Analysis

Logarithmic transformations of individual urine culture results were used to compute the mean values for different animal subgroups on specific days and these values were expressed as mean CFU/ml ± standard error. Different animal subgroups were compared using analysis of variance and Duncan's multiple range test (9).

RESULTS

Intrapelvic Bladder

Initial experiments were designed to determine the spontaneous rate of clearance of a standard bacterial inoculum in rats with bladders in the normal intrapelvic position and drinking tap wa-

Table 1. Effect of polyuria on clearance of intravesical inoculum of 10^8 E. coli in rats with intrapelvic bladders

Oral fluid	CFU/ml on specific post-inoculation day			
	3	5	7	9
Tap water	10^5	10^3	10^3	10^5
	500	10^3	10^3	0
	370	10^3	10^3	0
	330	130	210	0
	60	0	0	0
	20	0	0	0
	0	0	0	0
5% glucose water	10^5	10^5	10^5	10^5
	10^5	10^5	10^5	10^5
	10^5	10^5	10^4	10^5
	10^5	10^3	10^4	10^5
	10^4	0	10^3	0
	0		0	
	0			

0 signifies <10 CFU/ml

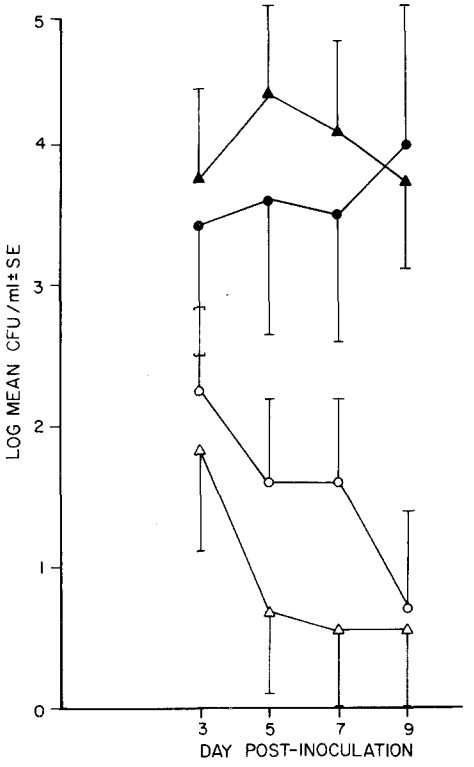

Fig. 1. Comparison of clearance of intravesical inoculum of 10^8 E. coli in rats with intrapelvic bladders or ventral bladder hernia and drinking tap water or 5% glucose water. o intrapelvic bladder/tap water; • intrapelvic bladder/glucose water; Δ ventral bladder hernia/tap water; ▲ ventral bladder hernia/glucose water

ter. Twenty-eight animals were sacrificed in groups on days 3, 5, 7 and 9 post-inoculation. Within 5 days of inoculation, cultures from 43% of the animals showed <10 CFU/ml and by day 9, 86% of the cultures had <10 CFU/ml (Table 1). The log mean CFU/ml for each group sacrificed decreased from 2.2 ± 0.6 on day 3 to 0.7 ± 0.7 by day 9 (Fig. 1).

The effect of polyuria on the clearance of bacteria from bladder urine was investigated in rats with intrapelvic bladders and unlimited access to 5% glucose water. Twenty-three animals were sacrificed in groups on days 3, 5, 7 and 9. At least 71% of the cultures obtained on any given day had 10^3 or more CFU/ml. The log mean CFU/ml for each post-inoculation day was at least 3.4 ± 1.0.

Ventral Bladder Hernia

To test the susceptibility of animals with ventral bladder hernias to urinary infection, 10^8 E. coli were inoculated intravesically into rats drinking tap water (9 animals) or 5% glucose water (8 animals). Suprapubic aspirated urine cultures were obtained daily for 9 days (Table 2). Of the 9 animals drinking tap water, 4 (44%) had sterile urine by day 2 and 8 (89%) had sterile urine by day 5. Bacteriuria decreased from a mean log CFU/ml of 1.8 ± 0.7 on day 3 to 0.6 ± 0.6 on day 9 (Fig. 1). Of the 8 animals drinking 5% glucose water, 7 (88%) had 10^3 or more CFU/ml for at least 6 days and 6 (75%) had 10^5 CFU/ml on day 9. The level of bacteriuria was maintained at a mean log CFU/ml of no less than 3.8 ± 0.6 for the entire 9 day period.

Statistical Analysis. Duncan's Multiple Range Test confirmed that the persistence of bacteriuria after inoculation was associated only with the drinking of glucose water and not with the surgical transposition of the bladder ($p<0.01$). When compared on specific post-inoculation days, this association was highly significant on day 9 ($p<0.01$), significant on days 7 and 5 ($p<0.05$) and not significant on day 3.

