Original Article

Bladder epithelium is abnormal in patients with neurogenic bladder due to myelomeningocele

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Objective: To describe the urothelium of the neurogenic bladder in patients with myelomeningocele on clean intermittent catheterization.

Setting: Outpatient practice.

Methods: Samples of bladder wall obtained from two groups of patients were examined for urothelium. The first group included 12 children and young adults with myelomeningocele and neurogenic bladder on intermittent catheterization for bladder emptying. The second group included eight children with vesicoureteral reflux and non-neurogenic bladder. Nine patients from the first group and four patients from the second group had urothelium. A contiguous section of each of the 13 samples with urothelium was stained for uroplakin expression, a marker of superficial bladder urothelium by immunohistochemistry.

Results: Samples from children with reflux revealed normal bladder epithelium and a uniform layer of umbrella cells (95% CI: 0–60%). In contrast, the epithelium from all patients with myelomeningocele was abnormal (95% CI: 66–100%). Epithelium from five patients (four children, one adult) revealed chronic inflammation. Three patients (two children, one adult) had squamous metaplasia. The apical surface of the epithelium in all patients with chronic inflammation had some reactivity with anti-uroplakin antibody, but the cells staining positive for uroplakin were scattered along the luminal surface of the epithelium. The apical surface of patients with squamous metaplasia was negative for uroplakin.

Conclusion: The urothelium of the neurogenic bladder in young patients with myelomeningocele is abnormal, with loss of uroplakin expression and altered urothelial proliferation.

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Introduction

Bacteriuria is frequently found in children with neurogenic bladder due to myelomeningocele on clean intermittent catheterization (CIC). In a 6-month observational study, we found that 70% of urine samples collected from children with myelomeningocele on CIC were positive for bacteria ($\geq 10^4$ colony-forming units/ml).¹ *Escherichia coli* and *Klebsiella* spp were the most common pathogens detected. Once acquired, *E. coli* and *Klebsiella* spp often remained in the urine for weeks in the absence of antimicrobial therapy. In an effort to decrease the high frequency of bacteriuria in this population, we conducted three randomized controlled treatment trials. In separate studies, we examined the effect of daily nitrofurantoin prophylaxis² and cranberry

juice³ prophylaxis on the rates of bacteriuria. We found that prophylaxis had no effect on the rate of bacteriuria in children with myelomeningocele on CIC. In addition, we examined whether the method of catheter care was responsible for the high rate of bacteriuria.⁴ We found that a single-use, sterile catheter for bladder-emptying did not decrease the frequency of bacteriuria when compared to reused clean catheters.

Previous work by Elliott *et al*⁵ provided a possible explanation for failure of these treatment trials. These investigators examined patients who had normal bladders and chronic bacteriuria. Scanning electron microscopy of bladder wall biopsies revealed that some of these patients had disrupted uroepithelium and bacteria attached to their urothelium. They proposed that bacteria may persist in underlying cells of abnormal urothelium, thereby avoiding being washed out by urine.

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The bladder urothelium of children with myelomeningocele has not been described. As a means of devising future strategies aimed at reducing bacteriuria in this population, we examined the urothelium of children with myelomeningocele.

The normal bladder epithelium (urothelium) is composed of three layers: basal, intermediate and superficial. The superficial layer is lined on the apical surface by umbrella cells. The presence of umbrella cells defines a normal superficial layer of the bladder epithelium. Recently, a marker for umbrella cells called uroplakin,⁶ was identified and provides a specific stain for umbrella cells on biopsy specimens of bladder epithelium using immunohistochemistry. For this study, patients with myelomeningocele on CIC with a history of chronic bacteriuria had bladder wall biopsies obtained during reconstructive bladder surgery. The bladder epithelium was described.

Material and methods

Patients and controls

Samples of bladder wall were obtained from two groups of patients. The bladder epithelium was described, then compared between the two patient groups. The first group comprised 12 patients with myelomeningocele, neurogenic bladder and a history of chronic bacteriuria. Samples of the bladder dome were obtained during primary reconstructive bladder surgery. All patients were on CIC for voiding. In all, 10 of the patients were children (10–12 years of age) and two were adults (44 and 46 years of age). The second group, eight children (1–16 years of age) with vesicoureteral reflux and non-neurogenic bladder, had samples of the bladder wall obtained at the time of primary ureteral reimplantation. All patients were selected when they underwent surgery. During surgery, all bladder samples were immediately fixed in 10% formalin and transferred to the immunohistochemistry lab. The use of bladder wall samples for research was approved by the human investigation committee at Children's Hospital and Medical Center, Seattle, WA and at the University of Virginia, Charlottesville, VA. This precluded obtaining any identifying patient information such as gender, specific age, urodynamic information or indication for reconstructive surgery.

