J Antimicrob Chemother 2022; **77**: 1706–1709
https://doi.org/10.1093/jac/dkac063 Advance Access publication 1 March 2022

Journal of Antimicrobial Chemotherapy

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 $\log_{10}$ cfu/g or $\log_{10}$ 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, $V = 0.63 \pm 0.02$ L/kg and $t_{½} = 1.16 \pm 0.03$ 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 $\log_{10}$ 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 $\log_{10}$ 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 low 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, fluroquinolones, 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

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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 $\log_{10}$ cfu were used for the transurethral inoculation and 10.98 $\log_{10}$ 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 $\log_{10}$ 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 $\log_{10}$ (kidney), 3.95 $\log_{10}$ (bladder) and 4.82 $\log_{10}$ (urine) below vehicle-treated animals. (Figure 1a). ED_{50} values were 7.7 mg/kg (kidney), 1.9 mg/kg (bladder) and 7.1 mg/kg (urine). STM-001

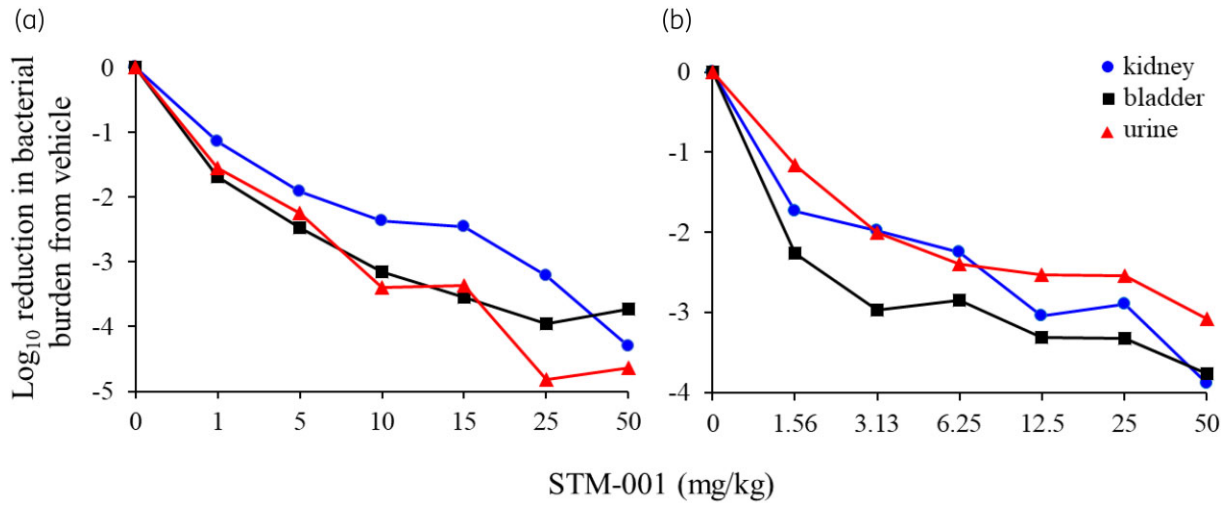

Figure 1. The effects of IV administered STM-001 (every 12 h over 3 days) on $\log_{10}$ reduction of bacterial burden (cfu/g or cfu/mL) compared with vehicle-treated animals in a murine model of cUTI driven by *E. coli* CTX-M-15 (a) and NDM-1 (b).

Table 1. Comparison of the effects of STM-001 on *E. coli* AR0055 bacterial burden (log_{10} cfu/g or mL ± SEM) following q12h or q24h administration

Total STM-001 dose (mg/kg/day)	Dosing regimen (mg/kg)	Kidney	Bladder	Urine
Vehicle	—	6.60 ± 0.20	6.58 ± 0.17	5.05 ± 0.14
50	25 q12h	3.70* ± 0.28	3.26* ± 0.27	2.51[a] ± 0.48
	50 q24h	3.22* ± 0.26	3.14* ± 0.42	2.51[b] ± 0.33
100	50 q12h	2.71* ± 0.16	2.82* ± 0.23	1.97* ± 0.48
	100 q24h	2.63* ± 0.32	2.81* ± 0.45	2.38[c] ± 0.55

Therapeutic intervention was initiated at 96 h post-infection with bacterial sampling on Day 7. *P* values shown are for comparisons with 7 day infection control group (vehicle).
*$P < 0.0001$;
[a]$P = 0.0015$;
[b]$P = 0.0011$;
[c]$P = 0.0054$.

