Vancomycin-arginine (STM-001) abrogates ESBL carrier and carbapenem-resistant *Escherichia coli* burden in a murine complicated urinary tract infection model

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Received 12 December 2021; accepted 11 February 2022

**Objectives:** STM-001, a retargeted glycopeptide, is active against MDR *E. coli* expressing ESBLs including carbapenemases. Herein, we assessed its capability to combat *E. coli* complicated urinary tract infections (cUTI) in mice driven by clinically important serine (CTX-M-15) and metallo-β-lactamases (NDM-1).

**Methods:** Plasma and urine pharmacokinetics following IV administration of STM-001 (1–50 mg/kg) were determined in mice via LC-MS/MS. The effects on bacterial burden (kidney, bladder and urine) were determined in a 7 day mouse cUTI model whereby STM-001 was administered q12h or q24h at 2–100 mg/kg/day from Day 4. Efficacy was assessed by the change in log10 cfu/g or log10 cfu/mL from vehicle-treated infected mice.

**Results:** MICs of STM-001 for CTX-M-15 and NDM-1 *E. coli* were 8 and 16 mg/L, respectively. Blood pharmacokinetic profile was linear and dose-dependent with low clearance of 9.49 ± 0.31 mL/min/kg, V = 0.63 ± 0.02 L/kg and t1/2 = 1.16 ± 0.03 h. High STM-001 concentrations were recovered in urine 0–8 h post-administration, reaching up to 120-fold above its MIC. In cUTI efficacy studies, STM-001 (1–50 mg/kg, q12h) reduced CTX-M-15 burden by log10 4.31 (kidney), 3.95 (bladder) and 4.82 (urine) compared with vehicle-treated animals (P < 0.0001). STM-001 also reduced NDM-1 burden by log10 3.89 (kidney), 3.76 (bladder) and 3.08 (urine) (P < 0.0001), with similar inhibitory effects following q24h dosing.

**Conclusions:** STM-001 was highly effective in reducing *E. coli* burden in kidney, bladder and urine in mouse cUTI models. The observed efficacy with either dosing regimen indicates potential humanized doses of 1–5 mg/kg. These data support further development of STM-001 as an innovative, carbapenem-sparing antibiotic to combat human cUTIs.

**Introduction**

Complicated urinary tract infections (cUTIs), which are often associated with MDR Enterobacteriaceae, are a significant global healthcare burden.1 In the USA, approximately 2.8 million cases annually result in total healthcare costs exceeding $6 billion, primarily due to the need for inpatient IV antibiotic therapy.2 This economic burden stems from the rise in antimicrobial resistance during the last one to two decades,3 especially due to the spread of ESBL *E. coli*, the major causative pathogen of cUTIs.4,5 Indeed, the prevalence of such genotypes, ranging from 10%–30% in the USA, has rendered oral treatments with cephalosporins, fluoroquinolones, sulphonamides and some β-lactam/β-lactamase inhibitors essentially obsolete, necessitating the use of IV therapies.6,7 Consequently, innovative antibiotics are keenly sought after to confront cUTIs driven by MDR *E. coli*, especially CTX-M-15, a globally important fluoroquinolone-resistant uropathogen and NDM-1, an urgent-threat, carbapenem-resistant pathogen.6,8,9

We recently reported the *in vitro* activity of vancomycin-arginine (STM-001) towards all Ambler classes of *E. coli* which was mediated via an acute bactericidal effect.10 In *vivo*, STM-001, but not vancomycin, eliminated thigh muscle infection driven by *E. coli* UTI89.10 We therefore hypothesized that STM-001 might afford significant protection in a murine cUTI model driven by clinically relevant CTX-M-15 and NDM-1 uropathogens.

**Methods**

**Ethics**

All husbandry aspects complied with the species-specific recommendations of The Guide for the Care and Use of Laboratory Animals (2011) and the Public Health Service Policy on humane care and use of laboratory
animals with appropriate internal IACUC procedures at the respective service providers.

**STM-001 manufacturing**

See Supplementary data (available at JAC Online).

**Antimicrobial susceptibility testing**

The MICs of STM-001,10 vancomycin, meropenem and colistin were determined for *E. coli* strains UNT057-1 (CTX-M-15 positive) and AR0055 (NDM-1 positive) following the CLSI M07-A10 microdilution procedure and the CLSI M100 interpretive criteria.

