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CASE REPORT

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Uroliths composed of antiviral compound GS-441524 in 2 cats undergoing treatment for feline infectious peritonitis

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Abstract

Feline infectious peritonitis (FIP) historically has been a fatal disease in cats. Recent unlicensed use of antiviral medication has been shown to markedly improve survival of this infection. An 8-month-old female spayed domestic short-haired cat undergoing treatment for presumptive FIP with the antiviral nucleoside analog GS-441524 developed acute progressive azotemia. Abdominal ultrasound examination identified multifocal urolithiasis including renal, ureteral, and cystic calculi. Unilateral ureteral obstruction progressed to suspected bilateral ureteral obstruction and subcutaneous ureteral bypass (SUB) was performed along with urolith removal and submission for analysis. A 2-year-old male neutered domestic medium-haired cat undergoing treatment for confirmed FIP with GS-441524 developed dysuria (weak urine stream, urinary incontinence, and difficulty expressing the urinary bladder). This cat also was diagnosed sonographically with multifocal urolithiasis requiring temporary tube cystostomy after cystotomy and urolith removal. In both cases, initial urolith analysis showed unidentified material. Additional testing confirmed the calculi in both cats to be 98% consistent with GS-441524. Additional clinical studies are required to determine best screening practices for cats presented for urolithiasis during treatment with GS-441524.

KEYWORDS
antivirals, coronavirus, feline, fip, GS-441524, urolith

1 | INTRODUCTION

Feline infectious peritonitis (FIP) historically has been a fatal disease. In recent years, nonlicensed antiviral medications sourced through nonclinical channels have improved survival.[1] Research and clinical evidence has shown them to be safe and effective.[2-4] We describe 2 cats treated with the nucleoside analog GS-441524,[2,3] a metabolite of the prodrug remdesivir (Veklury, Gilead, Foster City, California), that developed uroliths composed of GS-441524. Urolithiasis is a previously unreported adverse effect of GS-441524 in cats.

Abbreviations: AKI, acute kidney injury; FIP, feline infectious peritonitis viral infection; FTIR, Fourier transform infrared spectroscopy; LC/MS, mass spectrometry; NMR, nuclear magnetic resonance; SUB, subcutaneous ureteral bypass; XRD, x-ray powder diffractometry.

2 | CASE NO. 1

An 8-month-old female spayed domestic short-haired cat was presented to the emergency service at Charleston Veterinary Referral

Center for management of dehydration, weakness, and lethargy. Four months before presentation, the cat was presumptively diagnosed with FIP with intracranial involvement based on encephalopathic signs (seizures, altered mentation, generalized weakness), nonspecific pulmonary infiltrates, progressive anemia requiring transfusion, hyperbilirubinemia (2.5 mg/dL; reference interval [RI], 0-0.9), and hyperglobulinemia (7.6 g/dL; RI, 2.8-4.8).

After the presumptive FIP diagnosis, the owner obtained an antiviral medication through an online support group (FIP Warriors® 5.0). The unlabeled vials were reported to be GS-441524. The cat was treated with SC injections q8h (dose unavailable). Clinical status gradually improved and complete resolution of clinical signs was documented during a neurology consultation 1 month after the presumptive diagnosis.

The cat remained clinically normal at home for an additional 2 months. Injectable GS-441524 treatment was continued and approximately 6 weeks into treatment, decreased in frequency to q12h. Treatment then was transitioned to PO GS-441524 after approximately 3 months. Approximately 13 weeks into treatment, the cat developed lower urinary tract signs characterized by hematuria. The primary care veterinarian diagnosed ammonium magnesium phosphate crystalluria and a urinary tract infection. The cat was treated with enrofloxacin (5.6 PO mg/kg q24h).

At the time of referral to Charleston Veterinary Referral Center, approximately 4 months after the presumptive FIP diagnosis, the cat was presented in lateral recumbency with evidence of dehydration and hypovolemic shock. It was referred after being evaluated for reluctance to move, urinary incontinence, and hyporexia for 4 days. Serum biochemistry disclosed azotemia (BUN concentration, 161 mg/dL; RI, 16-33; serum creatinine concentration, 7.6 mg/dL; RI, 0.6-1.6), hyperphosphatemia (11.5 mg/dL; RI, 4.5-10.4), hypernatremia (166 mmol/L; RI, 150-165), hypokalemia (3.3 mmol/L; RI, 3.7-5.9), hyperchloremia (127 mmol/L; RI, 115-126), and hyperglobulinemia (4.9 g/dL; RI, 2.8-4.8). Laboratory results obtained 1 month before indicated a serum creatinine concentration of 1.5 mg/dL and BUN concentration of 40 mg/dL. The cat was admitted for treatment of acute kidney injury (AKI) and shock.

