A FimH Inhibitor Prevents Acute Bladder Infection and Treats Chronic Cystitis Caused by Multidrug-Resistant Uropathogenic Escherichia coli ST131

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Background. Escherichia coli O25b:H4-ST131 represents a predominant clone of multidrug-resistant uropathogens currently circulating worldwide in hospitals and the community. Urinary tract infections (UTIs) caused by E. coli ST131 are typically associated with limited treatment options and are often recurrent.

Methods. Using established mouse models of acute and chronic UTI, we mapped the pathogenic trajectory of the reference E. coli ST131 UTI isolate, strain EC958.

Results. We demonstrated that E. coli EC958 can invade bladder epithelial cells and form intracellular bacterial communities early during acute UTI. Moreover, E. coli EC958 persisted in the bladder and established chronic UTI. Prophylactic antibiotic administration failed to prevent E. coli EC958-mediated UTI. However, 1 oral dose of a small-molecular-weight compound that inhibits FimH, the type 1 fimbriae adhesin, significantly reduced bacterial colonization of the bladder and prevented acute UTI. Treatment of chronically infected mice with the same FimH inhibitor lowered their bladder bacterial burden by >1000-fold.

Conclusions. In this study, we provide novel insight into the pathogenic mechanisms used by the globally disseminated E. coli ST131 clone during acute and chronic UTI and establish the potential of FimH inhibitors as an alternative treatment against multidrug-resistant E. coli.

Keywords. E. coli ST131; uropathogenic E. coli; urinary tract infection; antibiotic resistance; type 1 fimbriae; mannoside; biofilm.

Uropathogenic Escherichia coli (UPEC) causes the majority (approximately 80%) of urinary tract infections (UTIs), resulting in an estimated 150 million cases globally per year [1]. Women are primarily affected, with almost 50% expected to experience 1 UTI in their lifetime and 20%–30% experiencing a recurrence within 6 months of an acute episode [2]. Recurrent UTI contributes significantly to UTI-associated morbidity and heavily imposes on public health resources [3]. The overwhelming success of antibiotics has led to the commonly held belief that cystitis is a self-limiting disease. However, placebo-controlled studies indicate that the natural course of cystitis can last for weeks in approximately half of all patients [4, 5]. Furthermore, many individuals have chronic recurrent UTI and require suppressive antibiotic therapy to prevent frequent recurrences. While, for the majority of cases, a short course of antibiotic therapy still remains an effective treatment, antibiotic resistance among UPEC strains is continually increasing. Despite large variation in UPEC antibiotic
resistance rates between different countries and among different UTI patient cohorts, large surveillance studies conducted during the last 20 years across Europe, North America, and South America highlight that 20%–50% of UPEC strains can be resistant to commonly prescribed antibiotics, such as trimethoprim-sulphamethoxazole (TMP-SMZ), fluoroquinolones, and β-lactams [3]. This trend has led to an increased rate of treatment failure with standard antibiotic therapies and the increased use of second- and third-line therapies, further promoting the emergence of multidrug-resistant UPEC. Taken together, the increasing antibiotic resistance among UPEC strains and the paucity of new antibiotics in development threaten to greatly complicate UTI management in the near future.