Tissue preparation and staining

A section from each bladder sample, mounted and stained with hematoxylin and eosin was examined to confirm the presence of urothelium. The sample from nine of the patients with neurogenic bladder (two adults, seven children) and samples from four of the children with vesicoureteral reflux had epithelium on one surface by hematoxylin and eosin stain. A contiguous section from each of the 13 samples with epithelium present was stained for uroplakin expression by immunohistochemistry. These 13 samples constitute the material described in the Results.

Immunohistochemistry

Rabbit polyclonal antibody raised by immunization with bovine urinary bladders and affinity purified against uroplakin III and Ib was kindly provided by TT Sun (PhD, New York University Medical School, NY, USA).⁷ Preimmune sera from the same rabbit prior to immunization were utilized as a negative control. Immunoperoxidase staining of slides was performed by the manufacturer's protocol using the Elite Vectastain ABC Kit (Vector Laboratories, Inc., Burlingame, CA, USA). A serum dilution of 1:1000 provided specific staining of the apical surface of the urothelium.

Results

The hematoxylin-and-eosin-stained samples from four children from the second group with vesicourethral reflux and non-neurogenic bladder (Table 1, nos. A1–A4) revealed normal bladder epithelium and lamina propria (95% CI: 0–60%) (Figure 1). The epithelium was three to four layers thick and displayed an orderly progression from the basal cell layer to the intermediate and superficial cell layers. Immunoperoxidase staining of sections contiguous to the hematoxylin and eosin sections in all four samples exhibited a strong and specific reactivity with anti-uroplakin antibodies in a uniform pattern indicating intact superficial epithelium (Figure 2).

In contrast, the bladder epithelium and lamina propria from all nine patients in the first group with myelomeningocele and neurogenic bladder (Table 1, nos. B1–B9) was abnormal (95% CI: 66–100%). The epithelium from one patient (B1) appeared almost normal with hematoxylin and eosin stain with minimal inflammation of the lamina propria (Figure 3). However, the apical surface of this epithelium showed no reactivity with anti-uroplakin antibody, indicating disruption of the superficial epithelium (Figure 4). Sections from five patients (B2–B6) revealed chronic inflammation in the epithelium and lamina propria with erosion (Figure 5) or hyperplasia of the surface epithelium (Figure 6). The lamina propria was infiltrated with lymphocytes and plasma cells (Figure 5). Two of these five patients had lymphoid follicles in the submucosa. The surface of the epithelium in all of these patients with chronic inflammation had some reactivity with anti-uroplakin antibody, but the superficial cells staining positive for uroplakin were scattered along the luminal surface of the epithelium (Figure 7). In three patients (B7–B9), there was squamous metaplasia of the bladder epithelium with keratinization of superficial epithelial cells (Figure 8). In addition, one child (B9) displayed mucinous metaplasia. The epithelium demonstrating squamous metaplasia was negative for uroplakin, indicating disruption of the superficial epithelium.

Table 1 Histology of urothelium and presence of intact superficial epithelium evidenced by uroplakin-stained cells in children with vesicoureteral reflux (a) and patients with chronic neurogenic bladder (b)

No	Patient	Histology of urothelium	Presence of uroplakin-stained cells
<i>Children with vesicoureteral reflux</i>			
1	Child	Normal bladder mucosa	Uniform layer of staining
2	Child	Normal bladder mucosa	Uniform layer of staining
3	Child	Normal bladder mucosa	Uniform layer of staining
4	Child	Normal bladder mucosa	Uniform layer of staining
<i>Patients^a with chronic neurogenic bladder</i>			
1	Child	Minimal inflammation	None
2	Child	Chronic inflammation	Yes; cells scattered along luminal surface
3	Child	Chronic inflammation	None
4	Child	Chronic inflammation with lymphoid follicles	None
5	Child	Acute and chronic inflammation with hyperplasia of surface epithelium	Yes, cells scattered along luminal surface
6	Adult	Chronic inflammation	Yes, cells scattered along luminal surface
7	Adult	Squamous metaplasia	None
8	Child	Squamous metaplasia	None
9	Child	Squamous metaplasia and mucinous metaplasia	None

^aChild is 1–12 years of age and adult is 44–46 years of age

Figure 1 Normal bladder epithelium and lamina propria from a child with vesicoureteral reflux (H&E, $\times 200$)