**Pharmacokinetic (PK) studies in blood plasma and urine of mice**

Naive female 7–9-week-old CD-1 mice (Beijing Vital River Laboratory Animal Technology Co. Ltd) were used for PK studies (WuXi AppTec, Shanghai, China) with *n* = 3 per group. Mice were dosed with STM-001 (IV bolus, 5 mL/kg) through the tail vein and blood samples (∼0.02 mL) were collected at 0, 0.25, 0.5, 1, 2, 4, 8 and 12 h following saphenous vein puncture. For urine collection, animals were placed individually in metabolic cages for 24 h and urine samples were pooled from two time intervals (0–8 h and 8–24 h). Lower limit of quantification (LLOQ) was 10 and 50 ng/mL in plasma and urine, respectively.

**In vivo efficacy studies (ascending cUTI model)**

The cUTI model for both infecting strains was established as previously described using 8–9 female C3H/HeJ mice (The Jackson Laboratory, Bar Harbor, ME, USA) per group of age 7–8 weeks.11 For the UNT057-1 strain (Pre-Clinical Services, University of North Texas Health Science Center, USA), 9.05 log10 cfu were used for the transurethral inoculation and 10.98 log10 for the AR0055 strain (Pharmacology Discovery Services, Taiwan). IV treatment with STM-001 (1–50 mg/kg/dose) was initiated 4 days post-infection for 3 days q12h with the addition of two further groups with q24h dosing for AR0055. For the UNT057-1 strain, meropenem (300 mg/kg/dose) and for AR0055, colistin (20 mg/kg/dose), were included as positive controls. Both were administered subcutaneously q12h. Two hours following the final drug administrations, tissues were harvested and homogenized in 1 mL sterile PBS, serially diluted (10-fold) and plated on Mueller-Hinton agar for quantitative bacterial counts. Urine samples were collected into sterile disposable plastic containers by gently pressing down the tail and lower back for urine induction. Bacteria in the urine samples were enumerated following identical dilutions and plating for tissues and cfu in kidney, bladder and urine were assessed for significance compared with vehicle groups using one-way ANOVA analysis. *P* < 0.05 was considered as significant.

**Results**

**Antimicrobial susceptibility testing**

MICs of STM-001 for UNT057-1 and AR0055 strains were 8 and 16 mg/L, respectively. In contrast, vancomycin MIC was ≥128 mg/L for both strains. The MIC of meropenem for UNT057-1 was ≤0.06 mg/L and that of colistin for AR0055, 1 mg/L.

**Mouse PK studies**

In blood, STM-001 (1–50 mg/kg), displayed first-order elimination with dose-dependent increases in *C* max (3.27–125.6 mg/L) and *AUC* (1.80–103.6 mg·h/L). At all doses, the mean half-life was 1.16 ± 0.03 h and *V* was 0.63 ± 0.02 L/kg (Figure S1, Table S1). The *AUC/MIC* ratio ranged from 0.11–12.96 (Table S1). In urinary excretion studies during the first 8 h, STM-001 concentrations reached almost 1000 mg/L (Table S2) at the 50 mg/kg dose, with the majority (∼90%) of material eliminated unmetabolized.

**STM-001 in vivo efficacy studies in murine cUTI models**

In the cUTI model with UNT057-1, mean bacterial burdens in vehicle-treated animals at 7 days post-infection were log10 7.27 ± 0.77 (kidney), 6.77 ± 0.40 (bladder) and 7.79 ± 0.48 (urine)—see Table S3. Following q12h administration of STM-001, dose-dependent reductions in bacterial burdens compared with vehicle-treated animals were observed, which peaked at 4.31 log10 (kidney), 3.95 log10 (bladder) and 4.82 log10 (urine) below vehicle-treated animals. (Figure 1a). ED50 values were 7.7 mg/kg (kidney), 1.9 mg/kg (bladder) and 7.1 mg/kg (urine). STM-001

![Figure 1. The effects of IV administered STM-001 (every 12 h over 3 days) on log10 reduction of bacterial burden (cfu/g or cfu/mL) compared with vehicle-treated animals in a murine model of cUTI driven by E. coli CTX-M-15 (a) and NDM-1 (b).](image-url)
also reduced NDM-1 burden by up to log_{10} 3.89 (kidney), 3.76 (bladder) and 3.08 (urine)—see Figure 1(b) and Table S4, with corresponding ED_{50} values of 3 mg/kg (kidney), 1.3 mg/kg (bladder) and 2.3 mg/kg (urine). In two additional experimental arms, the efficacy of q24h STM-001 at 50 and 100 mg/kg/dose was almost identical to q12h administrations of 25 and 50 mg/kg/dose (Table 1).