UPEC infection proceeds through a well-described pathogenic pathway in the lower urinary tract [6]. Numerous UPEC virulence factors, including adhesins, toxins, and iron-acquisition systems, have been identified [7]. Type 1 fimbiae mediate host-pathogen interactions critical in pathogenesis. The FimH adhesin, located at the tip of type 1 fimbiae, binds to mannosylated glycoproteins on human and mouse bladder epithelial cells and facilitates UPEC colonization and invasion of the bladder epithelium (urothelium) [8, 9]. After invasion, UPEC can escape the endocytic vesicle and rapidly replicate within the urothelial cell cytoplasm, forming intracellular bacterial communities (IBC) that resemble biofilms [10, 11]. IBC formation occurs primarily during acute bladder infection and allows bacteria to rapidly expand in numbers and establish infection in a host niche that is protected from neutrophil attack and antibiotics [12–14]. The IBC pathogenic cascade has been extensively characterized in a murine model of cystitis [15, 16], and exfoliated bladder epithelial cells containing IBCs have been significantly observed in urine from women with recurrent UTI but not from healthy controls [17]. Animal models have defined 2 distinct chronic outcomes to experimental UPEC infection of the bladder in immunocompetent hosts: spontaneous resolution of bacteriuria that is often accompanied by a persistent latent intracellular infection (ie, a quiescent intracellular reservoir [QIR]) [16] or by chronic cystitis [6]. UPEC within QIRs can reemerge months later to seed a recurrent infection [16]. On the other hand, the development of chronic cystitis can sensitize mice to recurrent UTI when they are challenged with a new bacterial strain after clearance of infection following antibiotic treatment [18]. Thus, UPEC can effectively colonize the host bladder and establish acute, chronic, or recurrent infections.

On the basis of murine models that mimic aspects of human disease, FimH is critical for UPEC pathogenesis. FimH is also under positive selection in human clinical isolates of UPEC, further supporting its role in human disease [19]. Inhibiting FimH function may thus represent a therapeutic strategy for the treatment and prevention of UTI. In this respect, novel biaryl mannoside FimH inhibitors, including compound ZFH-04269 (4’-[α-D-Mannopyranosyl]-N,3’-dimethylbiphenyl-3-carboxamide), were recently shown to attenuate UPEC virulence in mice by impeding FimH binding to the bladder epithelium, thereby preventing bacterial invasion and IBC formation and resulting in significantly reduced bladder bacterial titers during both acute and chronic cystitis [20, 21].

Recently, a clone of UPEC belonging to serotype O25b:H4 and sequence type 131 (E. coli ST131) has emerged as a leading multidrug-resistant pathogen causing urinary tract and bloodstream infections in hospitals and the community. Several reports have demonstrated the global distribution of this lineage, indicating that it constitutes a major threat to public health worldwide [22]. The successful dissemination of E. coli ST131 is thought to be due to a combination of antibiotic resistance and virulence. E. coli ST131 commonly harbor genes encoding several types of β-lactamases, particularly of the CTX-M family of extended-spectrum β-lactamases (ESBLs), and are typically associated with limited treatment options [23–26]. While the E. coli ST131 genes conferring resistance to multiple classes of antibiotics have been the focus of many recent studies, the virulence mechanisms used by this clone are less well understood. Although E. coli ST131 strains are derived from phylogenetic group B2, which includes several characterized pathogenic E. coli clonal groups, only a few virulence genes (eg, fimH, iutA, and sat) appear to be uniformly encoded in all E. coli ST131 strains [24, 25, 27, 28]. Clinical studies have demonstrated transmission of virulent E. coli ST131 strains between family members [29, 30], but the factors that contribute to the widespread dissemination of this lineage and the pathogenic mechanisms used during UTI remain poorly defined.

We have previously demonstrated that the genome sequence of a representative multidrug-resistant UPEC ST131 isolate, E. coli EC958, contains genes encoding a variety of potential virulence factors, including numerous adhesins, autotransporters, and siderophore receptors [28]. In this study, we examined the pathogenic lifestyle of E. coli EC958 during experimental UTI in mice with acute and chronic infection. We demonstrated that E. coli EC958 is able to invade the bladder epithelium and form IBCs. In addition, we showed that E. coli EC958 can persist in the bladder and establish chronic infection. We also demonstrated a key role for type 1 fimbiae in the ability of E. coli EC958 to establish bladder infection, as prophylactic treatment with an oral FimH inhibitor prevented acute cystitis. Moreover, a single oral dose of the same FimH inhibitor significantly reduced the bacterial load in the bladder of mice chronically infected with E. coli EC958. This study revealed the potential of FimH inhibitors as an alternative treatment against multidrug-resistant UPEC strains.