On days 1 and 2 of hospitalization, recurrent neurologic and renal viral disease was suspected based on persistently altered mentation, nonambulatory status, and persistent azotemia despite rehydration. On day 2, abdominal ultrasound examination indicated bilateral renal mineralization and mild loss of corticomedullary distinction, left-sided focal subcapsular hypoechoic thickening, right hydronephrosis and proximal ureteral dilatation (up to 0.52 cm) with multifocal ureteroliths, mild left pyelectasia (0.08 cm), renoliths within the pelvis, multiple ureteroliths within the proximal left ureter (0.11 cm), multiple cystoliths within the urinary bladder, and multiple punctate hyperechoic foci within the urethra. Urinalysis disclosed a USG of 1.008 (RI, 1.015-1.060), pH 7.0 (RI, 5.5-7.0), proteinuria (2+), and a sediment with pyuria, bacteriuria, and 21-50 struvite crystals per high powered field. Radiopaque uroliths were not observed in the kidneys or ureters, but indistinct radiopacities were visualized in the urinary bladder by survey abdominal radiography (Figure 1). Because of clinical concern regarding prognosis and owner financial limitations, medical management for ureteral obstruction and AKI was elected.

On day 4 of hospitalization, azotemia increased within a 24-hour period from a serum creatinine concentration of 3.5 mg/dL and BUN concentration of 110 mg/dL to 4.3 mg/dL and >140 mg/dL, respectively. Localized urinary ultrasound examination indicated progressive left renal pelvic dilatation. Surgical intervention was offered, with placement of a bilateral subcutaneous ureteral bypass (SUB) system recommended because of the multifocal proximal ureteroliths, and the owner elected to proceed.

Laparotomy was performed on day 4 and bilateral SUB systems were placed. A cystotomy was performed and urocystoliths were removed and submitted for analysis. The calculi were sharply marginated, irregularly shaped, and amber in color (Figure 2). Azotemia improved but persisted after SUB placement (BUN concentration, 91 mg/dL; serum creatinine concentration, 1.9 mg/dL on day 8). The cat remained stuporous to intermittently comatose postoperatively with persistent pressor-dependent hypotension. On day 9 of hospitalization, acute cardiopulmonary arrest occurred. Resuscitation was unsuccessful and necropsy was not performed. Urine cultures performed pre- and intraoperatively and returned postmortem were positive for *Escherichia coli*.

Initial urolith analysis indicated unidentifiable mineral (Minnesota Urolith Center, College of Veterinary Medicine University of Minnesota, 1352 Boyd Avenue, St Paul, Minnesota). The sample was sent to a second reference laboratory where initial analysis also indicated unidentifiable mineral (Louis C. Herring & Co, 1111 South Orange Avenue, Orlando, Florida). Because of clinical concern for potential medication-based urolithiasis, reference standard of GS-441524 was obtained, and the uroliths were found to be 98% consistent with GS-441524 (Louis C. Herring & Co). This finding was confirmed by the Minnesota Urolith Center.

3 | CASE NO. 2

A 2-year-old male neutered domestic medium-haired cat was presented to the Cumming's School of Veterinary Medicine at Tufts

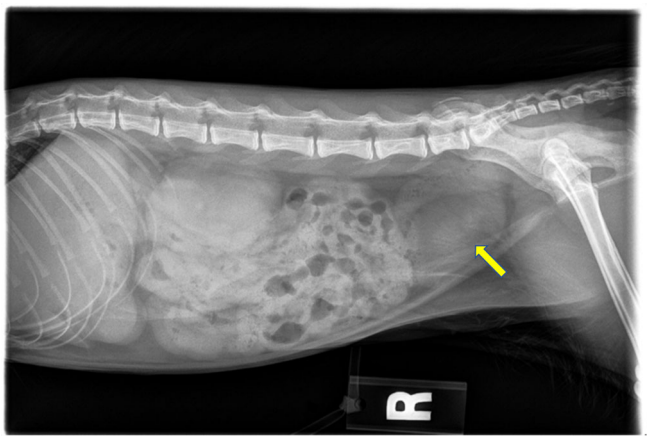

FIGURE 1 Indistinct radiopacities (arrow) within the urinary bladder depicted on a right lateral abdominal radiograph.

