Case Report

Staphylococcus xylosus Cystitis and Struvite Urolithiasis in Nude Mice Implanted with Sustained-release Estrogen Pellets

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Female nude mice (J:NU-*Foxn1*^{nu}; age, 6 wk) were injected with 1 million MCF7 human breast cancer cells in the fourth mammary fat pads and received a 21-d sustained-release estrogen pellet (0.25 mg) subcutaneously in the dorsum of the neck. All mice were maintained in sterile housing and provided sterile water and irradiated rodent chow. Approximately 6 wk after implantation, 4 of the 30 mice showed clinical signs of depression and dehydration. The 2 animals most severely affected were euthanized and presented for necropsy. The urinary bladders of these animals were distended with variable sized white, opaque uroliths. Urinalysis revealed coccal bacteria, erythrocytes, neutrophils and struvite crystals. Urine cultures from both necropsied animals grew heavy, pure growths of *Staphylococcus xylosus*. The organism was sensitive to all antibiotics tested except erythromycin (intermediate). Analysis of the uroliths revealed 100% struvite composition. Remaining mice in the study were evaluated clinically for hydration status, the ability to urinate, and the presence of palpable stones in the urinary bladder; one additional mouse had a firm, nonpainful bladder (urolithiasis suspected). Given the sensitivity of the organisms cultured from urine samples, the remaining mice were placed on enrofloxacin in the drinking water (0.5 mg/mL). All remaining mice completed the study without further morbidity or mortality. Previous studies have reported the association of estrogen supplementation with urinary bladder pathology, including infection and urolithiasis. Here we present a case of urolithiasis and cystitis in nude mice receiving estrogen supplementation that was associated with *Staphylococcus xylosus*, which previously was unreported in this context. When assessing these nude mice for urolithiasis, we found that visualizing the stones through the body wall, bladder palpation, and bladder expression were helpful in identifying affected mice.

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Breast cancer researchers rely on models that implant immortalized human tumor cell lines into immunocompromised mouse hosts. The addition of slow-release estrogen pellets supports the growth of the breast cancer cells. However, the use of sustained-release estrogen has been associated with complications including urinary retention,^{7,15,18,23} hydroneurter, ^{15,23} hydronephrosis (unilateral and bilateral),^{3,6,23} cystitis,^{3,18,23} pyelonephritis,³ perianal dermatitis,^{6,8} and urolithiasis.^{3,8} *Proteus mirabilis*, *Escherichia coli*, and *Staphylococcus intermedius* have all been cultured from infected bladders after the administration of sustained-release estrogen pellets.²³

Here we report cystitis due to *Staphylococcus xylosus* and struvite urolithiasis in intact female nude mice treated with sustained-release estrogen pellets.

Case Report

Animals. Reproductively intact female nude mice (J:NU-*Foxn1*^{nu}; age, 6 wk; The Jackson Laboratory, Bar Harbor, ME) were used for the study and maintained at Vanderbilt University, an AAALAC-accredited institution, in accordance with the

Guide for the Care and Use of Laboratory Animals.¹² All procedures were approved by the Vanderbilt University IACUC. Housing rooms were maintained on a 12:12 h light:dark cycle with ambient room temperatures of 72 ± 2 °F (23.3 ± 1.1 °C). Soiled-bedding sentinels were used for health monitoring and were tested quarterly for common murine pathogens including endoparasites, ectoparasites, ectromelia virus, epizootic diarrhea of infant mice virus, Theiler murine encephalitis virus, K virus (mouse pneumonitis virus), lymphocytic choriomeningitis virus, mouse adenovirus types 1 and 2, mouse norovirus, mouse hepatitis virus, *Mycoplasma pulmonis*, minute virus of mice, and mouse parvovirus. The room housing these mice had tested positive for EDIM and MNV within the previous 3 y. Prior to the shipment arrival of the mice on this study, the room had had a history of fur mite infestation that was eradicated through treatment and environmental decontamination. Mice were housed in sterile IVC and maintained on cob bedding with paper nesting rolls (Enrich-o-cobs, The Andersons Lab Bedding, Maumee, OH), with unrestricted access to food and water. Mice were fed an irradiated chow diet (Autoclavable Mouse Diet 5L0D, PMI Nutrition International, St Louis, MO) and received autoclaved water. All animal manipulations used microisolation techniques.

