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PHARMACOLOGY

# *In Vivo* Activity of QPX7728, an Ultrabroad-Spectrum Beta-Lactamase Inhibitor, in Combination with Beta-Lactams against Carbapenem-Resistant *Klebsiella pneumoniae*

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**ABSTRACT** Resistance to beta-lactams has created a major clinical issue. QPX7728 is a novel ultrabroad-spectrum cyclic boronic acid beta-lactamase inhibitor with activity against both serine and metallo-beta-lactamases developed to address this resistance for use in combination with beta-lactam antibiotics. The objective of these studies was to evaluate the activity of QPX7728 in combination with multiple beta-lactams against carbapenem-resistant *Klebsiella pneumoniae* isolates in a neutropenic mouse thigh infection model. Neutropenic mice were infected with strains with potentiated beta-lactam MICs of ≤2 mg/liter in the presence of 8 mg/liter QPX7728. Two strains of carbapenem-resistant *K. pneumoniae* were tested with aztreonam, biapenem, cefepime, ceftazidime, ceftolozane, and meropenem alone or in combination with 12.5, 25, or 50 mg/kg of body weight of QPX7728 every 2 hours for 24 hours. Treatment with all beta-lactams alone either was bacteriostatic or allowed for bacterial growth. The combination of QPX7728 plus each of these beta-lactams produced bacterial killing at all QPX7728 doses tested. Overall, these data suggest that QPX7728 administered in combination with different partner beta-lactam antibiotics may have utility in the treatment of bacterial infections due to carbapenem-resistant *K. pneumoniae*.

**KEYWORDS** beta-lactams, QPX7728, CRE

The beta-lactam class of antibiotics is one of the most valuable and widely utilized classes of antimicrobial agents used for the treatment of nosocomial and community-acquired bacterial infections (1, 2). This class of antibiotics not only has an excellent safety profile but also exhibits broad-spectrum activity against both Gram-positive and Gram-negative bacteria. Over the last three decades, resistance to beta-lactams has increased globally, threatening the utility of this important class of antibiotics (3–7).

Resistance to beta-lactams is primarily mediated by bacterial enzymes called beta-lactamases. These enzymes hydrolyze the beta-lactam ring which eliminates the antibacterial activity of the beta-lactam. Beta-lactam activity can be diminished by beta-lactamases from one or multiple enzymes classes. Beta-lactamases have been categorized into four classes (Ambler classifications) known as A, B, C, and D. The hydrolysis of beta-lactams by the enzymes of classes A, C, and D relies on an active site serine residue, whereas those of class B are metalloenzymes that use one or two zinc ions to coordinate hydrolysis. While serine enzymes from different classes may share some structural similarity, metalloenzymes are structurally dissimilar (9, 10). Given the differences in enzyme structure and binding sites, finding a single molecule that can inhibit both serine and metallo-beta-lactamases has been a daunting challenge.

As resistance to carbapenems, the most active and beta-lactamase-stable beta-

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**TABLE 1** Susceptibility of *K. pneumoniae* strains to beta-lactams alone and in combination with QPX7728

| | | MIC (mg/liter) of[a]: | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ATM | | BIA | | FEP | | CAZ | | TOL | | MEM | |
| Strain | Beta-lactamases | Alone | QPX7728[b] | Alone | QPX7728 | Alone | QPX7728 | Alone | QPX7728 | Alone | QPX7728 | Alone | QPX7728 |
| KP1096 | KPC-2, TEM, SHV-11 | >256 | 0.25 | >256 | 0.13 | 128 | 1 | 128 | 1 | 64 | 1 | >256 | 0.5 |
| KP1223 | KPC-2, SHV, TEM | >256 | 2 | >256 | ≤0.06 | >256 | 0.5 | >256 | 2 | >256 | 1 | >256 | ≤0.06 |

[a]ATM, aztreonam; BIA, biapenem; FEP, cefepime; CAZ, ceftazidime; TOL, 395 ceftolozane; MEM, meropenem.
[b]At a concentration of 8 mg/liter.

lactams, increased, new programs were initiated to develop novel beta-lactamase inhibitors with activity against carbapenemases. These programs were highly successful and led to the development and approval of Avycaz (ceftazidime-avibactam), Recarbrio (imipenem-cilastatin/relebactam), and Vabomere (meropenem-vaborbactam). These agents have broad activity against class A (TEM, CTX-M, SHV, and KPC) and class C (AmpC) enzymes. Avycaz has some activity against class D (OXA-48) enzymes. However, none of these agents have activity against metallo-beta-lactamases, such as NDM, VIM, and IMP or against class D enzymes found in *Acinetobacter baumannii*, such as OXA-23 (11–15). Consequently, there is a continued effort to discover and develop new beta-lactamase inhibitors that can inhibit both serine and metallo-beta-lactamases in *Enterobacteriaceae* as well as in nonfermenters (*Pseudomonas aeruginosa* and *A. baumannii*).