METHODS

Bacterial Strains and Culture Conditions
E. coli EC958 was isolated from the urine of a patient presenting with community UTI in the northwest region of England
and is a representative member of the United Kingdom epidemic strain A (pulsed-field gel electrophoresis type), one of the major pathogenic lineages causing UTI across the United Kingdom [31]. E. coli EC958 is a multidrug-resistant phylogenetic group B2 strain of serotype O25b:H4 and sequence type 131; its genome sequence has been recently determined [28]. For mouse infections, E. coli EC958 was typically cultured in Luria broth (LB) under type 1 fimbiae–inducing conditions (ie, 3 sequential 24-hour cultures incubated statically at 37°C). Functional expression of type 1 fimbiae by E. coli EC958 was confirmed by agglutination of yeast cells (Saccharomyces cerevisiae) as previously described [32].

**Mouse Infections With E. coli ST131**

All animal experimentation was conducted following the National Institutes of Health guidelines for housing and care of laboratory animals and was performed in accordance with institutional regulations after pertinent review and approval by the Animal Studies Committee at Washington University School of Medicine. C3H/HeN mice were obtained from Harlan Sprague Dawley (Indianapolis, IN). The mouse model of UTI was used as previously described [18]. Briefly, 7–8-week-old female C3H/HeN mice were inoculated with $2 \times 10^7$ colony forming units (CFU; acute infection) or $2 \times 10^8$ CFU (chronic infection) of E. coli EC958 directly into the bladder by transurethral catheterization. Bacterial titers in mouse urinary tract tissues were quantified by aseptically removing the bladder and kidneys at the time of euthanization, homogenizing the organs in phosphate-buffered saline (PBS), and plating serial dilutions on LB agar. In chronic infections, longitudinal urinalysis was performed prior to infection and at 1, 3, 7, 10, 14, 21, and 28 days after infection by urine collection and plating serial dilutions on LB agar.

**Detection of E. coli ST131 IBCs**

Bladders of C3H/HeN mice infected with E. coli EC958 were bisected, splayed, and fixed in 3% paraformaldehyde for 1 hour. For IBC enumeration, fixed bladders were washed and stained with 5-bromo-4-chloro-indolyl-β-D-galactopyranoside (X-gal) as previously described [33]. IBC size and morphology were examined by immunofluorescence and confocal laser scanning microscopy [34].

**FimH Inhibitor Studies**

Preparation and pharmacokinetic analysis of the FimH inhibitor 4′-(a-D-Mannopyranosyloxy)-N,3′-dimethylbiphenyl-3-carboxamide (compound ZFH-04269) has been previously described (compound 8 in [21]). One oral dose (50 mg/kg) of compound ZFH-04269 was administered to mice by oral gavage either 30 minutes prior to transurethral inoculation with E. coli EC958 (prophylactic therapy) or on day 14 after infection (treatment of chronic cystitis).

**Statistical Analyses**

Bacterial numbers in urinary tract tissues (bladder and kidneys) were compared between different groups of mice, using non-parametric tests; the Mann–Whitney U test was used to compare median bacterial titers between 2 groups of mice, and the Kruskal–Wallis test for comparisons involving ≥3 mouse groups. A statistical significance threshold was set at $P < .05$.

**RESULTS**

**E. coli ST131 Cause Acute Infection of the Mouse Urinary Tract.**

To map the pathogenesis of E. coli EC958 in vivo, we used a well-established mouse model of UTI [12]. Eight-week old C3H/HeN female mice were inoculated with $2 \times 10^7$ CFU of E. coli EC958 directly into the bladder, using a sterile Teflon catheter. The colonization ability of E. coli EC958 was examined over the course of acute infection by determining bacterial CFU in the mouse bladder and kidneys at 6, 16, and 24 hours after infection (Figure 1). Bladder colonization by E. coli EC958 remained high at all assessed time points of acute infection,

