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Struvite Urolithiasis and Chronic Urinary Tract Infection in a Murine Model of Urinary Diversion

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Abstract

OBJECTIVES—To characterize the clinical course following cutaneous vesicostomy (CV) in megabladder (*mgb*^{−/−}) mice with functional urinary bladder obstruction.

MATERIALS AND METHODS—Forty-five *mgb*^{−/−} males underwent CV at a median age of 25 days. The 34 animals that survived longer than 3 days after CV were evaluated by serial observation and renal ultrasound. Moribund animals were euthanized. Urinary bladders and kidneys were analyzed by histopathology, and urine biochemical studies were performed.

RESULTS—At a median of 11 weeks after CV, 35% (12/34) of *mgb*^{−/−} males became moribund with pelvic masses, which were identified as bladder stones at necropsy. Urine pH was alkaline and microscopy demonstrated struvite crystals. Urine contained Gram-positive cocci, while urine cultures were polymicrobial. Stone composition was chiefly struvite (88–94%) admixed with calcium phosphate. In 40% (2/5) of cases, retained intravesical polypropylene suture was identified as the presumed nidus. No stones were detected in over 100 males prior to CV or in 25 cases when CV was performed using polydioxanone suture. Kidneys from 33% (4/12) of animals with bladder stones contained staghorn calculi. Histopathology from animals with struvite stones demonstrated active cystitis, pyelitis, and chronic pyelonephritis.

CONCLUSIONS—These findings attest to the importance of the nidus in lithogenesis and provide a novel murine model for struvite urolithiasis and chronic infection of the diverted urinary tract.

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Keywords

urinary tract infection; pyelonephritis; urinary diversion; struvite; urolithiasis; mouse model

INTRODUCTION

Patients with impaired urinary bladder emptying are often initially managed with clean intermittent catheterization (CIC). Individuals who fail this conservative management require urinary diversion. In patients with congenital lower urinary tract obstruction, urinary diversion may be achieved through the creation of a surgical anastomosis between the bladder mucosa and skin, i.e., cutaneous vesicostomy (CV).

Complications of CV include pyelonephritis and urolithiasis.¹ In one recent study of children with myelomeningocele undergoing CV, the incidence of urolithiasis was 33% with concurrent pyelonephritis in 11% of patients.² These “infection stones” are composed of magnesium ammonium phosphate (struvite) formed by bacterial urease activity. When struvite stones occupy the renal pelvis and extend into the renal calices, the elevated likelihood of ureteral obstruction, recurrent pyelonephritis, and papillary necrosis places individuals at risk for progressive chronic kidney disease.³⁴ Evidence suggests aggressive treatment of staghorn calculi is associated with improved renal function.⁵

Patients with urinary diversion have multiple risk factors for infection stone formation. Individuals with spinal cord lesions and neurogenic bladder have ongoing urinary stasis and hypercalciuria from chronic immobilization.² Post-operatively retained intravesical suture can serve as a lithogenic nidus.^{6,7} Patients with indwelling catheters are at higher risk for developing recurrent UTI with urease(+) organisms, while alkaline urinary pH favors struvite lithogenesis.⁸

Experimental models of struvite stone formation entail administration of urease(+) bacteria to animals with an implanted material known to act as a nidus for stones.^{9–11} These inducible models of urolithiasis are limited by their reliance on a large inoculum of urease(+) bacteria. In contrast, a spontaneous model of struvite stone formation would be better suited to define early stages of infection, as well as to identify host and microbial factors that control infection and stone formation. Additionally, current animal models do not incorporate urinary diversion.

Megabladder mice possess a recessive mutation (*mgb*^{-/-}), the result of an unplanned gene disruption by a randomly inserted transgene, that prevents development of urinary bladder smooth muscle during embryogenesis.¹² Consequently, *mgb*^{-/-} bladders are atonic and exhibit urinary retention *in utero*. Male *mgb*^{-/-} animals are unable to void with development of bilateral hydroureteronephrosis, renal failure and death by 6 weeks of age.¹² Urinary diversion via CV prolongs survival of adult *mgb*^{-/-} males.^{13,14} In this study, we characterized the post-operative clinical course in this mouse model of urinary diversion.