As the part of this effort, our team discovered and initiated the development of QPX7728, an ultrabroad-spectrum beta-lactamase inhibitor with activity against all four beta-lactamase classes and the ability to restore activity to cephalosporins, carbapenems, penicillins, and monobactams (16). QPX7728 in combination with multiple different beta-lactams has *in vitro* activity against resistant organisms considered serious or urgent threats by the CDC, such as carbapenem-resistant *Enterobacteriaceae*, *P. aeruginosa*, and *A. baumannii* (17–19). The objective of these studies was to evaluate the *in vivo* activity of QPX7728 administered in combination with multiple beta-lactams in a neutropenic mouse thigh infection model of carbapenem-resistant *K. pneumoniae*.

## RESULTS

**Susceptibility studies.** The susceptibilities of carbapenem-resistant *K. pneumoniae* KP1096 and *K. pneumoniae* KP1223 to aztreonam, biapenem, cefepime, ceftazidime, ceftolozane, and meropenem were determined alone and combined with a fixed concentration of 8 mg/liter QPX7728. These strains produced various beta-lactamases as well as other non-beta-lactamase-mediated resistance mechanisms, including porin mutation(s) that are known to affect the potency of beta-lactams. As shown in Table 1, the *in vitro* potency of these beta-lactams was markedly improved when combined with QPX7728 at 8 mg/liter.

**Pharmacokinetics.** The pharmacokinetic parameters for aztreonam, biapenem, ceftazidime, meropenem, and QPX7728 are shown in Table 2. The pharmacokinetic

**TABLE 2** Pharmacokinetic parameters following single doses of compounds administered by the intraperitoneal route

| Compound | Dose (mg/kg) | $AUC_{0-\infty}$[a] (mg*h/liter) | CL/F[b] (liter/h/kg) | $C_{max}$[c] (mg/liter) | $T_{1/2}$[d] (h) |
|---|---|---|---|---|---|
| Aztreonam | 150 | 131.86 | 1.13 | 249.47 | 0.29 |
| Biapenem | 100 | 104.52 | 0.96 | 222.70 | 0.22 |
| Ceftazidime | 300 | 150.75 | 1.99 | 233.20 | 0.28 |
| Meropenem | 300 | 145.79 | 2.06 | 326.81 | 0.19 |
| QPX7728 | 10 | 28.52 | 0.35 | 39.20 | 1.55 |
| QPX7728 | 30 | 70.01 | 0.43 | 95.30 | 1.43 |
| QPX7728 | 100 | 223.08 | 0.45 | 337.00 | 1.27 |

[a]$AUC_{0-\infty}$, area under the concentration-time curve from 0 to infinity.
[b]CL/F, oral clearance.
[c]$C_{max}$, maximum concentration of drug in serum.
[d]$T_{1/2}$, half-life.

**TABLE 3** Pharmacokinetic parameters used for the simulations

| Drug by organism | $k_{01}$[a] (1/h) | $k_{10}$[b] (1/h) | V/F[c] (liter/kg) | V[d] (liter) | Protein binding (%) | Reference(s) |
|---|---|---|---|---|---|---|
| Human | | | | | | |
| Aztreonam | | 0.433 | | 12.6 | 56 | 29 |
| Biapenem | | 0.594 | | 18.5 | 7 | Unpublished, 30 |
| Cefepime | | 0.400 | | 18.0 | 20 | 31 |
| Ceftazidime | | 0.381 | | 18.1 | 5 | 32 |
| Ceftolozane | | 0.408 | | 13.3 | 20 | 33 |
| Meropenem | | 0.743 | | 20.2 | 2 | 34 |
| Mouse | | | | | | |
| Aztreonam | 28.56 | 2.37 | 0.48 | | 56.5 | This manuscript, 35 |
| Biapenem | 16.38 | 3.16 | 0.30 | | 2 | This manuscript, 30 |
| Cefepime | 16.00 | 2.24 | 0.50 | | 0 | 25–27 |
| Ceftazidime | 9.51 | 4.37 | 0.40 | | 0 | This manuscript, 36 |
| Ceftolozane | 3.00 | 3.09 | 0.43 | | 5 | 28 |
| Meropenem | 13.73 | 3.61 | 0.57 | | 10 | This manuscript, 37 |

[a]$k_{01}$, absorption rate constant.
[b]$k_{10}$, elimination rate constant.
[c]V/F, volume of distribution/fraction absorbed.
[d]V, volume of distribution.

parameters used for both the mouse and human pharmacokinetic treatment regimen simulations are provided in Table 3.

**Treatment regimens.** The comparison of the simulated percentage of the 24-h study duration that free-drug levels exceed the MIC (%24h $fT_{>MIC}$) of each beta-lactam in mice and that of humans is shown in Table 4. Based on the simulations, the dosage regimens were expected to produce %24h $fT_{>MIC}$ that were similar to human dosage regimens for each beta-lactam.

**Thigh infection model.** The activity of two carbapenems, three cephalosporins, and a monobactam alone or in combination with QPX7728 were evaluated against both strains of carbapenem-resistant *K. pneumoniae*. As shown in Fig. 1 and 2, treatment with ceftazidime, ceftolozane, or cefepime was ineffective at reducing bacterial counts for either strain compared with untreated control groups at the start of treatment. When the same exposures of these cephalosporins were combined with 12.5, 25, or 50 mg/kg of body weight of QPX7728, they produced about 2 log CFU reductions in bacterial counts for both strains compared with untreated controls at the start of treatment and all combinations with beta lactams alone ($P < 0.05$).