**Figure 1.** *Escherichia coli* ST131 causes acute urinary tract infection in female C3H/HeN mice. Scatter plots of E. coli EC958 titers (colony-forming units [CFU/organ]) in bladders (A) and kidneys (B) of mice at 6, 16, and 24 hours after infection. Horizontal bars represent median E. coli EC958 titers for each mouse group. A total of 8–10 mice were assessed per time point in each experiment. Plots show data from 2 experimental repeats.
with group median values of >10⁴ CFU/bladder. By 24 hours after infection, a bimodal distribution was observed for bacterial titers in the bladder (Figure 1A). The 2 bladder subpopulations observed at 24 hours after infection (each corresponding to approximately 50% of infected mice) displayed median bacterial CFU titers that were 2 logs higher or 2 logs lower than that observed at 6 hours after infection for all infected mice. C3H and CBA background mice are known to be genetically susceptible to vesicoureteral reflux [35], and therefore kidney colonization by 6 hours after infection has been shown to occur uniformly in these strains after intravesical inoculation of UPEC, even under conditions that minimize mechanical reflux during inoculation [36]. E. coli EC958 was also able to establish kidney infection with high titers (>10⁵ CFU/kidneys) by 6 hours after infection, and while the kidney bacterial load decreased over time, E. coli EC958 was still present in the kidneys of all infected mice by 24 hours after infection (Figure 1B). Taken together, our results demonstrate that E. coli EC958 is able to effectively colonize the bladder and kidneys of C3H/HeN mice and establish acute infection of the urinary tract.

**E. coli ST131 Proceed Through the IBC Pathway During Acute Bladder Infection**

To examine whether E. coli EC958 progresses through IBC development during acute bladder infection, the bladders of infected mice were extracted at 6 hours after infection, bisected, fixed, and stained with X-gal. IBC formation was observed in all infected bladders, with a median of 21 IBCs/bladder (range, 7–33 IBCs/bladder; Figure 2A), suggesting that E. coli EC958 is able to invade bladder urothelial cells and replicate intracellularly to form IBCs. Immunofluorescence labeling of E. coli EC958–infected bladders at 6 hours after infection and examination by confocal microscopy revealed large IBCs (Figure 2B–D). By 16 hours after infection, most IBC-containing urothelial cells had burst and released E. coli EC958 cells, in the form of free rod-shaped or long filamentous bacteria, into the bladder lumen (Figure 2E–G). This is the first demonstration of IBC formation by an E. coli ST131 strain during acute infection of the mouse bladder.

**E. coli ST131 Can Persist in the Bladder and Cause Chronic Infection**

Previous studies have shown that acute UTI due to UPEC can progress to chronic cystitis or infection resolution [18]. In agreement with these findings, we observed a bimodal distribution of bladder bacterial titers at 24 hours after infection with E. coli EC958, suggesting that mice with higher bacterial titers may develop chronic cystitis. To test whether E. coli EC958 can persist in the bladder beyond acute infection, we tested its ability to cause chronic cystitis in female C3H/HeN mice. Chronic cystitis was previously defined by the presence of high-titer (>10⁴ CFU/mL) persistent bacteriuria of at least 2-4 weeks.
duration and high-titer (>10^4 CFU/organ) bladder colonization with accompanying inflammation at 2 and 4 weeks after infection [18]. After experimental inoculation with 10^7 or 10^8 CFU of E. coli EC958, bacterial titers in urine were monitored over a period of at least 4 weeks (Figure 3A). We found that 2 of 20 mice (10%) and 8 of 20 mice (40%) infected with 10^7 and 10^8 CFU of E. coli EC958, respectively, developed high-titer (>10^4 CFU/mL) bacteriuria that was present at every time point through day 28 after infection (Figure 3B and 3C). A subset of mice infected with 10^8 CFU of E. coli EC958 were euthanized for analysis at day 28 (n = 10) and day 42 (n = 5) after infection. We found that the bladders recovered from mice with persistent bacteriuria (n = 5) were grossly enlarged and inflamed (data not shown) and had bacterial titers significantly higher than those of mice that had resolved bacteriuria (ie, mice with <10^4 CFU/mL in urine at least once during the infection period; n = 10), with a median difference in CFU per bladder of approximately 30 000-fold (P = .0026, by the Mann–Whitney U test; Figure 3D). Bacterial titers in the kidneys were also significantly higher in persistently bacteriuric mice as compared to those in mice with resolved bacteriuria, with a median difference in CFU per kidney of 10 000-fold observed (P = .0003, by the Mann–Whitney U test; Figure 3D). Taken together, our results demonstrate that E. coli EC958 can persist in the bladder of C3H/HeN mice and cause chronic infection.