Figure 2 Immunoperoxidase staining exhibits a strong and specific reactivity with anti-uroplakin antibody in a uniform pattern restricted to the apical surface of the umbrella cells ($\times 200$)

Discussion

This study provides the first description of the urothelium of patients with myelomeningocele on CIC. The bladder urothelium was surprisingly abnormal in all nine patients with neurogenic bladder. Abnormalities ranged from chronic inflammation with urothelial erosion or hyperplasia to squamous metaplasia. The superficial layer of the urothelium was disrupted. Our findings are similar to the work by Montgomerie *et al*⁸ who examined bladder biopsies from adults with spinal cord injury and an indwelling catheter using an antibody to a 28-kDa molecule present in umbrella cells. Biopsies revealed areas of squamous metaplasia usually associated with evidence of chronic inflammation.

Shapiro *et al*⁹ observed that the myelomeningocele human fetal bladder uroepithelium is highly differen-

tiated, including the superficial urothelium at 20 weeks of gestation. Our finding that children with myelomeningocele had abnormal urothelium suggests that there are ongoing changes to the bladder epithelium after birth.

Urothelial injury may occur by several mechanisms, one of which is desquamation of urothelial cells. The turnover of urothelium is 40 weeks in the normal adult bladder;¹⁰ however, desquamation of urothelial cells can be induced by injection of bacterial endotoxins into the bladder,¹¹ depletion of calcium from urothelial cells,¹² application of stress hormones to the bladder wall¹³ or by reduction of blood flow to the bladder.¹⁴

Lepilahti *et al*¹⁵ and others^{14,16} suggested that any condition that produces urinary retention resulting in

Figure 3 Minimal inflammation of the lamina propria in a child with neurogenic bladder due to myelomeningocele (H&E, × 200)

Figure 4 The apical surface of the epithelium shows no reactivity with anti-urolaklin antibody (immunoperoxidase stain, × 200)

Figure 5 Chronic inflammation with urothelial erosion in an adult with neurogenic bladder due to myelomeningocele (H&E, × 200)

Figure 6 Chronic inflammation with hyperplasia of the surface epithelium in a child with neurogenic bladder due to myelomeningocele (H&E, × 200)

Figure 7 Specific reactivity with anti-urolaklin antibody; however, cells staining positive for urolaklin are scattered and intermittent along the luminal surface of the epithelium (immunoperoxidase stain, × 200)

overdistention of the bladder may decrease blood flow to the bladder wall and result in a damaged urothelium. In a rat model,¹⁵ overdistention of the bladder for 3 h was associated with a disrupted urothelium and an influx of inflammatory cells. The integrity of the urothelium was restored only after three weeks.

Veranic and Jezernik¹² examined bacterial endotoxin, stress hormones and depletion of calcium as inducers of desquamation and found that bacterial endotoxin was the strongest inducer of desquamation. These authors placed lipopolysaccharide (LPS), the endotoxin derived from the cell wall of Gram-negative bacteria, into the bladder lumen of mice for 90 min. Scanning microscopy revealed desquamation of cells on the luminal surface of some regions of the bladder, while in other regions all three layers of epithelium down to the basal lamina

Figure 8 Squamous metaplasia with keratinization of the superficial epithelium in a child with neurogenic bladder due to myelomeningocele (H&E, $\times 200$)

desquamated. Uchida *et al*¹⁷ described the effect of long-term exposure of endotoxin to the urothelium. They instilled LPS, 10^9 live or heat-killed *E. coli* into a rat urinary bladder for a period of 4 weeks. Bladder instillation of live *E. coli* resulted in persistent infection, neutrophilic infiltration of the urothelium and diffuse urothelial hyperplasia. Instillation of killed *E. coli* or LPS induced the inflammatory reaction as well as urothelial hyperplasia. These authors concluded that LPS present over time causes cellular proliferation of the urothelium evidenced by hyperplasia.

The majority of children with neurogenic bladder have loss of control over voiding with urinary retention and bladder distension. Although CIC performed several times a day relieves bladder distension, there may be specific populations of children with hypertonic bladder who, as a result of high intravesical filling and contractile pressures, continue to be at risk for urothelial injury.¹⁸ A disadvantage of intermittent catheterization is that inoculation of bacteria into the bladder urine is inevitable in the course of repeated catheterization. Bacteriuria due to Gram-negative organisms often persists for weeks at a time in children with neurogenic bladder on intermittent catheterization.¹ Persistent Gram-negative bacteriuria may be one cause of urothelial hyperplasia in our population due to chronic exposure to endotoxins.