**TABLE 4** Comparison of the 24-h free AUC and %24h $fT>MIC$ of each beta-lactam in mice and in humans

| | | | %24h $fT>MIC$ at MIC (mg/liter) of: | | | | | | | | | | 24-h free AUC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Drug | Species | Dosage regimen[a] | 128 | 64 | 32 | 16 | 8 | 4 | 2 | 1 | 0.5 | 0.25 | mg*h/liter |
| Aztreonam | Human | 2,000 mg TID by 1-h infusion | 0 | 0 | 22.5 | 45 | 66.9 | 88.6 | 100 | 100 | 100 | 100 | 484 |
| | Mouse | 150 mg/kg q2h | 6 | 22 | 38 | 52.5 | 67 | 81.5 | 96.5 | 100 | 100 | 100 | 894 |
| Biapenem | Human | 1,000 mg TID by 3-h infusion | 0 | 0 | 0 | 27.5 | 60 | 74.4 | 88.6 | 100 | 100 | 100 | 254 |
| | Mouse | 100 mg/kg q2h | 16.5 | 29 | 40 | 50 | 62 | 73 | 84 | 95 | 100 | 100 | 1,229 |
| Cefepime | Human | 2,000 mg TID by 2-h infusion | 0 | 0 | 34.1 | 61.8 | 88.6 | 100 | 100 | 100 | 100 | 100 | 667 |
| | Mouse | 150 mg/kg q2h | 20 | 36.5 | 50.0 | 68.5 | 84 | 100 | 100 | 100 | 100 | 100 | 1,607 |
| Ceftazidime | Human | 2,000 mg TID by 2-h infusion | 0 | 8.8 | 41.9 | 70.6 | 97.5 | 100 | 100 | 100 | 100 | 100 | 826 |
| | Mouse | 300 mg/kg q2h | 25 | 41 | 55.5 | 69.5 | 83.5 | 98.0 | 100 | 100 | 100 | 100 | 1,809 |
| Ceftolozane | Human | 2,000 mg TID by 1-h infusion | 0 | 17.5 | 44.3 | 68.2 | 88.8 | 100 | 100 | 100 | 100 | 100 | 884 |
| | Mouse | 150 mg/kg q2h | 0 | 38 | 59 | 74.5 | 88.5 | 100 | 100 | 100 | 100 | 100 | 1,287 |
| Meropenem | Human | 2,000 mg TID by 3-h infusion | 0 | 0 | 18.8 | 44.4 | 60.0 | 75.1 | 87.1 | 100 | 100 | 100 | 392 |
| | Mouse | 300 mg/kg q2h | 20 | 30 | 41.5 | 51 | 60.5 | 70 | 79 | 89 | 100 | 100 | 1,575 |

[a]q2h, every 2 hours; TID, three times a day.

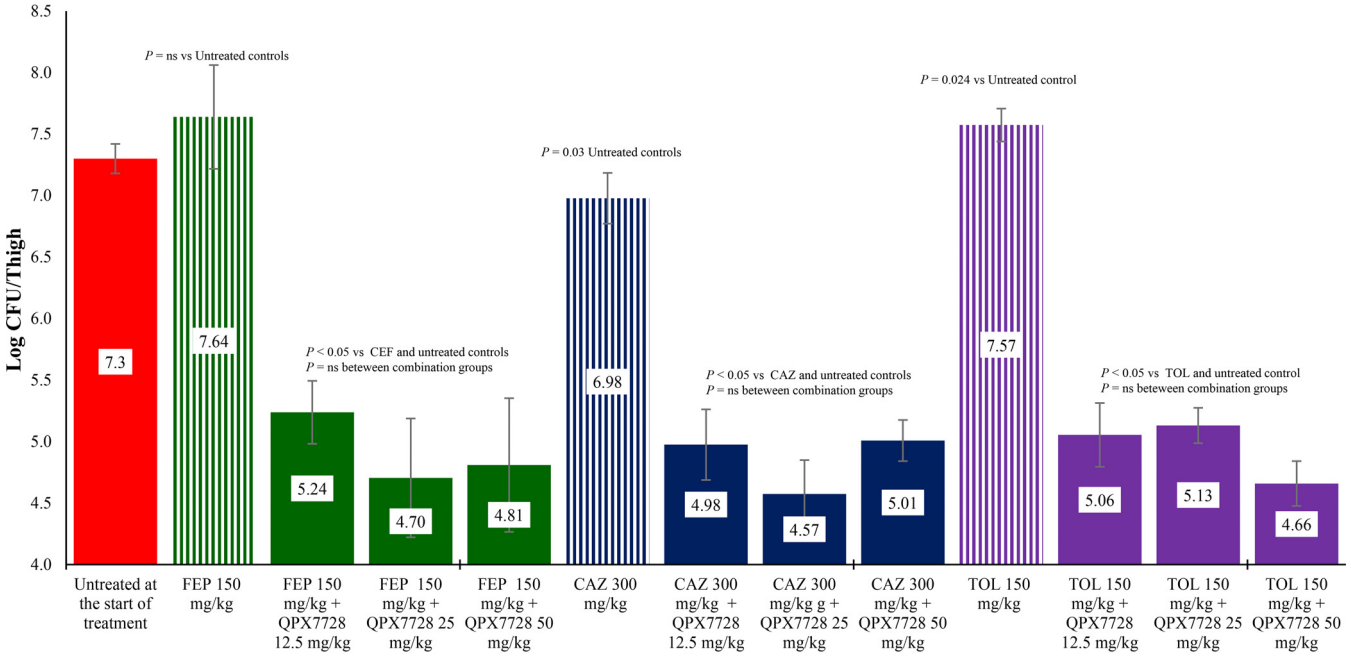


**FIG 1** Activity of cefepime (FEP), ceftazidime (CAZ), and ceftolozane (TOL) alone and in combination with QPX7728 against *K. pneumoniae* KP1096 in a neutropenic mouse thigh infection model. Treatments were administered every 2 hours for 24 hours by the intraperitoneal route.