Acute E. coli ST131 Bladder Infection Can Be Prevented Using an Orally Administered FimH Inhibitor

The multidrug resistance of E. coli ST131 poses a challenge for successful treatment and prevention of recurrent UTI, which may be exacerbated by the formation of IBCs. E. coli EC958 is resistant to 8 classes of antibiotics, including cephalosporins, fluoroquinolones, and aminoglycosides. Consistent with this phenotype, we showed that E. coli EC958 established acute bladder infection in C3H/HeN mice (n = 8) that were given prophylactic TMP-SMZ (54 or 270 μg/mL) for 3 days prior to infection (Figure 4A). This result, when considered in light of the widespread occurrence of multidrug-resistant UPEC infections, prompted us to evaluate alternative strategies for the treatment and prevention of E. coli ST131-mediated UTI. We have previously demonstrated that strains of the ST131 lineage, including E. coli EC958, rely on the FimH adhesin of type 1 fimbriae for colonization of the mouse bladder [28]. Given the key role of type 1 fimbriae in E. coli EC958 uropathogenesis, we evaluated the efficacy of a previously characterized FimH inhibitor [21] in preventing acute bladder infection by E. coli EC958. Two groups of 8 mice were administered 1 oral dose of compound ZFH-04269 (50 mg/kg) or PBS 30 minutes prior to intrarectal inoculation with E. coli EC958 (10^7 CFU), and bacterial titers in bladders and kidneys were assessed at 6 hours after infection. Mice treated with compound ZFH-04269 showed...
a median 100-fold decrease in *E. coli* EC958 CFU in their bladder, compared with PBS-treated control mice (P = .0009, by the Mann–Whitney U test; Figure 4B). No difference was observed in *E. coli* EC958 bacterial titers in the kidneys of mice treated with compound ZFH-04269 or PBS (Figure 4B). These data highlight the importance of type 1 fimbiae in the establishment of cystitis by *E. coli* EC958 and indicate that FimH inhibitors may be a promising strategy for preventing infection by multidrug-resistant *E. coli* ST131.

**A FimH Inhibitor Can Successfully Treat Bladder Infection in Mice Chronically Infected With E. coli ST131**

We also evaluated the efficacy of compound ZFH-04269 in treating established bladder infection in mice with chronic cystitis. Sixty mice were infected with *E. coli* EC958 (10⁸ CFU) and in 2 independent experiments and mice with chronic infection (urine bacterial titers of >10⁸ CFU over a 14-day period; n = 26) were treated with 1 oral dose of compound ZFH-04269 (n = 13) or PBS (n = 13) at day 14 after infection. Enumeration of bladder bacterial titers 6 hours after treatment revealed that the median number of *E. coli* EC958 CFU per bladder in mice treated with ZFH-04269 was >1000-fold less than that in PBS-treated controls (P = .0018, by the Mann–Whitney U test; Figure 4C). Moreover, the median *E. coli* EC958 load in the bladder of mannoside-treated mice was reduced to <10⁴ CFU (P = .0398, by the Wilcoxon signed rank test), which is closer to the median bacterial titer observed in the bladders of mice that resolved bacteriuria (Figure 3D). No effect was observed for compound ZFH-04269 treatment on kidney colonization (Figure 4C).

