

Experimental supratrigonal cystectomy: II—evaluation of urinary calculi, infection, and bladder dysfunction in the pathogenesis of renal failure

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Abstract The objective of this study was to evaluate the role of urolithiasis, infection, and bladder dysfunction in the pathogenesis of renal failure in rats subjected to supratrigonal cystectomy. One group of Sprague–Dawley rats was submitted to supratrigonal cystectomy, a second to cystectomy during which a suspension of *Proteus mirabilis* was injected into the bladder stump, and a third to sham surgery (controls). The animals were sacrificed two months after surgery. Blood pressure and serum urea and creatinine were measured before surgery and at sacrifice when a careful inspection of the urinary tract was performed to determine the presence of hydronephrosis and calculi. Microbiological analyses were performed on urine aspirated from the bladder and on the kidneys. Significant differences were found between values of systolic blood pressure and serum urea and creatinine recorded prior to the surgical procedure and those recorded at sacrifice in each group except the control

group. Renal failure was present in all animals subjected to cystectomy. Urinary calculi were documented in 5/10 animals subjected to cystectomy only and in all rats inoculated with *P. mirabilis*. Hypertension was documented in 43.75% of animals subjected to cystectomy. Pyelonephritis was diagnosed only in animals with urinary calculi, in each of which urine culture was also positive. No cases of renal failure, hypertension, calculi, and/or pyelonephritis were detected in the sham group. The findings of this study indicate that kidney failure in rats subjected to supratrigonal cystectomy is related to the severe bladder dysfunction induced by the surgical procedure.

Keywords Bladder dysfunction · Cystectomy · Infection · Lithiasis · Renal failure

Introduction

The normal bladder has important mechanisms that protect the urinary tract [1]. Its dysfunction, causing functional obstruction, affects the hydrodynamics of the urinary tract and increases intravesical pressure and residual urine volume, leading to vesicoureteral reflux and dilation of the urinary tract, predisposing to infection and to structural and functional kidney abnormalities [2–4].

Supratrigonal cystectomy is associated with accentuated reduction in bladder capacity and compliance and with bladder dysfunction related to the surgical

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procedure. In previous laboratory studies a high incidence of hydronephrosis, lithiasis, and pyelonephritis associated with renal failure and hypertension was found. It was suggested that the renal failure and hypertension were related to bladder dysfunction induced by the cystectomy and the presence of urolithiasis and pyelonephritis [5].

The objectives of this study were to evaluate the role of urolithiasis, with or without infection, and bladder dysfunction in the pathogenesis of renal failure in rats subjected to supratrigonal cystectomy.

Material and methods

Sprague–Dawley rats approximately two months old were anesthetized using pentobarbital and submitted to supratrigonal cystectomy, leaving both ureters intact. In order to study the role of infection in long-term complications, a second series of experiments was performed in which a group of animals was subjected to cystectomy, as already described; during surgery, however, 0.5 mL suspension containing 10^2 colony-forming units of *Proteus mirabilis* was slowly injected into the bladder stump. A third group of rats (control group) was subjected to sham surgery in which the bladder was exposed through a suprapubic incision but left intact. The surgical procedures were performed under rigorous aseptic conditions. During the study, regular laboratory rat chow and filtered water were provided ad libitum. All procedures were carried out in accordance with the Guidelines for the Care and Use of Experimental Animals (NIH publication no. 85–23).

The animals were sacrificed two months after the surgical procedure. Blood pressure, measured by the tail-cuff method, and serum levels of urea and creatinine were assessed before surgery and at the time of sacrifice. At the time of sacrifice, a careful inspection of the urinary tract was performed to determine the presence of hydronephrosis, defined as dilation of the renal pelvis and ureters, and calculi. In addition, microbiological studies were carried out on urine aspirated from the bladder. The kidneys were aseptically removed and transected. One half of each kidney was homogenized separately in 5 mL sterile normal saline solution (TRI-instruments). Dilutions (10^{-1} , 10^{-3} , 10^{-5}) of the tissue homogenates were cultured on plates and the number of bacteria were

determined after correction for the dilution factor [5, 6]. Standard microbiological techniques were used. The finding of more than 10^5 colony-forming units per mL (cfu mL⁻¹) of urine indicated the presence of a urinary tract infection. Pyelonephritis was defined as the presence of $\geq 10^5$ cfu per gram of kidney tissue. Animals with systolic blood pressure consistently above 140 mmHg were considered to be hypertensive. Renal failure was defined as serum urea ≥ 40 mg dL⁻¹ and/or serum creatinine > 1.0 mg dL⁻¹.