The activity of aztreonam alone and in combination with 12.5, 25, or 50 mg/kg of QPX7728 against both strains of carbapenem-resistant *K. pneumoniae* are shown in Fig. 3. The exposure of aztreonam allowed between 1 and 2 logs of bacterial growth in both *K. pneumoniae* strains. For *K. pneumoniae* KP1096, the combination of aztreonam with 12.5, 25, or 50 mg/kg of QPX7728 produced 1.47, 1.62, and 1.54 log CFU reductions

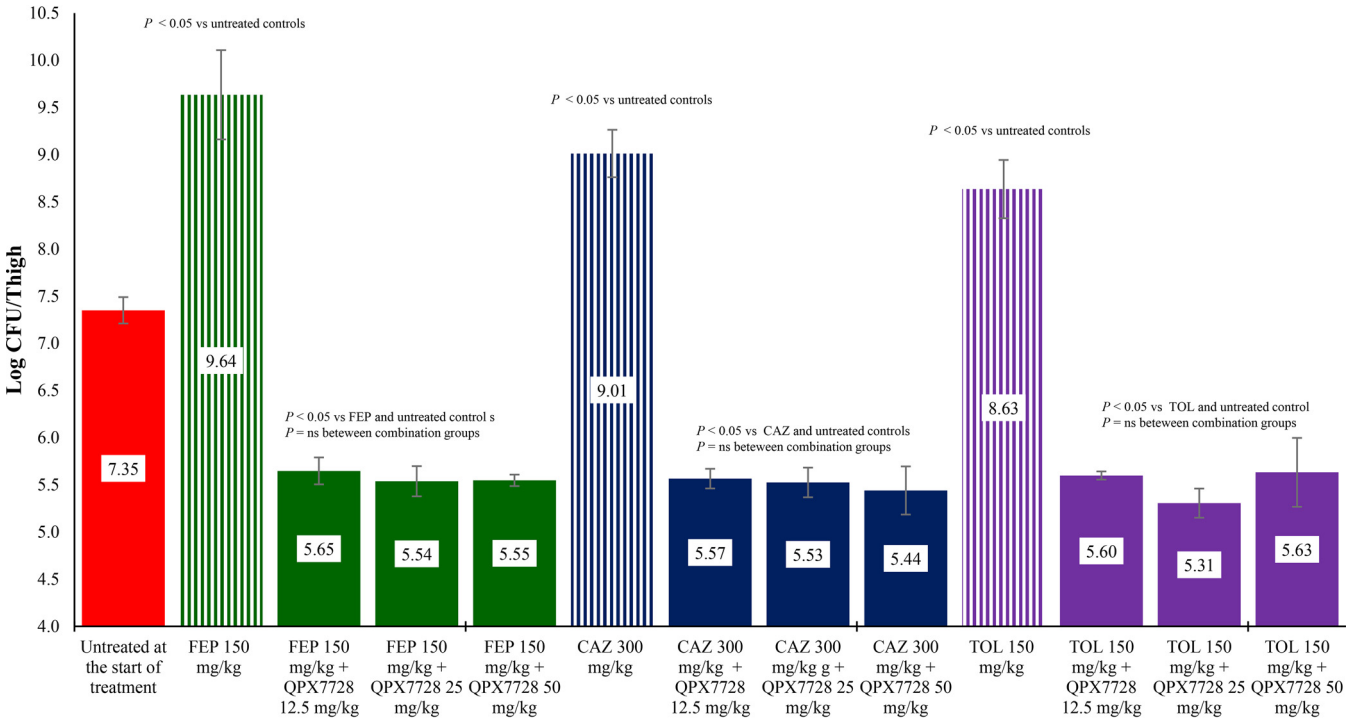


**FIG 2** Activity of cefepime (FEP), ceftazidime (CAZ), and ceftolozane (TOL) alone and in combination with QPX7728 against *K. pneumoniae* KP1223 in a neutropenic mouse thigh infection model. Treatments were administered every 2 hours for 24 hours by the intraperitoneal route.