**DISCUSSION**

Several recent publications, including reports from the Infectious Diseases Society of America, highlight the urgent need for new therapies to combat multidrug-resistant gram-negative pathogens, including *E. coli* ST131 [3, 37–39]. *E. coli* ST131 is a high-risk ESBL-producing clone and currently represents one of the
most dominant groups of multidrug-resistant *E. coli* strains internationally [40]. While many studies have examined the resistance profiles of *E. coli* ST131, little is known about the factors that contribute to the clone’s pathogenesis. We recently demonstrated that *E. coli* ST131 isolates establish acute bladder infection in C57BL/6 mice in a type 1 fimbiae–dependent manner [28]. In this study, we mapped the pathogenic lifestyle of *E. coli* ST131 in much greater detail, using a C3H/HeN mouse infection model, and we evaluated for the first time alternative strategies for the treatment of *E. coli* ST131–mediated UTI.

*E. coli* EC958 is a representative isolate from the ST131 lineage and is resistant to multiple classes of antibiotics, including cephalosporins, fluoroquinolones, sulfonamides, and aminoglycosides [28]. Using a well-established mouse model of UTI, we show here that *E. coli* EC958 invades urothelial cells of the mouse bladder and forms IBCs with a size and morphology comparable to those of the reference cystitis strain *E. coli* UTI89 [10]. Moreover, the progression of *E. coli* EC958 through the IBC developmental cycle in the C3H/HeN mouse bladder mirrored closely the timing previously observed for *E. coli* UTI89 in the same mouse model [12]. This study also describes the first analysis of *E. coli* ST131 infection in a chronic UTI model and will provide a framework to further understand these infections. Given that *E. coli* strains EC958 and UTI89 are as distantly related at the whole-genome level as *E. coli* EC958 is to *E. coli* strains belonging to other phylogroups (eg, A and D) and other pathotypes (eg, commensal and enterohemorrhagic *E. coli*; Totsika and Beatson, unpublished data), it is perhaps surprising that the pathogenesis of *E. coli* EC958 and UTI89 in the mouse bladder is so closely matched. This would support recent findings suggesting that UPEC strains that have different virulence profiles but express type 1 fimbiae induce a convergent host response involving pathways such as IBC formation that result in common symptoms of cystitis [41].

The inability to effectively combat infections caused by multidrug-resistant *E. coli* with currently available therapies is a pressing medical concern, and alternative therapeutic strategies are needed. Inhibiting the function of virulence factors required by pathogens to cause disease, such as the FimH adhesin in *E. coli*, is an attractive alternative approach to antibiogenic therapies [42]. A previously characterized biphenyl FimH inhibitor (previously termed compound 8 [21] and herein referred to by its official nomenclature as compound ZFH-04269) was selected for in vivo efficacy evaluation against *E. coli* ST131 strain EC958 because of its enhanced potency and oral bioavailability [21]. Administration of 1 oral dose of compound ZFH-04269 to female mice chronically infected with *E. coli* EC958 resulted in significant bacterial clearance in the bladder within 6 hours. In addition, 1 prophylactic dose of compound ZFH-04269 given orally to mice 30 minutes before intraurethral inoculation with *E. coli* EC958 was protective against acute cystitis, in contrast to TMP-SMZ, which was completely ineffective. As C3H mice are genetically susceptible to vesicoureteral reflux, kidney colonization occurs early in experimental UTI, independent of bladder colonization [35, 36]. Therefore, as expected, treatment with ZFH-04269 had no effect on kidney colonization, which is typically not dependent on type 1 fimbiae. However, in uncomplicated cystitis, which accounts for the vast majority of UTI, pylonephritis is a rare complication (incidence, <1% in placebo-treated patients [3]), and preventing and/or successfully treating initial bladder colonization would be expected to fully prevent or cure UTI.

The high efficacy of the ZFH-04269 FimH inhibitor as both a treatment and prevention strategy in small-animal models of cystitis paves the way for further work to test different dosing regimens, including extended therapy and combination therapy with antibiotics, and to examine the effect of this compound on gut colonization by both *E. coli* ST131 and other gram-negative bacteria. If our results are clinically translatable to humans, they provide strong evidence that FimH inhibitors could effectively prevent and treat bladder infections caused by multidrug-resistant *E. coli* ST131 and reduce the need for prescribing broad-spectrum antibiotics to treat community-acquired UTI.

**Notes**

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