MATERIALS AND METHODS

Mice

Mgb^{-/-} mice have been maintained for over 20 generations on a FVB/N genetic background.¹⁵ The use of *mgb*^{-/-} mice (Mouse Genome Informatics No. MGI:3772357) as well as age- and sex-matched FVB/N control animals was approved in advance by the Nationwide Children's Hospital Institutional Animal Care and Use Committee (Protocol No. 02105AR). All mice were given free access to drinking water and a standard, irradiated low-

fat chow (Harlan Laboratories, Indianapolis, IN) regardless of treatment. Hydration status of animals was monitored daily, but the exact volume of water consumed by different study groups was not recorded. Animals underwent CV at a median age of 25 days (range 16 to 65) using a previously published method.^{13,14} Polypropylene suture (Prolene™ 6-0, Ethicon) was used for 45 animals. Alternatively, polydioxanone suture was used for 25 animals (PDS™ II 6-0, Ethicon). Animals were observed daily for signs of morbidity, and moribund animals were euthanized by CO₂ inhalation.

Stone Analysis

Animals with urolithiasis were identified based on a palpable bladder stone during physical examination. If the animal was not moribund, stones were further evaluated by ultrasound using a previously described method.^{13,16} Next, urine obtained by aspiration from the CV stoma was centrifuged at 3000 g for 10 minutes, and the sediment was evaluated by light microscopy. Bladder stone composition was evaluated by x-ray diffractometry (Kidney Stone Analysis Laboratory, Louis C. Herring and Company, Orlando, FL).

Urine Biochemical Studies

Urine was obtained by sterile aspiration from the bladder at the time of CV or from the renal pelvis of animals with secondary hydronephrosis as a result of a stone fully occupying the bladder lumen at necropsy. Urine was assessed by microscopy, Gram stained, and cultured for 24 hours using standard techniques. Urine chemistries were evaluated as previously described (Litholink, Chicago, IL).¹⁷

Histologic Analysis

Four micron sections of 4% paraformaldehyde fixed, paraffin-embedded kidney and bladder were prepared from *mgb*^{-/-} animals with urolithiasis. Deparaffinized sections were rehydrated and subject to hematoxylin and eosin (H&E) or Masson's trichrome staining using standard techniques.

Statistical Analysis

An unpaired student's two-tailed *t* test assuming unequal variance was used to evaluate continuous variables (Excel, Microsoft Corporation, Redmond, WA). Categorical data were evaluated by Fisher's exact test (GraphPad, La Jolla, CA). In all cases, a *p* value of < 0.05 was considered statistically significant.

RESULTS

Effect of CV on *mgb*^{-/-} mice survival

A cohort of 45 male *mgb*^{-/-} mice was subject to urinary diversion by CV at a median age of 25 days using polypropylene suture. These animals were monitored post-operatively for clinical signs of deterioration. One animal died intraoperatively, and 10/45 (22%) died in the first three days following CV, consistent with previous observations.¹³ The remaining 34 mice survived for 13.4 ± 1.9 weeks (mean ± standard error) post-CV, with an average age of 17.6 ± 1.9 weeks at death. This lifespan compared favorably to historical un-diverted *mgb*^{-/-} male controls, which rarely lived past 6 weeks.¹²

Stone formation as a function of suture type

As early as 5 days following CV, 35% (12/34) of surviving *mgb*^{-/-} animals developed a new abdominal mass with a gritty, stone-like quality on palpation. The presence of bladder stones was confirmed by ultrasound and during post-mortem assessment (Figure 1). Of animals with bladder stones, 33% (4/12) also had staghorn calculi in the renal pelvis (Figure 1). In

contrast, no stones occurred in over 100 *mgb*^{-/-} males prior to CV (Fisher's exact test, $p < 0.0001$). Since these animals remained viable and reproductively active, they were retained and closely observed until they became moribund at a median interval of 11 weeks post-CV (range, 1 to 27 weeks). For those mice surviving the first 3 postoperative days, no significant difference existed in mean post-CV survival among animals with stones (16.3 ± 2.6 weeks) and those lacking clinical or sonographic evidence of stones (12.7 ± 2.5 weeks) (student's t test, $p = 0.348$). When a separate cohort of 25 males underwent CV with polydioxanone, all survived longer than 3 days post-CV and none developed stones (Fisher's exact test, $p = 0.003$).