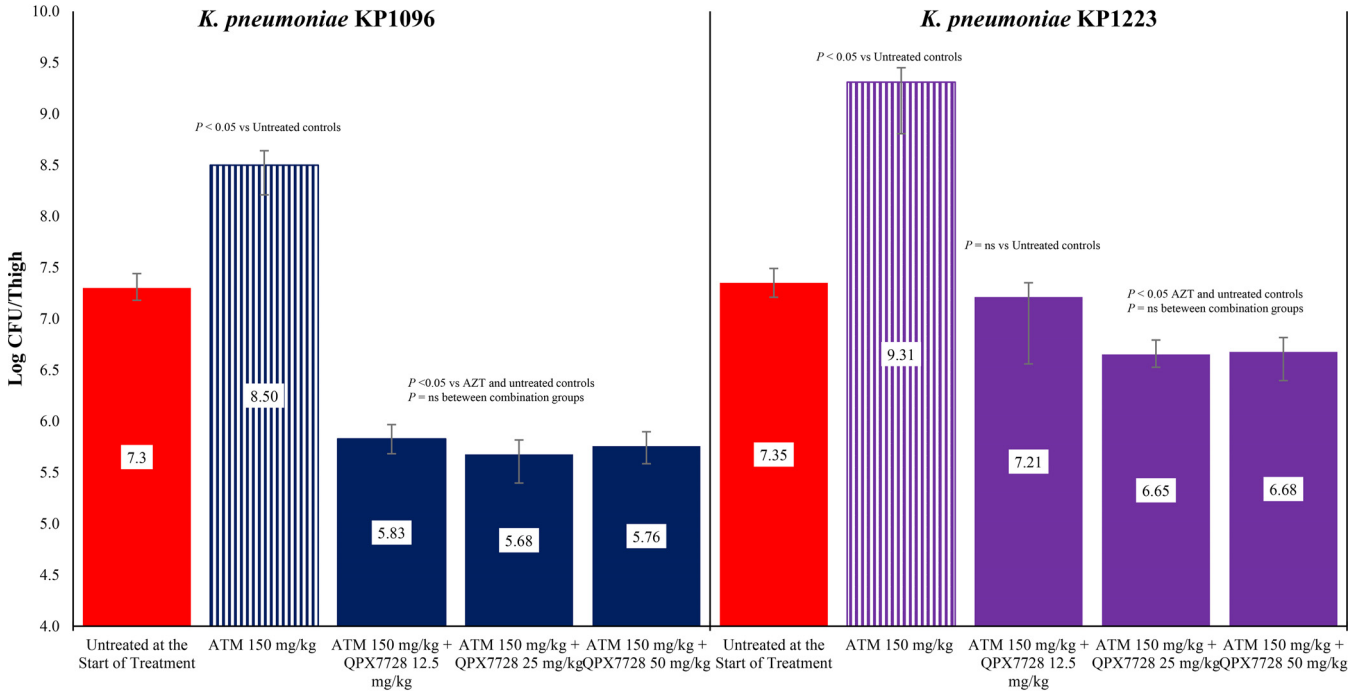


**FIG 3** Activity aztreonam (ATM) alone and in combination with QPX7728 against *K. pneumoniae* KP1096 and *K. pneumoniae* KP1223 in a neutropenic mouse thigh infection model. Treatments were administered every 2 hours for 24 hours by the intraperitoneal route.

in bacterial counts in the thigh, respectively. The reduction in bacterial counts with the combination therapy for all three dose regimens of QPX7728 was significantly greater than that observed for aztreonam alone ($P < 0.05$). For *K. pneumoniae* KP1223, the combination of aztreonam with 12.5, 25, or 50 mg/kg of QPX7728 produced 0.14, 0.70, and 0.67 log CFU reductions in bacterial counts in the thigh, respectively. The reduction in bacterial counts with aztreonam plus 12.5 mg/kg of QPX7728 was not significant compared with untreated controls at the start of treatment ($P > 0.05$). However, the bacterial counts with aztreonam in combination with 25 or 50 mg/kg of QPX7728 were significantly lower than that observed with aztreonam alone or untreated controls at the start of treatment ($P < 0.05$).

The activity of biapenem and meropenem alone and in combination with QPX7728 against *K. pneumoniae* strain KP1096 is shown in Fig. 4. Treatment with biapenem and meropenem allowed 0.73 and 0.49 log CFU, respectively, of bacterial growth compared with untreated controls at the start of treatment. The administration of biapenem in combination with 12.5, 25, or 50 mg/kg of QPX7728 produced 0.91, 1.21, and 1.37 log CFU reductions, respectively, in bacterial counts in the thigh compared with untreated controls at the start of treatment. Treatment with meropenem in combination with 12.5, 25, or 50 mg/kg of QPX7728 produced 0.94, 1.21, and 1.19 log CFU reductions, respectively, in bacterial counts in the thigh compared with untreated controls at the start of treatment, respectively. The reductions in bacterial counts observed with combination therapy for both carbapenems at all doses of QPX7728 were significantly greater than those observed with either biapenem or meropenem alone ($P < 0.05$).