All data are expressed as mean \pm standard deviation except where otherwise indicated. Student's *t*-test was used to compare continuous data, and the chi-square test or Fisher's exact test, as appropriate, for categorical data. One-way ANOVA was used to compare differences between groups followed by the Bonferroni correction. A *P*-value < 0.05 was considered to be statistically significant. The statistical analysis was performed using the SPSS software program for Windows, version 14.0.

Results

Sixteen rats submitted to supratrigonal cystectomy and six rats subjected to sham surgery (control group) were sacrificed two months after the surgical procedure. The mean systolic blood pressure and serum levels of creatinine and urea prior to the surgical procedure and at the time of sacrifice are shown in Table 1 for each group. The differences in these variables were statistically significant in both cystectomy groups but not in the control group. Analysis using one-way ANOVA indicated a statistically significant difference between the groups ($P < 0.05$). The Bonferroni correction revealed that the group of animals with urinary calculi unrelated to *Proteus mirabilis* inoculation had significantly higher systolic blood pressure levels and mean serum creatinine levels. Mean serum levels of urea, however, were higher in the group that in addition to being submitted to cystectomy had also been inoculated with *P. mirabilis*.

Renal failure was present in all the animals subjected to cystectomy, including those injected with *Proteus mirabilis*. Urinary calculi were documented in 5 of the 10 animals that were subjected to supratrigonal cystectomy alone, and these were analyzed as a separate group together with all the

Table 1 Mean systolic blood pressure and mean levels of serum creatinine and blood urea prior to surgery and at sacrifice in rats subjected to supratrigonal cystectomy and sham surgery

	<i>n</i>	Systolic blood pressure (mmHg)		Serum creatinine (mg dL ⁻¹)		Blood urea (mg dL ⁻¹)	
		Pre-surgery	Sacrifice	Pre-surgery	Sacrifice	Pre-surgery	Sacrifice
Cystectomy without calculi	5	101	122	0.72	1.28	37.4	36.4
Cystectomy with calculi	5	102	141	0.82	1.48	28.6	45.4
Cystectomy/ <i>P. mirabilis</i>	6	95.8	130	0.65	0.90	29.6	71.7
Sham surgery	6	103	110	0.83	0.85	24.5	25.8

n—number of animals

rats that in addition to undergoing cystectomy had also been inoculated with *Proteus mirabilis*. Hypertension was also frequent, being recorded in 43.75% of the animals subjected to surgery and in 54.5% of the animals with urinary calculi. Pyelonephritis was diagnosed in all animals with urinary calculi and in each of these animals urine culture was positive for the same bacteria: Staphylococcus sp. in three animals, *E. coli* in one, Staphylococcus sp. + *E. coli* in one and *Proteus mirabilis* in all six animals injected with the bacterial suspension. No case of pyelonephritis or urine infection was recorded in any of the rats without urinary calculi. There were no cases of renal failure, hypertension, calculus, and/or pyelonephritis in the sham group (Table 2).

There was a statistically significant association between the presence of calculi and pyelonephritis. There was also a statistically significant difference between the study groups and the control group with respect to renal failure. Although arterial hypertension was frequent in both study groups and absent in the sham group, the difference did not reach statistical significance in the group of animals that were subjected to supratrigonal cystectomy but that did not have urinary calculi as compared to the sham group.

Discussion

Previous studies carried out in rats subjected to supratrigonal cystectomy have shown that the first few weeks after the surgical procedure were associated with pyelonephritis, sepsis, and high mortality, while the long-term course was characterized by a high incidence of renal failure and arterial hypertension, and urolithiasis and pyelonephritis. Although it has been suggested that the renal failure may be related to the presence of urolithiasis and pyelonephritis, the actual cause of kidney damage was not investigated [5]. In the current study the same experimental model of cystectomy was used with the addition of a second series of experiments in which animals subjected to supratrigonal cystectomy, as already described, also received 0.5 mL of a suspension containing 10² cfu of *Proteus mirabilis*, which was slowly injected into the stump of the bladder in order to stimulate lithogenesis [7, 8].

The complications related to the surgical procedure that were recorded in this study were similar to those reported in previous studies. The presence of urinary calculi was documented in all the animals that were inoculated with *Proteus mirabilis* and in 50% of the animals subjected to cystectomy without bacterial

Table 2 Complications in rats subjected to supratrigonal cystectomy and to sham surgery

	<i>n</i>	Urinary calculi	PN/UTI	Renal failure	Arterial hypertension
Cystectomy	10	5	5	10	4
Without calculi	5	0	0	5	1
With calculi	5	5	5	5	3
Cystectomy/ <i>P. mirabilis</i>	6	6	6	6	3
Sham surgery	6	0	0	0	0

n—number of rats; PN—pyelonephritis; UTI—urinary tract infection

inoculation, which were analyzed as a separate group. As already noted, there was a strong association between the presence of calculi and pyelonephritis/urinary tract infection [5]. The association between infection, the formation of urinary calculi, and their presence as a contributing factor towards the maintenance of urinary tract infection are well-known [9].

