In Vivo Efficacy of Novel Monobactam LYS228 in Murine Models of Carbapenemase-Producing *Klebsiella pneumoniae* Infection

W. J. Weiss,^a M. E. Pulse,^a P. Nguyen,^a E. J. Growcott^{b*}

^aPre-Clinical Services, University of North Texas Health Science Center, Fort Worth, Texas, USA

^bNovartis Institute of BioMedical Research, Emeryville, California, USA

ABSTRACT LYS228 has potent antibacterial activity against carbapenem-resistant strains of *Enterobacteriaceae*. LYS228 was efficacious in neutropenic thigh models established with *Klebsiella pneumoniae* producing KPC-2 or NDM-1; pretreatment with uranyl nitrate considerably shifted calculated static doses of LYS228. In murine ascending pyelonephritis, LYS228 reduced bacterial burden in kidney, urine, and bladder. The successful treatment of murine infection models established with carbapenem-resistant *K. pneumoniae* further supports the clinical development of LYS228.

KEYWORDS LYS228, neutropenic thigh model, pyelonephritis

Multidrug-resistant bacteria, such as carbapenem-resistant *Enterobacteriaceae* (CRE), are a serious health care problem (1). LYS228 is a novel monobactam that has the potential to be developed to treat complicated infections caused by *Enterobacteriaceae* that are susceptible to the agent (2). The molecule has potent antibacterial activity against β -lactamase-producing strains of *Enterobacteriaceae*, including those expressing metallo- β -lactamases (e.g., NDM-1), extended-spectrum β -lactamases (ESBLs) (e.g., CTX-M-15), and serine carbapenemases (e.g., KPC) (3, 4). In this report, we evaluated the efficacy of LYS228 against carbapenem-resistant *K. pneumoniae* in murine models of neutropenic thigh infection and ascending pyelonephritis.

The MICs for LYS228 (synthesized at Novartis), meropenem (meropenem for injection; USP, Hospira, Lake Forest, IL), and levofloxacin (Sigma-Aldrich, St. Louis, MO) were tested using broth microdilution based on the methods outlined by Clinical and Laboratory Standards Institute (CLSI) guidelines and are recorded in Table 1 (5). *K. pneumoniae* strains UNT170-1 and UNT-184-1 were part of the University of North Texas (UNT) Health Science Center collection and expressed KPC-2 and NDM-1, respectively. LYS228 MIC₅₀ and MIC₉₀ values have been reported as 0.25 and 1 μ g/ml, respectively (4), and the MIC range measured against UNT170-1 is within the LYS228 MIC₉₀, while against UNT184-1, the range is on the elevated end of the LYS228 MIC spectrum (4). Both strains were resistant to meropenem (16 to 32 μ g/ml for both strains) and levofloxacin (8 to 16 μ g/ml for UNT170-1 and 16 to 32 μ g/ml for UNT184-1) as defined by European Committee on Antimicrobial Susceptibility Testing (EUCAST) and CLSI breakpoints for these antibiotics (6, 7).

Next, the efficacy of LYS228 was determined *in vivo*, and all studies were performed in accordance with protocols approved by the UNT Health Sciences Center Institutional Animal Care and Use Committee. The compound was first tested in a neutropenic murine thigh infection model using female 5- to 6-week-old CD-1 mice (18 to 20 g; Envigo Laboratories). The model was run in a manner similar to one previously described (8). LYS228, dosed subcutaneously every 4 hours (q4h), reduced the thigh

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Address correspondence to E. J. Growcott, ellie.growcott@novartis.com.

* Present address: E. J. Growcott, Novartis Institute for Biomedical Research, Cambridge, Massachusetts, USA.

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TABLE 1 Calculated LYS228 doses required for bacterial stasis or 1 log₁₀ bacterial kill

<i>K. pneumoniae</i> strain	Relevant characteristic	Uranyl nitrate pretreatment	Dose (mg/kg/day)		LYS228 MIC (μg/ml)
			Stasis	1 log ₁₀ kill	
UNT170-1	KPC-2	No	1,180	DNR ^a	0.5–1
		Yes	55	351	
UNT184-1	NDM-1	No	192	DNR	8–16
		Yes	16	109	

^aDNR, did not reach.

bacterial burden of UNT170-1 and UNT184-1 in a dose-dependent manner (Fig. 1A and B). The highest dose (1,620 mg/kg/day) of LYS228 significantly reduced bacterial load compared with vehicle controls against both strains tested (UNT170-1, 9.14 ± 0.79 log₁₀ CFU/thigh, $P < 0.01$ [$n = 5$ /group]; UNT184-1, 8.99 ± 0.29 log₁₀ CFU/thigh, $P < 0.001$ [$n = 5$ /group]). The dose needed to maintain the bacterial load at the same level as when treatment began (static dose) was calculated; 1,180 or 192 mg/kg/day LYS228 was necessary to achieve bacterial stasis when treating UNT170-1 and UNT184-1, respectively. These data demonstrate that LYS228 can treat CRE *in vivo*. This may be extremely important clinically given the facts that KPCs have caused many carbapenem-resistant epidemics around the world and that NDM-1 has spread rapidly and had many global outbreaks (9). Additionally, resistance to colistin, one of the last lines of antibiotic defense, is emerging against β -lactamase-expressing strains in Eu-

FIG 1 Change (Δ) in log₁₀ CFU/thigh of UNT170-1 (A, C) and UNT184-1 (B, D) *K. pneumoniae* strains present in the thigh after 24 h of treatment with LYS228. (C and D) Bacterial load in the thigh after pretreatment with 5 mg/kg intraperitoneal uranyl nitrate. LYS228 treatment was administered subcutaneously every 4 h. Data are presented as means $\pm$ SD; $n = 5$ animals/group. Kruskal-Wallis one-way analysis of variance followed by Dunn's multiple-comparison test was used for statistical analysis, with $P < 0.05$ considered significant. **, $P < 0.01$; ***, $P < 0.001$, compared to vehicle-treated animals.

rope (10–12), and there is a correlation between β -lactamase production and colistin resistance (13).