The activity of biapenem and meropenem alone and in combination with QPX7728 against *K. pneumoniae* strain KP1223 is shown in Fig. 5. Treatment with biapenem or meropenem allowed for, roughly, 2 log CFU of bacterial growth compared with untreated controls at the start of treatment. The administration of biapenem in combination with 12.5 mg/kg of QPX7728 did not produce a reduction in bacterial counts compared with untreated controls at the start of treatment ($P > 0.05$), but the bacterial counts were significantly lower with 12.5 mg/kg of QPX7728 than treatment with biapenem alone ($P < 0.05$). The administration of biapenem in combination of 25

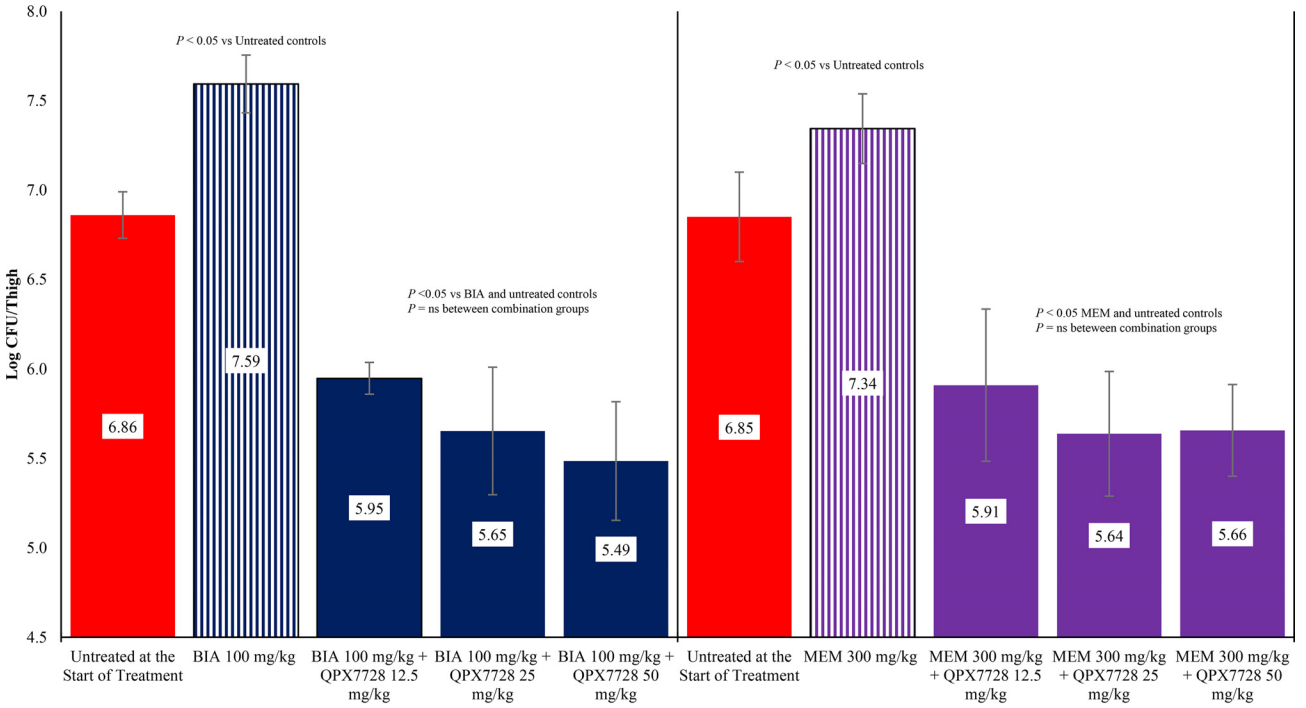


**FIG 4** Activity of biapenem (BIA) and meropenem (MEM) alone and in combination with QPX7728 against *K. pneumoniae* KP1096 in a neutropenic mouse thigh infection model. Treatments were administered every 2 hours for 24 hours by the intraperitoneal route.

or 50 mg/kg of QPX7728 produced 1.56 and 1.62 log CFU reductions, respectively, in bacterial counts in the thigh. These reductions in bacterial counts were significantly lower than that observed with biapenem alone and untreated controls at the start of treatment ($P < 0.05$). The administration of meropenem in combination with 12.5, 25,