Gross dissection of bladders with ventral hernias demonstrated moderate adhesions to the rectus muscle and dermis. The ventral portion of the bladder appeared thickened, but histological sections showed only minimal hypertrophy when

Table 2. Effect of polyuria on clearance of intravesical inoculum of 10^8 E. coli in rats with ventral bladder hernias

Oral fluids	Animal number	Colony forming units/ml on specific post inoculation day 1	2	3	4	5	6	7	8	9
Tap water	1	0	10^3	20	10^4	0	0	0	0	0
	2	10^5	10^5	10^3	0	0	0	0	0	0
	3	10^4	70	120	0	0	0	0	0	0
	4	70	0	0	0	0	0	0	0	0
	5	10^5	10^5	10^5	10^5	10^5	10^5	10^5	10^5	10^5
	6	10^4	10^5	10^5	500	0	0	0	0	0
	7	10^3	0	0	0	0	0	0	0	0
	8	0	0	0	0	0	0	0	0	0
	9	0	0	0	0	0	0	0	0	0
5% glucose water	1	10^5	10^5	10^5	10^5	10^5	10^5	10^5	10^5	10^5
	2	10^4	10^5	10^5	10^5	10^5	10^5	10^5	10^5	10^5
	3	10^5	10^5	10^5	10^5	10^5	10^5	10^5	10^5	10^5
	4	10^3	10^3	10^3	10^5	10^5	10^5	500	0	0
	5	10^5	10^5	10^3	10^3	10^5	10^5	10^5	10^5	10^5
	6	10^5	10^5	10^5	10^5	10^5	10^5	10^5	10^5	10^5
	7	10^5	10^5	10^5	10^5	10^5	10^5	10^5	10^5	10^5
	8	0	0	0	0	0	0	0	0	0

0 signifies <10 CFU/ml

compared to the portion of the bladder below the rectus muscle. The catgut suture was seen in the bladder wall and did not intrude into the lumen.

DISCUSSION

This study describes a simple and reliable technique for creating a ventral bladder hernia. In addition to permitting repeated, safe sampling of bladder urine, percutaneous aspiration of urine via a ventral bladder hernia assures even small variations in bacterial counts can be interpreted with a high degree of confidence. Other investigators, who relied on voided specimens, reported that at least 3 successive cultures with similar colony counts were required to accurately assess the degree of bacteriuria (2). Failure to differentiate experimental bacteria from urethral or perineal contaminants could account in part for the discrepancy observed in successive voided specimens.

Surgical creation of a ventral bladder hernia did not alter the susceptibility of rats to urinary tract infection. Injection of 10^8 E. coli into the bladder lumen of tap water drinking animals demonstrated the remarkable efficiency with which both normal rats and rats with ventral bladder hernias cleared bacteria from the urinary tract. Within 5 days, only 4 of 7 rats (57%) with intrapelvic bladders had any bacteriuria and in none of the animals did the count exceed 10^3 CFU/ml. Eight (89%) of the rats with ventral bladder hernias had sterile bladder urine within 5 days. Similar results were reported by Freedman who demonstrated that within 4 to 5 days of an intravesical inoculum of 10^5 to 10^7 E. coli, only 3 of 19 rats (15%) had any bacteriuria and in 2 of these animals the counts were only 10^2 CFU/ml (5).

Conversely, rats drinking 5% glucose water and undergoing diuresis were highly susceptible to persistent bacteriuria. Four of 5 (80%) rats with normal bladders and 6 of 8 (75%) rats with ventral bladder hernias had 10^5 CFU/ml 9 days after inoculation. Freedman showed that polyuric

rats were unable to clear as few as 10 viable bacteria from their urine and that large numbers of bacteria were recovered up to 3 weeks later (5).

Statistical analysis of our data confirmed that animals drinking glucose water had a significantly impaired ability to clear a bacterial inoculum ($p < 0.01$) but that this impairment was only evident after the 4th post-inoculation day. These results suggest that animals drinking tap water require 4 days to spontaneously clear an inoculum. Aronson also found that the introduction of bacteria into the bladders of mice under conditions of tap water intake that did not produce infection (as judged by histologic examination) nonetheless resulted in shedding of some bacteria in the urine over 3 to 4 days (2).

The possibility that water diuresis impedes the antibacterial properties of urine or the antibacterial activity of the bladder mucosa has received considerable attention. Kaye, for example, demonstrated that first voided urine from normal women was often inhibitory and sometimes bactericidal for small inocula of E. coli (7). Dilution of the first voided urine by only 4-fold obliterated any differences in the inhibitory activity. Urea appeared to be one of the major antibacterial factors since its removal eliminated antibacterial activity. Urine pH, osmolality and glucose content can also influence bacterial growth in urine. Andriole (1) showed that the pH of urine samples from control rats had a mean value of 7.14 as compared to an average of 7.88 for animals undergoing water diuresis. Urine osmolality dropped below 200 and frequently below 100 mOsmole/kg. Growth of E. coli in urine is reduced at pH greater than 7.5 and osmolality less than 200 mOsmole/kg (3). Glucose did not appear in the urine or in abnormal amounts in the blood (1). A mucosal antibacterial defense mechanism has been suggested by studies of intact rat bladders (6). Bactericidal activity could be shown only when the bladder was empty. Freedman observed that it was generally possible to aspirate 1.0 to 2.0 ml of urine from the bladders of rats drinking glucose water, whereas less than 0.5 ml was usually obtained from animals drinking tap water (5). Such distension may lessen the opportunity for surface phagocytosis of bacteria (10) and provide a constant pool of residual urine conducive to bacterial growth.

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