LYS228 exhibits high renal clearance in rat and dog (14). To examine the effect of this parameter on LYS228 activity, animals were pretreated with uranyl nitrate (5 mg/kg subcutaneously 3 days before infection), a single administration of which causes necrosis in the proximal tubules and is associated with a decline in renal function (15). This renal impairment has been used to simulate a humanized pharmacokinetic profile of antibiotics in mice (16–19). Pretreatment with uranyl nitrate enhanced the efficacy of LYS228 in neutropenic thigh models (Fig. 1C and D), as indicated by a considerable shift in calculated static dose, with lower doses of LYS228 required to achieve bacterial stasis (Table 1). Additionally, in combination with uranyl nitrate pretreatment, LYS228 induced a 1- $\log_{10}$ reduction below stasis against both *K. pneumoniae* strains tested (Table 1). Comparing the bacterial load of vehicle-treated animals ($9.83 \pm 0.12 \log_{10}$ and $10.04 \pm 0.25 \log_{10}$ CFU/thigh for UNT170-1 and UNT184-1, respectively) with LYS228-treated animals, there was a significant reduction (Fig. 1C and D). The improved efficacy of LYS228 in mice that received uranyl pretreatment was presumably the result of reduced clearance of LYS228, which in turn helped maintain drug concentrations above those that were microbiologically active. Although plasma concentrations were not measured, uranyl nitrate has been used to slow the clearance of the clinically available monobactam aztreonam (18). In comparison, against UNT170-1, 300 mg/kg/day meropenem was not efficacious, as evidenced by $1.41 \pm 0.12 \log_{10}$ CFU growth over 24 h compared with the bacterial load in the thigh at the time treatment began ($7.80 \pm 0.07 \log_{10}$ CFU/thigh). In contrast, the same dose of meropenem reduced UNT184-1 bacterial burden in the thigh to below static levels ($7.73 \pm 0.22 \log_{10}$ CFU/thigh) by $1.21 \pm 0.65 \Delta \log_{10}$ CFU/thigh, despite a high MIC for meropenem against this isolate. Meropenem is also eliminated renally (20), and, in a similar manner, utilizing a neutropenic thigh model pretreated with uranyl nitrate, a humanized dosing regimen of meropenem has been shown to be effective against a number of VIM-1-producing *Enterobacteriaceae* strains regardless of MIC (19).

In both sets of thigh studies, LYS228 was more effective against UNT184-1 than against UNT170-1, with the calculated static dose lowered by ~3- to 6-fold even though the MIC against UNT184-1 was 16-fold higher than that against UNT170-1. Differences in LYS228 *in vivo* efficacy versus *in vitro* activity were previously observed (8). Meropenem was also more efficacious against UNT184-1 despite having the same MIC against both strains. A similar observation was made of increased *in vivo* susceptibility compared with *in vitro* activity against NDM-1-expressing isolates (21). A reason behind these differences may be reduced expression of activity of the enzyme *in vivo* due to metal ion availability, thus accounting for greater observed efficacy of certain treatments.

After demonstrating LYS228 efficacy in a neutropenic thigh model of UNT170-1, efficacy was further examined in a murine model of pyelonephritis established with this isolate. The model was run in a similar manner to that already described using female C3H/HeJ mice (20 ± 2 g; Jackson Laboratories) (22). Treatment started 4 days after transurethral inoculation and lasted 3 days. Over the course of therapy, bacterial levels in the kidney, urine, and bladder of vehicle-treated animals increased by ~0.5 to 1 $\log_{10}$ CFU. Treatment with meropenem 150 mg/kg q12h reduced bacterial burden in kidney and urine by 2.32 ± 0.92 and $2.65 \pm 0.20 \log_{10}$ CFU, respectively, compared to that at the start of treatment. The reduction observed in the kidney was similar to the $\log_{10}$ CFU reduction previously reported for meropenem against this strain in this model ($1.51 \pm 0.55 \log_{10}$ CFU), although the compound was dosed at 300 mg/kg q2h for 2 days (22). When administered using a q12h dosing regimen, LYS228 dose dependently reduced UNT170-1 CFU present in the kidney, urine, and bladder compared with bacterial levels at the start of treatment (Fig. 2). The highest dose of LYS228 (1,620 mg/kg daily) reduced the bacterial burden to the limit of quantification in all

FIG 2 Log₁₀ CFU of strain UNT170-1 *K. pneumoniae* present in the kidney (A), bladder (B), and urine (C) after 3 days of treatment with LYS228 beginning on day 4 postinfection (4 dpi). Treatment was administered subcutaneously every 12 h. Bacterial levels were measured at the start of treatment, 4 dpi. Data are presented as means $\pm$ SD; $n = 5$ to 9 animals/group. Kruskal-Wallis one-way analysis of variance followed by Dunn's multiple-comparison test was used for statistical analysis, with $P < 0.05$ considered significant. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$, compared to vehicle treatment 7 dpi. LOQ, limit of quantification.

three compartments ($\log_{10}$, 2.35, 2.05, and 2.35), a reduction of 3.85 ± 0.14 , 2.92, and 0.74 $\log_{10}$ CFU, respectively.

Here, we demonstrated the efficacy of LYS228 against carbapenem-resistant *K. pneumoniae* in murine models of thigh infection and ascending pyelonephritis, further supporting the clinical development of LYS228. Indeed, LYS228 is currently in phase 2 clinical trials for the treatment of complicated urinary tract infections.

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