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EXPERIMENTAL THERAPEUTICS

In Vivo Efficacy of Relebactam (MK-7655) in Combination with Imipenem-Cilastatin in Murine Infection Models

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ABSTRACT The World Health Organization has identified antimicrobial resistance as a global public health threat since the prevalence and spread of antibiotic resistance among bacterial pathogens worldwide are staggering. Carbapenems, such as imipenem and meropenem, have been used to treat multidrug-resistant bacteria; however, since the development of resistance to carbapenems, β-lactam antibiotics in combination with β-lactamase inhibitors (BLI) has been one of the most successful strategies to enhance the activity of β-lactam antibiotics. Relebactam (REL) is a new BLI which has been found to inhibit class A and class C β-lactamases *in vitro*. REL has been reported to restore imipenem's activity against both imipenem-resistant *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. Reported here are the *in vivo* efficacy studies of the imipenem-cilastatin (IMI)-REL combination in mouse models of disseminated and pulmonary infection caused by imipenem-resistant clinical isolates of *P. aeruginosa* and *K. pneumoniae*. The combination was also evaluated in a *P. aeruginosa* delayed pulmonary model of infection. IMI-REL was found to be effective in the disseminated model of infection with log reduction in *P. aeruginosa* CFU of 3.73, 3.13, and 1.72 at REL doses of 40, 20, and 10 mg/kg, respectively. For *K. pneumoniae*, log reductions in CFU of 2.36, 3.06, and 2.29 were reported at REL doses of 80, 40, and 20 mg/kg, respectively. The combination was less effective in the delayed pulmonary model than in the immediate pulmonary model; however, overall REL was found to be effective against these imipenem-resistant strains.

KEYWORDS β-lactamase inhibitors, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, imipenem resistant, multidrug resistance, relebactam

According to a recent Centers for Disease Control and Prevention report, each year more than 2 million people in the United States alone acquire serious infections with bacteria that are resistant to commonly used antibiotics (1). Worldwide statistics for antibacterial resistance are staggering, prompting the World Health Organization to identify antimicrobial resistance as a global public health threat (2).

There are many mechanisms of bacterial resistance to antibiotics. For β-lactam antibiotics, one of the most important resistance mechanisms is production of β-lactamase enzymes (3). These enzymes cleave the β-lactam ring of the molecule, rendering it inactive. Given that many of the resistance genes are encoded on a plasmid, the spread of resistance is rapid (4). *Enterobacteriaceae* often acquire resistance through such plasmids, but some strains encode a cephalosporinase AmpC on the chromosome (5). In other opportunistic pathogens, such as *Pseudomonas aeruginosa*, resistance is often intrinsic (6). Apart from harboring β-lactamases, these bacteria gain resistance by upregulation of endogenous efflux pumps that export antibiotics (7) or by changing the outer membrane permeability, reducing exposure to the antibiotic (6). Carbapenems, such as imipenem, meropenem, and ertapenem were used as a last resort to treat multidrug-resistant (MDR) bacteria; however, the development of resis-

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TABLE 1 *In vivo* preclinical efficacy following immediate treatment in mice[a]

Model of infection	IMI-REL therapy (mg/kg/dose)	Total $\log_{10}$ CFU	Log change from control	REL plasma exposure (AUC_{0-24} in μg·h/ml)
P. aeruginosa (CLB 24228) disseminated model of infection	Control	6.78		
	5 (IMI)	6.33	−0.45	
	5 (IMI), 10 (REL)	5.06	−1.72	30.0
	5 (IMI), 20 (REL)	3.65	−3.13	59.2
	5 (IMI), 40 (REL)	3.05	−3.73	120.5
K. pneumoniae (CL 6339) disseminated model of infection	Control	6.15		
	5 (IMI)	6.67	+0.52	
	5 (IMI), 20 (REL)	3.86	−2.29	37.0
	5 (IMI), 40 (REL)	3.09	−3.06	76.2
	5 (IMI), 80 (REL)	3.79	−2.36	164.5
P. aeruginosa (CLB 24228) pulmonary model of infection	Control	6.59		
	5 (IMI)	6.70	+0.11	
	5 (IMI), 20 (REL)	4.22	−2.37	37.8
	5 (IMI), 40 (REL)	3.00	−3.59	106.6
	5 (IMI), 80 (REL)	2.00	−4.59	155.8