University for management of urethral obstruction and recent weight loss. The cat was sedated and the obstruction successfully relieved. Results of a CBC were consistent with a stress leukogram. Serum biochemistry was largely unremarkable other than abnormal liver function test results (serum alkaline phosphatase [ALP], 188 U/L; RI, 10-79; total bilirubin concentration, 1.0 mg/dL; RI, 0.1-0.3; serum aspartate transaminase [AST], 146 U/L; RI, 5-42; serum alanine transaminase [ALT], 59 U/L; RI, 25-145). The cat was hospitalized for supportive care.

The cat was persistently inappetent in the hospital, and peritoneal effusion and multifocal lymphadenopathy were found by abdominal ultrasonography. Samples of the lymph node were obtained by fine needle aspiration and coronavirus PCR testing was positive with 426 400 mRNA copies/mL (Auburn University Clinical Pathology Laboratory, Auburn, Alabama).

While hospitalized, progressive neurologic signs developed (nonambulatory paraparesis, focal head tremors). The owner obtained GS-441524 from an online support group (FIP Warriors® 5.0) and began SC administration at 10 mg/kg twice daily. The cat then was transitioned to remdesivir 20.6 mg/kg IV once daily but subsequently restarted on GS-441524 because of progressive neurologic signs. The cat improved and was discharged from the hospital. During hospitalization, liver function test results remained abnormal (ALT, 226 U/L; RI, 25-145 U/L; ALP, 119 U/L; RI, 10-79 U/L; total bilirubin concentration, 0.2 mg/dL; RI, 0.1-0.3; AST, 72 U/L; RI, 5-42 U/L) but resolved before discharge. After discharge, the GS-441524 dose was increased to 15 mg/kg SC q12h and GC-376, another antiviral used off-label in cats with FIP, concurrently was started at a dosage of 20 mg/kg SC q12h. Neurologic signs improved, but manual expression of urine was required q8h.

Approximately 2 weeks after the diagnosis of FIP, urinalysis disclosed a pH of 7.0, many magnesium ammonium phosphate crystals, few bacteria, and many unidentified needle-like crystals. A urine culture was positive for *Corynebacterium amycolatum* and the cat was treated with amoxicillin/clavulanic acid. At that time, the cat was transitioned to PO GS-441524 at a dosage equivalent to 16 mg/kg q12h.

Approximately 6 weeks later, the cat presented to The University of Georgia Veterinary Medical Center for a weak urine stream and dribbling urine between bladder expressions. It was sedated and the urinary bladder was expressed with difficulty. Urinalysis disclosed USG 1.016, 1+ proteinuria, hematuria, and magnesium ammonium phosphate crystals. Urine culture was positive for *Enterococcus faecalis*. Mineralized bladder sediment and a possible proximal urethrolith were observed ultrasonographically. The cat was treated supportively, but on day 3 serum biochemistry disclosed azotemia (serum creatinine concentration, 7.3 mg/dL; RI, 0.7-1.8; BUN concentration, 124 mg/dL; RI, 18-35; hyperkalemia, 7.4 mmol/L; RI, 0.7-5.3; and hyperphosphatemia, 10.8 mg/dL; RI, 2.8-5.7). On day 4, a repeat ultrasound examination indicated bilateral pyelectasia, left ureteral dilatation with suspected mineral debris, nephroliths, mineral debris in the urinary bladder and uroliths in the proximal urethra. The cat then developed progressive bilateral hydronephrosis. At that time, GS-441524 administration was changed to injectable GS-441524 at a dose of 15 mg/kg SC q12h.

Uroliths in the urinary bladder and proximal urethra, improved hydronephrosis and ureteral dilatation, and chronic degenerative renal changes (hypoechoic cortical wedge-shaped lesions consistent with infarcts) were found ultrasonographically on subsequent examination during hospitalization. Cystotomy was performed to remove the uroliths (Figure 3) and place a temporary cystostomy tube. The uroliths