In our study, we were unable to differentiate whether abnormal urothelium resulted from chronic bacteriuria or high bladder pressures. It is conceivable that once abnormal urothelium develops, it may be very difficult to eradicate bacteriuria using standard antimicrobial therapies. The results of this study have shifted our focus away from treatment trials toward examination of specific mechanisms (bacterial endotoxin, overdistension, local trauma from intermittent catheterization) that induce urothelial injury in patients with myelomeningocele.

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References

- Schlager TA, Dilks S, Trudell J, Whittam TS, Hendley JO. Bacteriuria in children with neurogenic bladder treated with intermittent catheterization: natural history. *J Pediatr* 1995; **126**: 490–496.
- Schlager TA, Anderson S, Trudell J, Hendley JO. Nitrofurantoin prophylaxis for bacteriuria and urinary tract infection in children with neurogenic bladder on intermittent catheterization. *J Pediatr* 1998; **132**: 704–708.
- Schlager TA, Anderson S, Trudell S, Hendley JO. Effect of cranberry juice on bacteriuria in children with neurogenic bladder receiving intermittent catheterization. *J Pediatr* 1999; **135**: 698–702.
- Schlager TA, Clark M, Anderson S. Effect of a single-use sterile catheter for each void on the frequency of bacteriuria in children with neurogenic bladder on intermittent catheterization for bladder emptying. *J Pediatr* 2001; **108**: 1–4.
- Elliott TA, Reed L, Slack RGB, Bishop MC. Bacteriology and ultrastructure of the bladder in patients with urinary tract infections. *J Infect* 1985; **11**: 191–199.
- Wu XR, Manabe M, Yu J, Sun TT. Large scale purification and immunolocalization of bovine uroplakins I, II, and III. Molecular markers of urothelial differentiation. *J Biol Chem* 1990; **265**: 19170–19179.
- Moll R, Wu XR, Lin JH, Sun TT. Uroplakins, specific membrane proteins of urothelial umbrella cells, as histological markers of metastatic transitional cell carcinomas. *Am J Pathol* 1995; **147**: 1383–1397.
- Montgomerie JZ, Holshuh HJ, Keyset AJ, Bennett CJ, Schick DG. 28 K in squamous metaplasia of the bladder in patients with spinal cord injury. *Paraplegia* 1993; **31**: 105–110.
- Shapiro E *et al*. Altered smooth muscle development and innervation in the lower genitourinary and gastrointestinal tract of the male human fetus with myelomeningocele. *J Urol* 1998; **160**: 1047–1053.
- Jost SP. Cell cycle of normal bladder urothelium in developing and adult mice. *Virchows Arch B* 1989; **57**: 27–36.
- Aronson M, Medalia O, Amichay D, Nativ O. Endotoxin-induced shedding of viable uroepithelial cells is an antimicrobial defense mechanism. *Infect Immun* 1988; **56**: 1615–1617.
- Veranic P, Jezernik K. The response of junctional complexes to induced desquamation in mouse bladder urothelium. *Biol Cell* 2000; **92**: 105–113.
- Dalal E, Medalia O, Harari O, Aronson M. Moderate stress protects female mice against bacterial infection of the bladder by eliciting uroepithelial shedding. *Infect Immun* 1994; **62**: 5505–5510.
- Korosec P, Jezernik K. Early cellular and ultrastructural response of the mouse urinary bladder urothelium to ischemia. *Virchows Arch* 2000; **436**: 377–383.
- Lepilahti M, Kallioinen M, Tammela TLJ. Duration of increased mucosal permeability of the urinary bladder after

- acute overdistension: an experimental study in rats. *Urol Res* 1999; **27**: 272–276.
- 16 Siroky MB, Azadzi KM, Pontari M, Vlachiotis J. Canine bladder blood flow and oxygenation: changes induced by filling, contraction and outlet obstruction. *J Urol* 1996; **155**: 1459–1465.
- 17 Uchida K, Samma S, Kenji R, Warren JR, Oyasu R. Stimulation of epithelial hyperplasia in rat urinary bladder by *Escherichia coli* cystitis. *J Urol* 1989; **142**: 1122–1126.
- 18 Kasabian N, Bauer S, Dyro F, Colodny A, Mandell J, Retick A. The prophylactic value of clean intermittent catheterization and anticholinergic medication in newborns and infants with myelodysplasia at risk of developing urinary tract deterioration. *Am J Dis Child* 1992; **146**: 840–843.