[a]*P. aeruginosa* strain CLB 24228 was administered as 2.2×10^6 CFU intraperitoneally in the disseminated model of infection and as 1.4×10^5 CFU intranasally in the pulmonary model of infection. *K. pneumoniae* strain CL 6339 was administered 5.5×10^5 intraperitoneally. DBA/2n ($n = 5$ per dose) mice were used. The plasma AUC_{0-24} values are reported here. IMI, imipenem-cilastatin; IMI-REL, imipenem-cilastatin-relebactam combination; REL, relebactam.

tance to carbapenems has created an urgent need for alternative options to treat MDR Gram-negative infections (7, 8). Use of β-lactam antibiotics in combination with β-lactamase inhibitors (BLI) has been one of the more successful strategies to enhance the activity of β-lactam antibiotics, as well as an effective therapeutic option against resistant bacteria (9, 10).

A study reported the discovery of a new BLI, relebactam (REL; MK-7655) (11), which was found to inhibit two different classes of β-lactamases *in vitro*: class A (serine-containing β-lactamases, such as the *Klebsiella pneumoniae* carbapenemase [KPC]) and class C (such as AmpC cephalosporinases). REL effectively restored imipenem's activity against both imipenem-resistant *P. aeruginosa* and *K. pneumoniae* by reducing the MIC (12–15).

The aim of these studies is to describe the *in vivo* efficacy of the imipenem-cilastatin (IMI)-REL combination in murine models of disseminated and pulmonary infection caused by imipenem-resistant clinical isolates of *P. aeruginosa* and *K. pneumoniae*. Since imipenem is generally coadministered with cilastatin in humans to prevent metabolism of imipenem, the *in vivo* efficacy studies utilized an IMI-REL combination.

RESULTS

Preclinical efficacy. The efficacy of REL was evaluated in combination with subefficacious doses of imipenem (5 mg/kg) in the treatment of antibiotic-resistant strains of *P. aeruginosa* and *K. pneumoniae* in two different models of infection: disseminated and pulmonary. The efficacy of IMI-REL was further assessed under the following two conditions: when treatment was administered immediately after infection and when treatment was delayed for 16.5 h. Log reductions in the CFU of the pathogen from animals treated with the antibiotic were compared with those from the untreated controls. In the disseminated model of infection, treatment with IMI-REL showed log reductions in *P. aeruginosa* (strain CLB 24228) CFU of 3.73, 3.13, and 1.72 at REL doses of 40, 20, and 10 mg/kg, respectively (Table 1). For *K. pneumoniae* (strain CL 6339), REL doses of 80, 40, and 20 mg/kg were associated with log reductions in CFU of 2.36, 3.06, and 2.29, respectively (Table 1). Studies in the pulmonary model of infection caused by *P. aeruginosa* (strain CLB 24228) showed similar results (log reductions in CFU of 4.59, 3.59, and 2.37 at REL doses of 80, 40, and 20 mg/kg, respectively; Table 1).

In the delayed pulmonary model of infection, mice were infected with *P. aeruginosa* strain CLB 24228, and treatment was initiated 16.5 h postinoculation. At 40 h (the time of termination of the study), there was an increase in organ burden of nearly 3 logs in the

TABLE 2 *In vivo* preclinical efficacy following delayed treatment in mice

Harvest time postinfection (h)[a]	IMI-REL therapy (mg/kg/dose)[d]	Total $\log_{10}$ CFU	Log change in CFU from 16.5 h	REL plasma exposure (AUC_{0-24} in μg·h/ml)	Log reduction at 40 h relative to the control
16.5	None	5.04			
24	None	5.99	+0.95		
40	None	7.92	+2.88		
40	5 (IMI)	7.14	+2.10		−0.78
40	5 (IMI), 20 (REL)	4.98[b]	−0.06	40.9	−2.94
40	5 (IMI), 40 (REL)	5.80[b,c]	+0.82	84.4	−2.06
40	5 (IMI), 80 (REL)	5.80[b]	+0.76	185.3	−2.12