Mice were anesthetized by using isoflurane and were injected subcutaneously and bilaterally into the fourth mammary fat pads with 1 × 10⁶ MCF7 human breast cancer cells suspended in 1:1 Matrigel (Corning, Corning, NY) and Opti-MEM (Gibco,

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Grand Island, NY). At the same time, mice received a 21-d sustained-release estrogen pellet (0.25 mg) implanted subcutaneously in the dorsum of the neck by using a trocar. Animals were examined every other week for palpable tumors. Once tumors were identified on palpation, calipers were used to measure tumor development every other day.

Clinical presentation. Approximately 6 wk after implantation, 4 of the 30 mice showed clinical signs of lethargy and dehydration. The 2 animals most severely affected were euthanized and presented for necropsy. The 2 mice that were less severely affected were treated with subcutaneous fluids (Lactated Ringers Injection USP, Hospira, McPherson, KS) until necropsy results were received.

Gross pathology. Two mice were submitted for necropsy. The distended urinary bladder was easy to visualize through the abdominal wall (Figure 1 A). The urinary bladders of both animals were markedly distended with variable sized, white, opaque uroliths (Figure 1 B). One mouse had marked unilateral ureteral dilation with abundant white material within the ureteral lumen (Figure 1 C). Urinalysis revealed coccal bacteria, erythrocytes, neutrophils, and struvite crystals (Figures 2 A and B). After necropsy evaluation, the remaining mice in the study were evaluated for hydration status, and their bladders were palpated for uroliths. One of the 2 less affected animals had a firm urinary bladder but was urinating on its own. All mice were placed on enrofloxacin (Baytril Bayer, Pittsburgh, PA) in the water at a concentration of 0.5 mg/mL. All remaining animals were monitored until experimental termination and showed no further progression of clinical signs.

Microbial culture. Swabs of the urine from the bladders of the 2 necropsied mice were submitted to the Athens Veterinary Diagnostic Laboratory (AVDL-UGA) for culture and antimicrobial sensitivity evaluation.

Tissues (heart, lungs, liver, reproductive tract, kidneys, ureters, urinary bladder, liver, intestines, spleen, and pancreas) were fixed in 10% neutral buffered formalin, routinely processed by dehydration, embedded in paraffin, sectioned at 5 μ m, placed on charged slides, and stained with hematoxylin and eosin.

Histopathologic findings. Multifocal neutrophilic tubulointerstitial nephritis along with focally extensive interstitial fibrosis and tubular loss was present variably in the kidneys of both necropsied animals. One animal had less severe changes within the kidneys but showed evidence of bilateral, neutrophilic tubulointerstitial nephritis with unilateral, suppurative pyelonephritis and hydronephrosis. Tubules were distended with large numbers of neutrophils and coccal bacteria (Figure 3 A). Severe, unilateral, suppurative pyelonephritis was present in the other necropsied mouse (Figure 3 B). The pyelonephritis was severe enough to cause adjacent renal pelvis atrophy. Unilaterally, one ureter was markedly dilated with an admixture of neutrophils, necrotic debris, and coccal bacteria. Small colonies of coccal bacteria were seen within the renal pelvis, ureter, and renal tubules.

Urinary bladder walls were markedly thickened and edematous. The bladder lumen contained multiple masses of amorphous, basophilic material admixed with neutrophils and coccal bacteria (Figure 3 C). Gram staining of the urinary bladder section revealed large numbers of gram-positive cocci. Multifocal ulceration of the urothelium was present also, with neutrophilic infiltration of the ulcerated urothelium and underlying submucosa (Figure 3 D).

Uteri from both mice showed moderate to severe cystic endometrial hyperplasia. Rare neutrophils and desquamated epithelial cells were present within the lumen.