Although urinary lithiasis and pyelonephritis are associated with reduced kidney function [10, 11], in the current study renal failure occurred in all animals submitted to supratrigonal cystectomy and was unrelated to the presence of calculi and urinary infection/pyelonephritis. Moreover, renal failure was also diagnosed in the absence of arterial hypertension. It is interesting that the frequency of hypertension varied between the groups, being diagnosed in 50–60% of the animals with lithiasis and in 20% of the animals without it. This variation may be related to the severity of the renal insult [12], since the mean levels of blood pressure and of serum creatinine and urea were higher in the groups of animals with calculi. This finding suggests that the formation of calculi in the urinary tract of animals subjected to cystectomy aggravates the renal failure that is already present and the accompanying complications.

Supratrigonal cystectomy is a surgical procedure associated with extensive section of muscle fibers and nerves, causing severe bladder dysfunction. Abnormalities in bladder function and contractility secondary to structural alterations or neurological disease cause important pathologies of the urinary tract and of the kidney [3, 13–15]. Alterations in bladder contractility lead to an elevation of the hydraulic pressure of the urinary system that, if persistent, results in renal lesions and failure [16]. In addition, bladder dysfunctions increase the residual volume of urine predisposing to infection, formation of calculi, vesicoureteral reflux, and hydronephrosis, aggravating the renal parenchymal lesion already present [4, 11, 15]. It should be stressed, however, that the findings of this study are the result of animal experiments and should not be extrapolated to human clinical conditions.

Conclusion

In conclusion, the findings of this study indicate that renal failure in rats subjected to supratrigonal

cystectomy is related to severe bladder dysfunction and a reduction in compliance induced by the surgical procedure, which involves extensive section of muscle and nerve fibers. These findings are based on experimental studies and may not be extrapolated to humans.

References

- Giebisch G, Windhager E (2003) Organization of the urinary system. In: Boron WF, Boulpaep EL (eds) Medical physiology. Saunders, Philadelphia
- Su X, Stein R, Stanton MC, Zderic S, Monland RS (2003) Effect of partial outlet obstruction on rabbit urinary bladder smooth muscle function. *Am J Physiol Renal Physiol* 284:F644–F652
- Klahr S (2001) Urinary tract obstruction. *Semin Nephrol* 21:133–145
- Hodson CJ, Maling TMJ, McManamon PJ, Lewis MG (1975) The pathogenesis of reflux nephropathy (chronic atrophic pyelonephritis). *Br J Radiol Suppl* 13:1–26
- Barros M, Martinelli R, Rocha H (2006) Experimental supratrigonal cystectomy. Evaluation of long-term complications. *Int Braz J Urol* 32:350–354
- Poljakovic M, Persson K (2003) Urinary tract infection in iNOS-deficient mice with focus on bacterial sensitivity to nitric oxide. *Am J Physiol Renal Physiol* 284:F222–F231
- Ciftcioglu N, Bjorklund M, Kuorikoski K, Bergstrom K, Kajander EO (1999) Nanobacteria: an infectious cause of kidney stone formation. *Kidney Int* 36:1893–1898
- Goldfarber DS (2004) Microorganisms and calcium oxalate stone disease. *Nephron Physiol* 98:48–54
- Johnson DE, Russell RG, Lockatell V, Zulty JC, Warren JW, Mobley HLT (1993) Contribution of *Proteus mirabilis* urease to persistence, urolithiasis, and acute pyelonephritis in a mouse model of ascending urinary tract infection. *Infect Immun* 61:2748–2754
- Gambaro G, Favaro S, D'Angelo A (2001) Risk for renal failure in nephrolithiasis. *Am J Kidney Dis* 37:233–243
- Jahnukainen T, Chen M, Celsi G (2005) Mechanisms of renal damage owing to infection. *Pediatr Nephrol* 20:1043–1053
- Campese VM, Park J (2006) The kidney and hypertension: over 70 years of research. *J Nephrol* 19:691–698
- Anderson W-E, Arner A (2004) Urinary bladder contraction and relaxation: physiology and pathophysiology. *Physiol Rev* 84:935–986
- Turner WH, Brading AF (1997) Smooth muscle of the bladder in the normal and disease state: pathophysiology, diagnosis and treatment. *Pharmacol Ther* 75:77–110
- Klahr S, Morrissey J (2002) Obstructive nephropathy and renal fibrosis. *Am J Physiol Renal Physiol* 283:F861–F875
- Wright FS (1982) Effects of urinary tract obstruction on glomerular filtration rate and renal blood flow. *Semin Nephrol* 2:5–16