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.[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.[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,[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.[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.[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.[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.[10,16] Pre-clinical safety studies with appropriate biomarkers of acute kidney injury are ongoing to scrutinize any effects following STM-001 dosing.[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/taniborbactam to curb cUTIs despite MICs of up to 32 mg/L for different Enterobacteriaceae.[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,[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.

References

1 Murray BO, Flores C, Williams C *et al.* Recurrent urinary tract infection: a mystery in search of better model systems. *Front Cell Infect Microbiol* 2021; **11**: 691210.

2 Carreno JJ, Tam I, Meyers JL *et al*. Longitudinal nationwide cohort study to assess incidence, outcomes and costs associated with complicated urinary tract infection. *Open Forum Infect Dis* 2019; **6**: ofz446.

3 De Oliveira DMP, Forde BM, Kidd TJ *et al*. Antimicrobial resistance in ESKAPE pathogens. *Clin Microbiol Rev* 2020; **33**: e00181-19.

4 Zowawi HM, Harris PNA, Roberts MJ *et al*. The emerging threat of multidrug resistant Gram-negative bacteria in urology. *Nat Rev Urol* 2015; **12**: 570–84.

5 Medina M, Castillo-Pino E. An introduction to the epidemiology and burden of urinary tract infections. *Ther Adv Urol* 2019; **11**: 3–7.

6 Critchley IA, Controneo N, Pucci MJ *et al*. The burden of antimicrobial resistance among urinary tract isolates of *Escherichia coli* in the United States in 2017. *PLoS One* 2019; **14**: e0220265.

7 Talan DA, Takhar SS, Krishnadasan A *et al*. Emergence of extended-spectrum β-lactamase urinary tract infections among hospitalized emergency department patients in the United States. *Ann Emerg Med* 2021; **77**: 32–43.

8 Johnson JR, Johnston B, Clabots C *et al*. *Escherichia coli* sequence type ST131 as the major cause of serious multidrug-resistant *E. coli* infections in the United States. *Clin Infect Dis* 2010; **51**: 286–94.

9 Doi Y, Paterson DL. Carbapenemase-producing Enterobacteriaceae. *Semin Respir Crit Care Med* 2015; **36**: 74–84.

10 Neville LF, Shalit I, Warn PA *et al*. *In-vivo* targeting of *Escherichia coli* with vancomycin-arginine. *Antimicrob Agents Chemother* 2021; **65**: e02416-20.

11 Weiss WJ, Pulse ME, Nguyen P *et al*. Activity of meropenem-vaborbactam against carbapenem-resistant Enterobacteriaceae in a murine model of pyelonephritis. *Antimicrob Agents Chemother* 2018; **62**: e01439-17.

12 FDA. Guidance Document: Complicated Urinary Tract Infections: Developing Drugs for Treatment. 2018. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/complicated-urinary-tract-infections-developing-drugs-treatment.

13 Rybak MJ. The pharmacokinetic and pharmacodynamic properties of vancomycin. *Clin Infect Dis* 2006; **42** Suppl 1: S35–39.

14 FDA Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers. 2005. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/estimating-maximum-safe-starting-dose-initial-clinical-trials-therapeutics-adult-healthy-volunteers.

15 Bader MS, Loeb M, Leto D *et al*. Treatment of urinary tract infections in the era of antimicrobial resistance and new antimicrobial agents. *Postgrad Med* 2020; **132**: 234–50.

16 Antonopolis A, Zang X, Wegner T *et al*. Vancomycin-arginine conjugate inhibits growth of carbapenem-resistant *E. coli* and targets cell-wall synthesis. *ACS Chem Biol* 2019; **14**: 2065–70.

17 Avedissian SN, Pais GM, O'Donnell JN *et al*. Twenty-four hour pharmacokinetic relationships for intravenous vancomycin and novel urinary biomarkers of acute kidney injury in a rat model. *J Antimicrob Chemother* 2019; **74**: 2326–34.

18 Lasko MJ, Nicolau DP, Asempa TE. Clinical exposure–response relationship of cefepime/taniborbactam against Gram-negative organisms in the murine complicated urinary tract infection model. *J Antimicrob Chemother* 2022; **77**: 443–7.

19 Lasko MJ, Nicolau DP. Carbapenem-resistant Enterobacterales: considerations for treatment in the era of new antimicrobials and evolving enzymology. *Curr Infect Dis Rep* 2020; **22**: 6.

20 van Duin D, Paterson DL. Multidrug-resistant bacteria in the community: an update. *Infect Dis Clin North Am* 2020; **34**: 709–22.