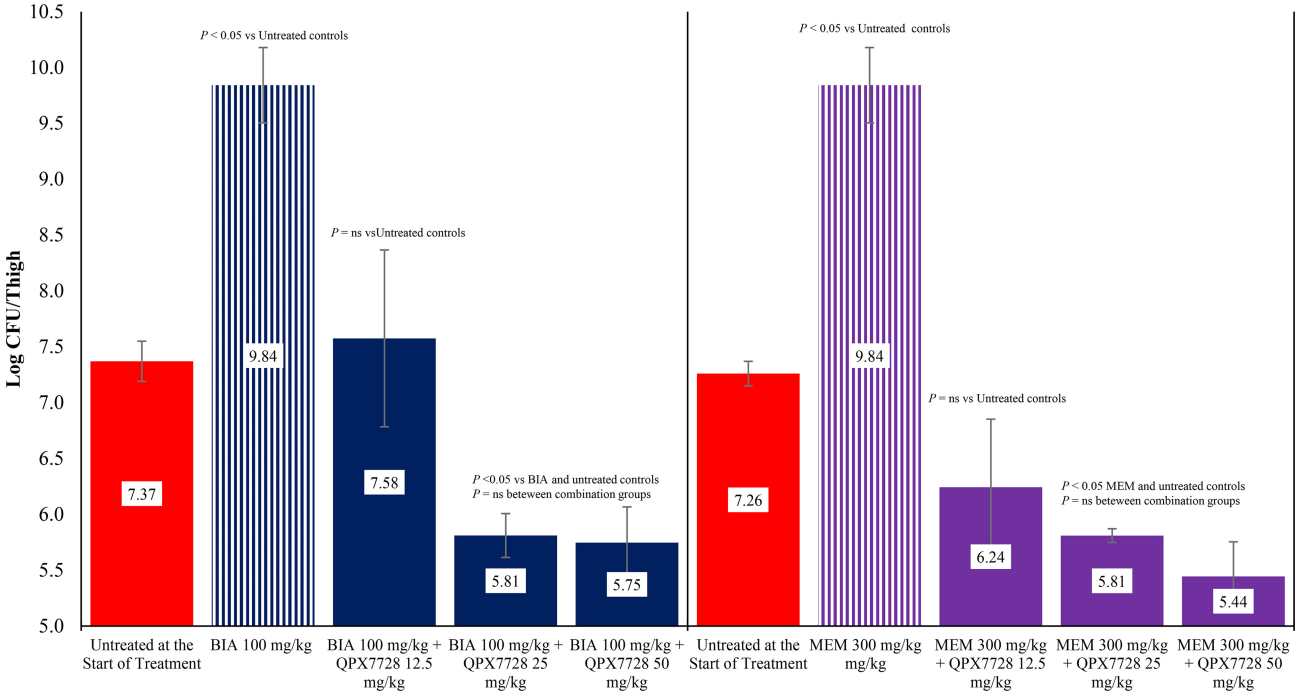


**FIG 5** Activity of biapenem (BIA) and meropenem (MEM) alone and in combination with QPX7728 against *K. pneumoniae* KP1223 in a neutropenic mouse thigh infection model. Treatments were administered every 2 hours for 24 hours by the intraperitoneal route.

or 50 mg/kg of QPX7728 produced between 1.02, 1.45, or 1.82 log CFU reductions, respectively, in bacterial counts in the thigh. These reductions in bacterial counts were significantly lower than that observed with meropenem alone and untreated controls at the start of treatment ($P < 0.05$).

QPX7728 alone did not have any antibacterial activity against *K. pneumoniae* KP1096. Treatment with QPX7728 at 50 mg/kg every 2 hours allowed for 1.14 log CFU/thigh of bacterial growth.

For all experiments, untreated control groups for both *K. pneumoniae* strains were sacrificed after 12 hours due to signs of distress. Over the 12-hour period, *K. pneumoniae* KP1096 grew by 0.7 log CFU/thigh and *K. pneumoniae* KP1223 grew by 2.0 log CFU/thigh.

## DISCUSSION

The steady rise of bacterial resistance due to beta-lactamases in *Enterobacteriaceae* is threatening the utility of beta-lactams against these organisms (20–22).

In response, our laboratory has been investigating novel cyclic boronic acid beta-lactamase inhibitors for over a decade. Our first discovery and successful development candidate, vaborbactam, was specifically designed to inhibit serine carbapenemases, particularly the *K. pneumoniae* carbapenemase (KPC) enzyme. Vaborbactam is now approved in the United States and Europe in combination with meropenem as Vabomere (United States) or Vaborem (European Union). Since that discovery, our focus has been on improving the potency and spectrum of these inhibitors, with an emphasis on metallo-beta-lactamases. Through these investigations, we have discovered QPX7728, an ultrabroad-spectrum beta-lactamase inhibitor with activity against both serine (class A, C, and D) and metalloenzymes (class B) in *Enterobacteriaceae*. In addition, QPX7728 is less susceptible to non-beta-lactamase resistance mechanisms, such as efflux and porin mutations (16–19). Given this ultrabroad spectrum activity, QPX7728 was tested *in vitro* and *in vivo* in combination with multiple beta-lactams possessing various degrees of intrinsic stability to beta-lactamases against two strains of carbapenem-resistant *K. pneumoniae*. Both strains used in these studies produced SHV, TEM, and KPC beta-lactamases and had mutations in the outer membrane porins, OmpK 35 and OmpK 36 (23). The combination of resistance mechanisms rendered these strains highly resistant to each beta-lactam alone (all MICs were $\geq$64 mg/liter). The addition of QPX7728 at 8 mg/liter reduced the MICs of each beta-lactam at least 32-fold, with the highest MIC to the combination being 2 mg/liter. The dosage regimens in mice for each beta-lactam were selected to approximate the %24h $fT_{>MIC}$ of human regimens. The exposure simulations were based on single-dose pharmacokinetic (PK) studies from our laboratory with the exceptions of ceftolozane and cefepime. The PK profiles for both of these drugs were referenced from the literature and not replicated in our laboratory; as such, the simulated profiles reported may not reflect the exposures generated in the efficacy studies.

Based on the simulations, the selected dosage regimens for aztreonam, biapenem, cefepime, ceftazidime, ceftolozane, and meropenem alone resulted in bacterial stasis or bacterial growth compared with untreated controls at the start of treatment. In contrast, the combination of each beta-lactam with QPX7728 produced at least 1 log of bacterial killing against both strains of carbapenem-resistant *K. pneumoniae*, apart from aztreonam against *K. pneumoniae* strain KP1223.

Overall, these data demonstrate that QPX7728 restores the activity of multiple different beta-lactams, including cephalosporins, carbapenems, and monobactams, against highly resistant strains of *K. pneumoniae*. While these data were generated with only two strains and only cover a limited number of mechanisms that confer resistance to beta-lactams, the data suggest that QPX7728 administered in combination with multiple different beta-lactams could have clinical utility in the treatment of infections due to carbapenem-resistant *Enterobacteriaceae*.