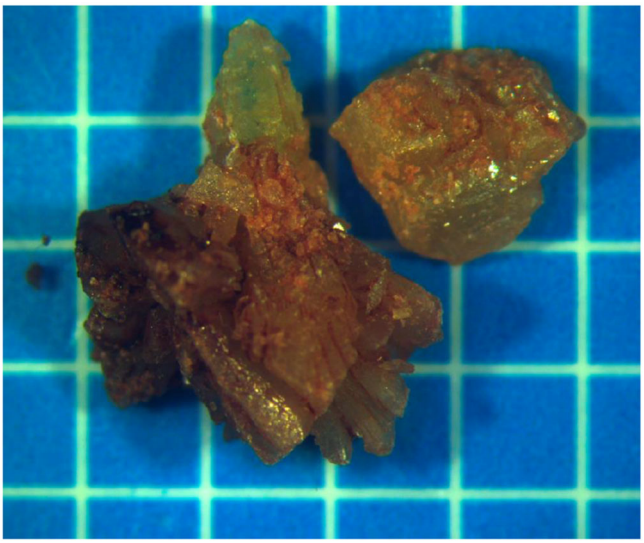

FIGURE 2 Case 1, cystolith from an 8-month-old female spayed domestic short-haired cat presumptively diagnosed with feline infectious peritonitis and treated with GS-441524. Louis C. Herring & Co.

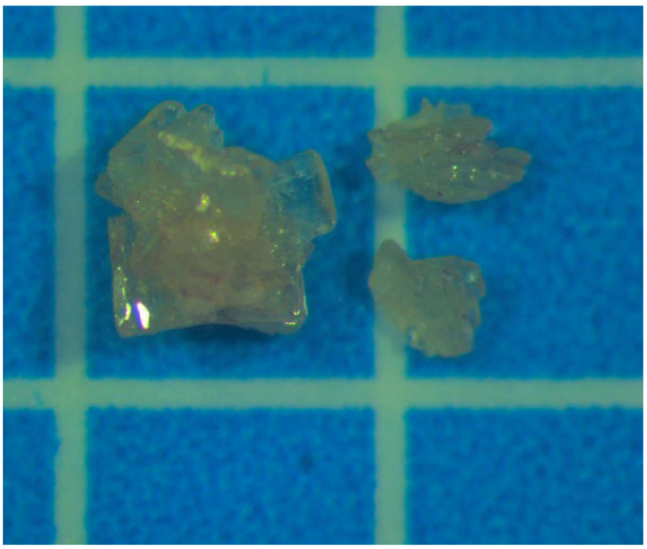

FIGURE 3 Case 2 cystolith from a 2-year-old male neutered domestic medium-haired cat diagnosed with feline infectious peritonitis and treated with GS-441524. Louis C. Herring & Co.

were submitted for analysis (Minnesota Urolith Center, College of Veterinary Medicine University of Minnesota, 1352 Boyd Avenue, St Paul, Minnesota). The cat improved clinically and was discharged after azotemia plateaued (serum creatinine concentration, 3.1 mg/dL; BUN concentration, 75 mg/dL). Two days later, the cat was re-presented for progressive clinical decline (inappetence, lethargy). At that time, a CBC indicated a normocytic normochromic anemia (HCT, 15%; RI, 33%-49%) and leukopenia (WBC, 1.1 K/μL; RI, 4.8-15.3) characterized by a neutropenia (0/μL; RI, 1.85-9.99 K/μL). Serum biochemistry disclosed static azotemia. Because of concerns regarding overall prognosis, euthanasia was elected but necropsy was not performed.

Initial urolith analysis indicated 100% unidentified mineral. Subsequent analysis confirmed the composition to be 98% GS-441524.

4 | STONE ANALYSIS

GS-441524 reference standard was subjected to Fourier transform infrared spectrophotometry (FTIR) and x-ray powder diffractometry (XRD) to obtain identifying FTIR and XRD references for GS-441524. Urolith 1 (Case 1) then was subjected to FTIR and XRD, and patterns confirmed urolith composition of 98% GS-441524. Urolith 2 (Case 2) composition subsequently was identified as 98% GS-441524 using FTIR (Louis C. Herring & Co).

Further confirmatory analyses were performed at a second laboratory (Department of Drug Discovery and Biomedical Sciences, Medical University of South Carolina, 70 President Street, Charleston, South Carolina) and included ultrahigh-performance-liquid chromatography, mass spectrometry (LC/MS), and ^{1}H- and ^{13}C nuclear magnetic resonance (NMR).