[a]Five animals were tested per group (unless specified otherwise). *P. aeruginosa* strain CLB 24228 was administered at 1.8×10^4 CFU intranasally in DBA/2n mice ($n = 5$ per dose). The plasma AUC_{0-24} values are reported here.
[b]Not significantly different from burden at 16.5 h.
[c]Three animals were tested per group.
[d]IMI, imipenem-cilastatin; IMI-REL, imipenem-cilastatin-relebactam combination; REL, relebactam.

sham control animals. When IMI was administered at the subefficacious dose of 5 mg/kg, the organ burden increased by 2.1 logs. All tested doses (80, 40, and 20 mg/kg) of REL in combination with 5 mg/kg of IMI reached a static response at all doses: +0.76, +0.82, and −0.06 $\log_{10}$ CFU, respectively, versus the organ burden at the start of therapy (5.04 $\log_{10}$ CFU). This corresponded to a decrease in organ burden versus the 40-h $\log_{10}$ CFU of the sham control of −0.78 $\log_{10}$ CFU for the IMI of 5 mg/kg, and −2.12, −2.06, and −2.94 $\log_{10}$ CFU, respectively, for the 5 mg/kg IMI in combination with 80, 40, and 20 mg/kg of REL.

In order to determine the plasma exposure of REL, samples were taken at 20 and 40 min into infusion, as well as at 15 and 45 min postinfusion of the drug. The values for the plasma area under the concentration-time curve from 0 h to 24 h (AUC_{0-24}) are reported in Tables 1 and 2.

DISCUSSION

In this study, the *in vivo* efficacy of IMI-REL was tested and shown to be effective against imipenem-resistant strains of *P. aeruginosa* and *K. pneumoniae*, each of which express β-lactamase enzymes of different Ambler classes. The *P. aeruginosa* strain CLB 24228 used in the study overexpresses AmpC and lacks OprD porin (16), while the *K. pneumoniae* strain CL 6339 harbors KPC-2 and extended-spectrum β-lactamases (8, 16). Second, we utilized the disseminated and pulmonary mouse models of infection, with different methods for inoculation of the pathogen (intravenous injection and intranasal administration). Prior to the challenge, the mice were rendered neutropenic (17), so that the host immune system did not interfere with the interaction of the antibiotic and the bacteria within the host. Subefficacious doses of imipenem (5 mg/kg) in combination with REL at concentrations of 10 to 80 mg/kg showed corresponding increases in log reductions in the CFU of the pathogens in treated compared with untreated animals. This reduction in the multiplication of the pathogen was seen in both the pulmonary and the disseminated models of infection, confirming that IMI-REL was effective against a spectrum of antibiotic resistance. These *in vivo* studies are consistent with previous *in vitro* studies that indicate promising antibacterial activity for IMI-REL (16). A number of additional strains have been tested with IMI-REL in these mouse infection models and will be published in the future.

In studies using mouse models of infection, pathogen challenge tends to be immediately followed by antibiotic treatment; however, this is almost never the case clinically, wherein the pathogen multiplies within the host for hours or sometimes days before manifestation of the disease. Therefore, we studied the therapy in a delayed model of infection where treatment was delayed for 16.5 h after mice were challenged with *P. aeruginosa*. REL showed a static effect at a 20 mg/kg dose in combination with IMI in this delayed pulmonary model of infection caused by imipenem-resistant *P. aeruginosa*. Follow-up delayed-therapy studies confirmed these results (data not published). Plasma exposure of the drugs was determined at four different time points: 20 and 40 min into infusion and 15 and 45 min postinfusion.

Overall, we have shown that REL in combination with imipenem was efficacious against infections caused by imipenem-resistant clinical isolates of *P. aeruginosa* and *K. pneumoniae* in both the disseminated and the pulmonary murine models of infection. Thus, IMI-REL may prove to be a valuable addition to the armamentarium of antibacterial agents against the increasing threat of resistant bacteria.

MATERIALS AND METHODS

Bacterial strains (inoculum and dose preparation). Strains of *P. aeruginosa* (CLB 24228), with an MIC for imipenem of 32 μg/ml, and *K. pneumoniae* (CL 6339), with an MIC of 64 μg/ml, were used in all preclinical studies. The resistance mechanisms of the *P. aeruginosa* strain CLB 24228 include the overexpression of AmpC and absence of OprD porin (16), while the *K. pneumoniae* strain CL 6339 harbors KPC-2 and extended-spectrum β-lactamases (8, 16). The MICs of imipenem for these isolates in the presence of 4 μg/ml REL were 8 and 1 μg/ml, respectively. The *in vitro* susceptibility concentration of 4 μg/ml REL was chosen based on a phase 2 average plasma concentration in patients of 4.94 μg/ml (18).