### Discussion

We previously described the selectivity and efficacy of STM-001 in reducing bacterial burden in an *E. coli* thigh muscle model.\textsuperscript{10} Herein, we addressed the efficacy of STM-001 in mouse cUTI models driven by clinically relevant MDR *E. coli* CTX-M-15 and NDM-1 ESBLs.

A major impetus for the efficacy studies was the favourable plasma and urine PK of IV STM-001 with low clearance and high concentrations of intact drug in urine. In both cUTI efficacy models, STM-001 caused dose-dependent and significant reductions in bacterial burden in kidney, bladder and urine from vehicle-treated mice. Indeed, at many doses, especially with the NDM-1 strain, bacterial burden in urine was <1000 cfu/mL, which defines microbiological clinical success in cUTI patients.\textsuperscript{12}

A key finding from both cUTI studies was significant efficacy at low doses of STM-001. This was presumably due to high renal elimination, in alignment with the features of vancomycin,\textsuperscript{13} which constitutes ~91% of STM-001 by molecular weight. Consequently, putative humanized doses of STM-001 based on allometric scaling might be as low as ~1–5 mg/kg.\textsuperscript{14} Assuming a 5–10 day course of cUTI treatment, the total amount of STM-001 required would be expected to be significantly less than other IV antibiotic therapies.\textsuperscript{15} A second key finding from the NDM-1 study was that equivalent efficacies were observed irrespective of whether STM-001 was given q12h or q24h similar to the AUC-driven efficacy of vancomycin.\textsuperscript{13} Thus, following effective breaching of the outer membrane via the LPS-disrupting properties of the cationic arginine amino acid, STM-001 is free to engage with d-Ala-d-Ala target sites located within the thin peptidoglycan layer to elicit a rapid bacterial kill.\textsuperscript{10,16} Pre-clinical safety studies with appropriate biomarkers of acute kidney injury are ongoing to scrutinize any effects following STM-001 dosing.\textsuperscript{17}

It is noteworthy that the efficacy of STM-001 was achieved despite its modest MIC potency. The described cUTI data are aligned with a recent report of the effectiveness of cefepime/ taniborcactam to curb cUTIs despite MICs of up to 32 mg/L for different Enterobacteriaceae.\textsuperscript{18} These findings underscore the importance of appropriate pharmacokinetic/pharmacodynamic profiles of antibiotics in targeting cUTIs, especially those with high-level elimination of intact drug via the kidneys.

In summary, we have described the promising properties of STM-001 to combat cUTIs driven by clinically important ESBL *E. coli* uropathogens with limited treatment options especially towards the NDM-1 variant. The low dose efficacy of STM-001 provides a potential novel targeted therapy to treat human cUTIs in terms of both the predominant causative pathogen and site of infection. In an era of worrisome bacterial infections globally,\textsuperscript{3,19,20} STM-001 might represent a highly meaningful, carbapenem-sparing antibiotic to counteract drug-resistant *E. coli* ESBLs mediating such human infections.

**Acknowledgements**

We would like to thank Michael Gassen, Jing Li, Liping Ma (WuXi AppTec, China), William Weiss, Mark Pulse (Pre-Clinical Services, University of North Texas Health Science Center, USA) and Lynn Miesel, Pony Lee, Kun-Yuan Lin, Lucy Chia, Tim Yeh, Jui-Che Chien, Chin-Wei Chiang, Mei-Lee Hsieh, Candace Lee, David Lo, Gin-Feng Lai, Timmy Huang and Wei-Ting Lao (Eurofins Pharmacology Discovery Services Taiwan) for their outstanding and dedicated efforts in the conduct of the described studies. We are also grateful to Noam B. Neville for expertise in graphical and data analysis support. Part of this work utilized NIAID’s suite of pre-clinical services for in vivo assessment (Contract No. HHSN272201700020I/75N93021F00001). STM-001 and related molecular transporter technologies constitute part of a worldwide exclusive licensing agreement acquired by SuperTrans Medical from Stanford University, CA, USA.

**Funding**

The studies described were supported by SuperTrans Medical’s internal funding.

**Transparency declarations**

L.F.N. and J.T.R. have none to declare. I.S. and P.A.W. serve as consultants to SuperTrans Medical. The sponsor provided support and did not exercise control over the conduct or reporting of the research.

**Supplementary data**

Supplementary data, including Figure S1 and Tables S1 to S4, are available at JAC Online.

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