Stone composition and urine chemistries

We submitted the 5 largest bladder stones for X-ray diffractometry to ensure that sufficient material was available for analysis. Given the degree of similarity in stone composition among these samples (Table 1), no further stones were analyzed. Stone composition was primarily struvite (88–94%), admixed with calcium phosphate and protein (Table 1). In 40% (2/5) of evaluated stones, retained polypropylene suture material was identified as a nidus (Figure 1 and Table 1). To determine if *mgb*^{-/-} males were predisposed toward struvite crystalluria, urine chemistries were evaluated before and 1–4 weeks following CV (Table 2). Post-CV urine was significantly more alkaline, with reduced levels of magnesium, phosphorus, and citrate. Mean ammonium/Cr ratios were higher post-CV, although this change did not reach statistical significance.

Cystitis and chronic pyelonephritis in *mgb*^{-/-} animals post-CV

In contrast with pre-CV urine, urine microscopy demonstrated struvite crystals as early as 7 days post-CV (Figure 1). Urine harvested from animals with stones contained Gram-positive cocci in clusters or chains (Figure 1). Urine cultures obtained from animals with stones at the time of necropsy were polymicrobial, with *Staphylococcus* species accompanied by either *Enterococcus casseliflavus* or *Pasteurella* species, as well as *Escherichia coli* in combination with *Pasteurella* species. In contrast, urine cultured from six *mgb*^{-/-} males prior to CV was sterile and demonstrated no crystalluria on microscopy. Bladders post-CV exhibited mucosal hyperplasia, typically with squamous metaplasia or papillomatous extensions, in association with chronic multifocal inflammation and fibrosis of the submucosa (Figure 2). Aplasia of the detrusor muscle in *mgb*^{-/-} mice was obvious as was previously reported.¹² The bladder lumen and renal pelvis were filled with cocci and myriad degenerating neutrophils, reflecting active cystitis and pyelitis (Figure 2). Kidneys of animals with staghorn calculi demonstrated hydronephrosis and chronic pyelonephritis, with multifocal lymphocyte and plasma cell aggregates near large arteries, as well as prominent peripelvic fibrosis (Figure 2).

DISCUSSION

Mgb^{-/-} mice post-CV model struvite urolith formation and ascending UTI

The urinary tract is the second-most common site of infection in the human body.¹⁸ The high capacity, atonic bladder of *mgb*^{-/-} animals is reminiscent of patients with areflexic neurogenic bladder as well as children with prune belly syndrome. These individuals are at risk for recurrent UTI and stone formation secondary to urinary stasis and repeated instrumentation of the urinary tract. Thus, CV in *mgb*^{-/-} mice offers the opportunity to test potential clinical interventions to prevent stone formation and ascending UTI, and ultimately to develop surgical and medical management strategies for reducing morbidity in patients with urinary diversion.

Role of lower urinary tract dysfunction in struvite lithogenesis

Patients with lower urinary tract dysfunction – whether functional or anatomic – are at risk for struvite stone formation.² This risk is further enhanced by the presence of intravesical suture material, foreign bodies (including indwelling catheters), recurrent UTIs, and non-adherence to routine CIC regimens resulting in urinary stasis. Struvite stones remain a significant cause of stone-related morbidity and chronic kidney disease.³ In humans with high struvite stone burden affecting the upper urinary tract, percutaneous nephrolithotomy has achieved comparable stone-free rates as traditional open surgery.¹⁹ However, incomplete stone removal and/or UTIs with urease-splitting organisms result in frequent stone recurrence, and open surgery may be required for complete surgical removal of certain struvite stones. Use of extracorporeal shockwave lithotripsy (ESWL) is an alternative approach to eradicating staghorn calculi, particularly in children (who have a lower incidence of sepsis following treatment).⁸ Nonetheless, multiple rounds of ESWL are required in these patients. Urease inhibitors have been developed to prevent stone formation, but dose-limiting systemic toxicity has impaired widespread use.⁸ Development of novel, better tolerated urease inhibitors, urine acidification agents, and antimicrobial agents would be welcome adjunctive strategies to surgical removal of struvite stones. The *mgb*^{-/-} CV model can be used to discover and test such therapies.