Uroliths were submitted to the Minnesota Urolith Center (St Paul, MN) for quantitative analysis. In both animals, analysis revealed 100% struvite composition. Urine culture and sensitivity revealed a pure growth of *Staphylococcus xylosum*, which was immediately resistant only to erythromycin.

Discussion

Sustained-release estrogen pellets (especially the 0.18 mg, 60-d pellets) have been shown to yield variable serum concentrations after implantation.¹¹ Initial spikes well above physiologic range were noted, along with rapid depletion.¹¹ Another study showed unreliable serum estradiol levels from sustained-released pellets throughout an 8-wk study period, with peak plasma concentrations occurring at week 2.³ In addition, the method of handling of the pellets can affect the release of estrogen from the implant. Reports of variability in the rate of estradiol release from pellets can depend on the delivery method used. Oral dosing (with estrogen placed in a commercially available chocolate-hazelnut spread) and subcutaneous silastic capsules were noted as reliable methods to deliver and maintain consistent physiologic plasma levels of 17 β -estradiol in ovariectomized mice.¹¹ The same study indicated that sustained-released pellets provided a significantly increased plasma concentration and were unpredictable regarding day-to-day levels.¹¹ Administration of estrogen in drinking water at doses of 200 and 1000 nM has been demonstrated to provide uterine responses in the absence of urinary retention.¹⁴ Estradiol can increase urethral resistance and the volume of residual urine in the bladder after micturition and decreases urinary frequency in mice.⁷ The mechanism of urinary retention in mice is thought to be associated with urethral occlusion due to increased urethral resistance, submucosal fibrosis, and mucosal epithelial stratification.¹³ These estrogenic effects on urethral physiology can affect each animal differently as a function of variable levels of estrogen, and estrogen supplementation is thought to be a key factor in the development of cystitis and urolithiasis, as seen in the mice we report here.

Estrogen supplementation itself has been associated with urinary stasis and bladder luminal distention, which can lead to subsequent cystitis.^{7,13} Only a few mice in our study showed clinical signs of dehydration, lethargy, and urinary bladder distention. We presume that the remaining mice had some degree of pathology, but this assumption was not confirmed because the animals were not submitted for necropsy evaluation at the end of the study. However, previous studies have indicated that a 6-wk treatment with 1.7-mg estrogen pellets led to retention of urine.¹⁴ Another study using 0.18-mg sustained-release estrogen pellets showed microscopic evidence of pyelonephritis, urethritis, and cystitis in some animals as early as 4 wk.³ We suspect that some of the remaining mice in this cohort likely had some pathologic change in the urinary tract, but the antibiotic therapy prevented subsequent cystitis and urolithiasis. Cystitis has been shown to serve as a nidus for the development of urolithiasis.^{18,23} Struvite urolithiasis in animals is associated with the presence of urease-producing bacteria, including *Staphylococcus* and *Proteus* species.

Urine cultures from mice treated with estrogens and subsequently developing cystitis have implicated several bacteria, including *Proteus mirabilis*, *Escherichia coli*, and *Staphylococcus intermedius*.^{6,23} In addition, *S. intermedius* has been associated with mild, perineal ulcerative dermatitis and cystitis with struvite crystalluria in female nude mice that received 1.7-mg subcutaneous sustained-release 17 β -estradiol pellets.⁸ *S. xylosum* had not previously been reported in association with cystitis in mice, but this organism is a common commensal bacteria of

Figure 1. (A) Ventral abdomen of mouse. The urolith-distended urinary bladder can be visualized through abdominal wall (circled). (B) Urinary bladder has been incised to show multiple uroliths. (C) Unilaterally, one of the ureters was distended with urine and thick, white material (arrow). Both uterine horns are markedly enlarged.