5 | DISCUSSION

We describe medication-based urolithiasis in 2 cats treated with unlicensed GS-441524. Uroliths in cats are commonly encountered in veterinary practice. Struvite and calcium oxalate uroliths occur most commonly, but other urolith types may be encountered.[5] The most common techniques used for urolith analysis are optical crystallography and infrared spectroscopy; in difficult to evaluate uroliths, more advanced techniques such as XRD may be used.[6]

Medication-based urolithiasis in humans has been defined as "stones formed by direct crystallization of a poorly soluble, renally excreted medication or its metabolites, as well as … stones formed when medications crystallize around previously formed urinary stones."[7] These uroliths are infrequent in humans (1%-2%).[7,8] Few reports of medication-based uroliths exist in small animals, and <0.1% of uroliths in cats have been reported to be drug metabolites.[5] Antiviral drugs have been reported in humans as causes for medication-based urolithiasis.[7,9,10] Drugs that have been reported to cause medication-based urolithiasis in veterinary patients include sulfonamide antimicrobials, fluoroquinolones, primidone, and doxycycline.[11] To our knowledge, uroliths comprised of antiviral medications or their metabolites have not been reported previously in cats.

In these cases, uroliths were precipitations of the medication being used as confirmed by LC/MS, FTIR, XRD, UPLC, and NMR. Urolith analysis was possible because of clinical suspicion of GS-441524 medication-based urolithiasis and subsequent comparison with a laboratory reference standard. Our initial differential diagnosis for the azotemia in cat 1 was FIP with renal involvement and subsequent dysfunction or AKI secondary to systemic illness from FIP with a prerenal contribution. Urinary tract ultrasonographic evaluation in patients treated with GS-441524, especially with high doses, should be considered to evaluate for the presence of this complication. Contrast radiography also may be considered (particularly when ultrasonography is not available) because these uroliths are radiolucent on abdominal radiographs.

Because of the unlicensed status, adequate variables for evaluating drug safety of GS-441524 have not been established in cats. In human patients, remdesivir and its metabolite GS-441524 are recommended to be used with caution in patients with impaired renal function.[12] With no previous reports of this complication in cats receiving GS-441524, it is possible that predisposing factors (including renal dysfunction) may make some cats more likely than others to form these medication-based uroliths.[2-4] It seems clinically reasonable to monitor renal function in cats with FIP that are receiving GS-441524, with consideration for dose or frequency reduction in patients with renal dysfunction although additional controlled clinical studies are needed.

Multiple patient and drug-related factors have been suggested as predisposing factors for medication-based urolithiasis in humans. Patient factors can include history of uroliths, obesity, urinary stasis, underlying metabolic abnormalities, and urinary abnormalities including infection or altered pH.[7] It is unclear in our cases if infection could have predisposed to urolith formation, or if the presence of the uroliths was a contributing factor in the development of infection. Risk factors for obstructive upper urinary tract uroliths in cats include female sex, bilateral uroliths, and young age.[13] Patient 1 met all of the criteria, including being <1 year of age. Both cats in our study had crystalluria, lower urinary tract signs and either documented or suspected lower urinary tract infections. Both also developed azotemia during their clinical course. The contribution of these factors is uncertain but should be considered in future studies as potential risk factors.

Drug-related factors include higher doses, longer course of treatment, high urinary excretion, low water solubility of the drug or its metabolites, as well as size and morphology of the drug crystals. Because of the lack of a licensed product, it is impossible to state the exact dose each patient received. Likewise, there is no standardized treatment duration. Marked urinary excretion of antivirals has been noted in humans, rats, and dogs.[14] Solubility is a known concern, as noted in previous literature.[2] Use of an unlicensed product, particularly without veterinary oversight, results in the potential for additive risks to patients undergoing FIP treatment. Additionally, cat 2 was treated with both GS-441524 and remdesivir. A recent study found

no clinically relevant adverse effects of these medications in the treatment of FIP.[15] It is uncertain if the concurrent use of the drugs potentially contributed to urolith formation.

Increasing hydration, dietary modifications such as salt restriction and alterations in urinary pH also have been suggested as preventive measures in human patients receiving antiviral drugs to decrease risk of medication-based urolithiasis.[7] Along with monitoring laboratory variables, including renal function and urinalysis, increasing hydration (SC or enteral fluid administration) where it is not contraindicated may be considered. Renal histopathology by biopsy or necropsy in future cases may be useful in evaluating the role of crystal precipitation in the pathogenesis of AKI. Clinicians may consider screening for urolithiasis in cats that become azotemic or develop lower urinary tract signs during treatment with GS-441524. A larger retrospective or prospective study would facilitate assessment of risk factors and additional preventive measures.

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CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

GS-441524, Remdesivir, and GC-376 used off label.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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