Role of suture composition in struvite stone formation

We did not detect struvite stones or pyelonephritis in over 100 male *mgb*^{-/-} kidneys without CV or in 25 *mgb*^{-/-} males who underwent CV using polydioxanone suture. These observations support a paramount role for suture material in the genesis of struvite stones and chronic UTI in these animals. These data are consistent with literature reports identifying polypropylene suture as a nidus for bladder struvite and calcium oxalate stones in patients undergoing ureteroneocystostomy at the time of renal transplantation,⁶ and bladder calculi associated with intravesical polypropylene suture in women following urethrovesical suspension.^{7,20} It remains to be determined why *mgb*^{-/-} mice undergoing CV with polydioxanone suture failed to develop stones. It is unlikely that the differences in stone burden are related to reabsorption of polydioxanone suture, since stones formed in animals with polypropylene suture as early as five days post-CV and previous studies of polydioxanone demonstrate persistence in rat and beagle bladders after 8 weeks.^{21,22} Instead, we hypothesize that polydioxanone possesses anti-lithogenic properties in diverted *mgb*^{-/-} bladders. Previous studies support such a role, although the mechanism remains poorly defined.^{20,22} In a direct comparison of polypropylene and polydioxanone suture closure of rabbit cystotomies, calculi formed on both suture types, but persistent calculi were associated with polypropylene suture.²⁰ In comparing polydioxanone with polyglactine 910 and chromed catgut in a rat cystotomy model, bladder stones were uniquely absent in the polydioxanone cohort.²² We speculate that differences in the lithogenic properties of polypropylene and polydioxanone suture may relate to the ability of each to prevent mucosal epithelialization, activate the host inflammatory response, serve as a substrate for urease(+) bacteria biofilms, and/or function as a nidus for struvite encrustation. Further research to define the complex relationship between suture, bladder microenvironment, urease(+) bacteria and lithogenesis will be greatly facilitated by the diverted *mgb*^{-/-} mouse model.

Proposed pathologic sequence in *mgb*^{-/-} mice post-CV

Prior to CV, *mgb*^{-/-} mice may be protected from struvite calculi by virtue of their slightly acidic urine, sterile urinary tract, and absence of intravesical material capable of serving as a stone nidus. CV provides a direct portal for bacterial invasion of the *mgb*^{-/-} bladder from a variety of sources including the skin (coagulase-negative *Staphylococcus* species), gastrointestinal tract (*E. casseliflavus*, *E. coli*), and oral mucosa (*Pasteurella* species). Of

these, *Staphylococcus* species are well-recognized sources of urease production,⁸ while *E. coli* may produce urease in rare instances.²³ We hypothesize that intravesical polypropylene serves as a substrate for bacterial biofilm formation. Bacterial urease hydrolyzes urea to ammonia, thereby increasing urine pH and favoring supersaturation of struvite and calcium phosphate (apatite) crystals. Alkaline urine may also reflect an inability of the renal collecting system to acidify urine following CV. Precipitation of struvite crystals likely accounts for reduced magnesium and phosphate levels in diverted *mgb*^{-/-} urine. Invading bacteria may also degrade citrate in *mgb*^{-/-} urine post-CV, resulting in hypocitraturia and thereby favoring formation of apatite crystals. Polypropylene suture also appears to serve as a nidus for struvite stone formation. Renal staghorn calculi may form through ureteral propagation of an obstructing bladder stone, or alternatively may occur *de novo*.

Of note, we did not detect bladder stones in 65% of animals undergoing CV with polypropylene suture. We attribute this primarily to under-detection as stones were initially identified by palpation and verified by ultrasound and at necropsy. Eleven of 22 (50%) of animals without palpable stones died suddenly and due to rigor mortis did not undergo necropsy. These animals may have had a low degree of stone burden. Alternatively, a difference in urinary inhibitors, retained suture in the bladder lumen, local immune response, and/or bladder inoculum of urease(+) bacteria could account for absence of bladder stones in these animals. Future studies with *mgb*^{-/-} mice will help to clarify the risk factors for bladder stones and serial ultrasound of all animals following CV will provide a more accurate estimate of stone burden

CONCLUSIONS

In summary, we describe the first mouse model that reliably develops UTI and stone formation following urinary diversion by CV. The *mgb*^{-/-} CV model closely mimics humans at risk for chronic UTI and struvite stones including individuals with obstructive uropathy. Furthermore, this model presents an opportunity to characterize underlying pathogenic mechanisms responsible for upper and lower urinary tract inflammation and scarring in the context of chronic infection. Finally, diverted *mgb*^{-/-} mice present an opportunity to devise and test potential therapeutic measures to delay stone formation, eradicate existing stones, and prevent disseminated UTI in subjects with urinary diversion.