Figure 2. Urine. (A) Transitional epithelial cells, neutrophils with intracytoplasmic bacteria (arrow), and extracellular coccidial bacteria are seen in this photomicrograph. (B) Urine. Abundant struvite crystals are present. Giemsa stain; magnification, 100 $\times$.

vivarium-housed mice. *S. xylosus* typically lives on the skin and mucosal surfaces of mice and rarely induces evidence of pathogenicity. When the organism does cause clinical concern, most of the problems are seen in immunodeficient strains of mice and primarily as dermatitis and abscesses.¹⁰ A fatal epizootic dermatitis was reported in athymic nude mice infected with *S. xylosus*.^{2,20} In NADPH oxidase-deficient strains, soft tissue abscessation was present and spread to regional lymph nodes, lungs, muscle, bones and meninges.⁷ Rag^{-/-}Tp12^{-/-} mice developed a severe dermatitis, and PCR analysis detected *S. xylosus* in feces, skin, lymph nodes, and lungs.¹ In addition to its recovery from mice, *S. xylosus* has been isolated as a causative agent of mastitis in sheep⁵ and cattle,^{4,16} nasal dermatitis in gerbils,²¹ and cystitis and peritonitis in a calf.¹⁹ In humans, *S. xylosus* has been associated with 2.2% of the coagulase-negative staphylococci urinary tract infections in one hospital's published study.⁹ This organism has also been associated with acute pyelonephritis in humans.²²

We suspect that the cystitis and urolithiasis present in our female nude mice is secondary to therapy with sustained-release estrogen. However, the bacterial etiology of the associated cystitis differs from previous reports of *P. mirabilis*, *E. coli*, and *S. intermedius*.^{6,23} *Staphylococcus xylosus* is a commensal organism that usually does not cause disease in immunocompetent mice. *S. xylosus* was cultured directly from the urine of our mice, and microscopic evidence of cystitis was detected. Therefore *S. xylosus* must be considered as a potential pathogen in immunodeficient mice. Estrogen supplementation in the form of sustained-release pellets in nude mice enhances the risk of urinary retention, thereby increasing the potential for cystitis and the development of urolithiasis. Daily estrogen supplementation through delivery in drinking water or commercial nut paste is effective at maintaining consistent physiologic hormone levels, and no cystitis or urolithiasis has been reported in association with these methods.^{11,14} Frequent monitoring of the urinary bladder (through both visualization and palpation) can be instituted

Figure 3. (A) Multifocal, neutrophilic tubulointerstitial nephritis with intralumenal coccoid bacteria are present in this kidney section. Hematoxylin and eosin stain; magnification, 5 $\times$. (B) This histologic section of the renal hilus and ureter illustrates the large number of neutrophils and amorphous debris in found in the ureteral lumen (*). Hematoxylin and eosin stain; magnification, 5 $\times$. (C) Bladder wall. This section shows a markedly thickened urinary bladder, with neutrophils and debris in the lumen. Hematoxylin and eosin stain; magnification, 5 $\times$. The inset (Gram stain) is a higher magnification of the bladder luminal contents and shows large numbers of gram-positive coccoid bacteria and abundant neutrophils. (D) Higher magnification (10 $\times$) of the bladder wall, showing ulcerative and neutrophilic cystitis.

as an aide in determining distention and urolithiasis. Because struvite uroliths are radiopaque, radiographic evaluation might be used to detect affected animals noninvasively.¹⁷ In addition, clinical scoring systems using perineal fur staining, abdominal

distention, hair coat quality, hydration, and urination have been developed to identify problems early during estrogen supplemented models.³ Our findings show that staff should be aware of the potential for cystitis and urolithiasis in J:NU-*Foxn1*^{nu} mice

implanted with sustained-release estrogen pellets. This propensity actually may extend to other strains of immunodeficient mice, because we subsequently have seen the same presentation and identified the same bacteria in NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ mice that received subcutaneous sustained-release estrogen according to this same model. Given the association of *S. xylosum* with infections in immunoincompetent mice, the organism might need to be identified as an excluded pathogen in those populations. The use of alternative routes of administration of estrogen (orally in water or in a favored foodstuff) and closer monitoring should be considered during the development of these experiments. In addition, we subsequently have recommended use of enrofloxacin in the water for studies at our institution that use sustained-release estrogen, to reduce the potential for urinary tract infection secondary to urine stasis.

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