Frozen stocks of the bacteria were reconstituted in a 10 ml tube of Trypticase soy broth and incubated at 35°C for 6 h. A volume of 1 ml was then transferred into 49 ml of Trypticase soy broth, followed by incubation overnight at 35°C. Inocula were prepared by diluting the overnight cultures to the required concentration based on the optical density at 600 nm. Aliquots were serially diluted and plated for quantification on MacConkey II agar plates. The inoculum for each bacterial species and the infection route were predetermined in an *in vivo* titration study, in which the inoculum was defined as the bacterial dose at which all mice succumbed to infection in 48 h (data not shown). Imipenem was added to a solution of 10 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS) buffer, containing 50 mg/kg cilastatin, for use as a control at a subefficacious dose of 5 mg/kg in all experiments. REL was dissolved in sterile water at the highest test concentration and serially diluted for remaining concentrations.

Preclinical efficacy models. All *in vivo* experiments were conducted in accordance with the recommendations outlined by the Association for Assessment and Accreditation of Laboratory Animal Care and approved by the Merck Institutional Animal Care and Use Committee. Two murine models of infection were used: disseminated and pulmonary. Jugular-vein-cannulated female DBA/2n mice ($n = 5$ per dose tested) weighing 20 g were used in all experiments. Mice were rendered neutropenic 4 days prior to the experimental infection with a single intraperitoneal injection of 250 mg/kg of cyclophosphamide. A single-level inoculum of 4.4×10^6 CFU/ml of *P. aeruginosa* was introduced as an intraperitoneal injection in a disseminated model of infection. For the pulmonary model of infection, 7.0×10^6 CFU/ml of the same strain was introduced intranasally. In both models, treatment with a 5 mg per kg of body weight/dose imipenem-titration of REL (10, 20, 40, or 80 mg/kg) was initiated 15 min after infection. In the *K. pneumoniae* disseminated model of infection, a single-level inoculum of 1.1×10^6 CFU/ml was introduced via intraperitoneal injection. Treatment was initiated after 15 min with 5 mg/kg imipenem and titration of REL (20, 40, and 80 mg/kg). All doses were administered via dual infusion using Lomir infusion systems. Four 1-h infusions were given every 6 h (0.3 ml/h). During the final infusion, mice were bled via tail snip for determination of the compound level. Samples for plasma pharmacokinetic (PK) analysis were collected at 20 and 40 min into the infusion, as well as at 15 and 45 min postinfusion.

In the delayed therapy model, neutropenia-induced mice were inoculated with 9.0×10^5 CFU/ml of *P. aeruginosa* intranasally. Treatment was initiated 16.5 h postinfection, with 5 mg/kg imipenem and titration of REL (20, 40, and 80 mg/kg) administered as described previously.

At 6 h after the final infusion, target tissue (e.g., spleen or lung) was aseptically removed and homogenized. Serial dilutions of the homogenate were plated on MacConkey agar plates, followed by incubation for 24 h. Log CFU/g of tissue values from untreated and treated mice were compared and used to determine the net log reduction in treated groups.

Mouse PK modeling. The pharmacokinetic (PK) data from *in vivo* efficacy murine lung studies was used to build a mouse population PK (popPK) model. Using the popPK model, the exposure at minimum dose of REL required to achieve stasis in each study was computed using the post hoc individual PK parameters for that study. The properties of REL in mice used for mouse popPK modeling were determined as follows: the extent of mouse plasma protein binding was 20.8%, and the mouse blood/plasma ratio was 0.61. An average weight of 20 g was assumed for each mouse in the modeling. Since the mice PK was measured in blood, the blood/plasma ratio determined above was used for conversion to plasma PK. The exposures (AUC_{0-24}) in mice for the various REL doses in studies were obtained from the mouse popPK model. Detailed results for exposure are presented in Tables 1 and 2.

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