ABBREVIATIONS

<i>mgb</i> ^{-/-}	<i>Megabladder</i>
CV	Cutaneous Vesicostomy
CIC	Clean Intermittent Catheterization
UTI	Urinary Tract Infection
H&E	Hematoxylin and Eosin
Cr	Creatinine

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Figure 1. Crystalluria and struvite stone formation in *mgb*^{-/-} males following CV
 (A) A struvite stone (arrow) occupies the decompressed bladder of a *mgb*^{-/-} male with severe bilateral hydroureteronephrosis following CV. (B) A struvite stone with embedded polypropylene suture (arrow) serving as an apparent nidus; white line: 5 mm. (C) Urine sediment from a *mgb*^{-/-} male post-CV demonstrating struvite crystals with an orthorhombic (“coffin-lid”) appearance on light microscopy (400x). (D) Urine Gram stain from a *mgb*^{-/-} male with a struvite stone post-CV shows linear clusters of Gram-positive cocci (1000x). (E) Staghorn calculus in a *mgb*^{-/-} kidney (thick white arrow) as shown by ultrasonography. Thin white arrows indicate acoustic shadowing.

Figure 2. Cystitis and chronic pyelonephritis in *mgb*^{-/-} animals with urolithiasis following CV (A) Low-magnification view of a *mgb*^{-/-} bladder post-CV demonstrates detrusor aplasia, multifocal dense inflammatory infiltrates (#), squamous metaplasia of the mucosa, and debris (intermingled bacteria, crystals, and sloughed cells) in the lumen (H&E, 40x). (B) Papillomatous mucosal hyperplasia with extensive submucosal inflammation and fibrosis (H&E, 200x). (C) Neutrophils and bacteria (pale blue granular clusters) in renal pelvis (H&E, 1000x). (D) Lymphocyte and plasma cell aggregates (arrows) surrounding a large muscular artery (*) near the renal pelvis are enlarged in a *mgb*^{-/-} male with a struvite stone (H&E, 40x). (E) Renal fibrosis (arrows) accompanies the intense inflammation in the peri-pelvic area (H&E, 100x). (F) Trichrome stain demonstrates progression of fibrosis (blue columns) and intense inflammatory cell infiltrates expanding the boundaries of the renal pelvis (P)(100x).

Table 1**Stone analysis**

The composition of five large bladder stones isolated from *mgb*^{-/-} male mice post-CV was determined by X-ray diffractometry.

Composition	Stone 1	Stone 2	Stone 3	Stone 4	Stone 5
Struvite (%)	93	88	94	94	93
Calcium phosphate (%)	2	10	3	3	2
Protein (%)	3	2	3	3	3
Suture (%)	2	0	0	0	2

ND = Not Detected.

Table 2
Change in $mgb^{-/-}$ urine chemistries following CV

Urine chemistries from $mgb^{-/-}$ males prior to CV (n=4) or following CV (n=5), compared to a pooled urine sample from 25 wildtype (WT) age-matched FVB/N males. Analytes in mM were adjusted to mg/dL based on molecular mass before normalization to urine creatinine (Cr) concentrations.

Analyte	WT	Pre-CV	Post-CV	<i>P</i> value
pH	5.9	5.9 ± 0.1	7.9 ± 0.2	0.0003
Calcium / Cr	0.4	0.4 ± 0.05	0.3 ± 0.2	0.5
Sodium / Cr	5.6	10 ± 2.2	12 ± 3.9	0.7
Potassium / Cr	28	36 ± 6.9	27 ± 6.7	0.4
Phosphate / Cr	6.5	12 ± 1.6	3 ± 0.7	0.008
Magnesium / Cr	2.4	3.9 ± 0.7	0.2 ± 0.1	0.01
Ammonium / Cr	3.1	7.4 ± 0.6	16 ± 3.6	0.07
Sulfate / Cr	3.1	18 ± 2.5	11 ± 0.4	0.09
Oxalate / Cr	0.3	0.6 ± 0.1	0.5 ± 0.03	0.5
Citrate / Cr	15	17 ± 2.6	1.4 ± 0.9	0.006

Values represent mean ± standard error of the mean. *P* values from unpaired 2-tailed Student's *t*-tests are shown for each analyte.