## MATERIALS AND METHODS

**Mice.** Female Swiss mice (age, 5 to 6 weeks) were obtained from Envigo (Livermore, CA). All studies using animals were performed under protocols approved by an Institutional Animal Care and Use Committee (IACUC).

**Antimicrobial agents.** Cefepime for injection (lot number 9D01006A41), ceftazidime for injection (lot number 108225C), and meropenem for injection (lot number DF-3297) were obtained from commercial sources. Cefepime, ceftazidime, and meropenem were prepared according to their package inserts. Aztreonam (lot number S07F022) powder was obtained from a commercial source and solubilized in 1% $NaHCO_3$. Biapenem, ceftolozane, and QPX7728 were synthesized by Qpex Biopharma, Inc. (San Diego, CA) and solubilized in 0.9% saline.

**Bacterial strain testing and MIC determination.** Two *K. pneumoniae* clinical isolates were used in these studies. The susceptibility of these isolates to aztreonam, biapenem, cefepime, ceftazidime, ceftolozane, and meropenem alone and in combination with 8 mg/liter of QPX7728 was determined by a broth microdilution assay according to CLSI reference methods (24). Assays were performed using a final volume of 100 $\mu$l. The bacterial suspensions were adjusted to yield a final cell density of ca. $5 \times 10^5$ CFU/ml. Each beta-lactam was prepared at a concentration equivalent to 2-fold the highest desired final concentration in culture medium and was then diluted directly into 96-well microtiter plates. For the determination of MICs in the presence of QPX7728, QPX7728 was added at a fixed concentration of 8 mg/liter. Microtiter plates were incubated for 16 to 18 h at 37°C and were read by using a microtiter plate reader (Molecular Devices, Sunnyvale, CA) at 600 nm, as well as by visual observation by using a microtiter plate reading mirror. The MIC was defined as the lowest concentration of antibiotic at which the visible growth of the organism was completely inhibited.

**Neutropenic mouse thigh infection model.** Female mice ($n = 2$/group) were rendered neutropenic by an intraperitoneal (i.p.) injection of 150 mg/kg cyclophosphamide (Baxter, IL) on day 1 and day 4. On day 5, mice were infected by intramuscular injection of 0.1 ml of inoculum ($10^7$ CFU/ml) into both thigh muscles while under isoflurane anesthesia (5% isoflurane in oxygen running at 4 liters/min) (21). The infecting bacterial suspensions were prepared as follows: strains were grown in Mueller-Hinton broth (MHB) at 37°C under constant aeration (300 rpm) for 20 h. The infecting inoculum was prepared by removing an aliquot and diluting it into fresh MHB at 37°C, under constant aeration, for 3 h to reach an absorbance at 600 nm of 0.30 to 0.35. The bacterial suspensions were diluted in fresh MHB to make $\sim 10^7$ CFU/ml by correlation of absorbance at 600 nm with predetermined plates.

**Pharmacokinetics.** The pharmacokinetics of cefepime and ceftolozane in mice were referenced from the literature (25–28). The pharmacokinetics of aztreonam, biapenem, ceftazidime, meropenem, and QPX7728 were determined for this work in female Swiss mice. Mice were rendered neutropenic and infected in both thighs as described above. Two hours following infection, mice were administered a single dose by the i.p. route. At 0.08, 0.25, 0.5, 1, 2, 4, and 6 hours following administration, 3 mice/time point were euthanized and their blood collected by cardiac puncture and transferred to EDTA-containing tubes. Blood samples were centrifuged within 5 min of collection at $12{,}000 \times g$ for 5 min to obtain plasma. An equal volume of morpholinopropanesulfonic acid (MOPS) buffer (pH 7) was added to plasma samples containing aztreonam and meropenem. All samples were stored at −80°C until analyzed.

**Bioanalytical analysis.** Aztreonam standard curves were prepared in 50% 1 M MOPS/50% plasma at concentrations of 0.05 to 50.0 $\mu$g/ml. Twenty-five-microliter aliquots of sample were placed in 1.5-ml microcentrifuge tubes containing 100 $\mu$l of 4 $\mu$g/ml QPX7015 in 10%, 45%, 45% of water-methanol-acetonitrile (vol/vol/vol). Biapenem standard curves were prepared in mouse plasma at concentrations of 0.01 to 100 $\mu$g/ml. Fifty-microliter aliquots of sample were placed in 1.5-ml microcentrifuge tubes containing 200 $\mu$l of 2 $\mu$g/ml gatifloxacin and 2 $\mu$g/ml QPX7015 in 100 mM ammonium acetate in methanol. Ceftazidime standard curves were prepared in mouse plasma at concentrations of 0.01 to 100 $\mu$g/ml. Fifty-microliter aliquots of sample were placed in 1.5-ml microcentrifuge tubes containing 200 $\mu$l of 2 $\mu$g/ml gatifloxacin and 2 $\mu$g/ml QPX7015 in 100 mM ammonium acetate in methanol. Meropenem standard curves were prepared in 50% 1 M MOPS/50% plasma at concentrations of 0.04 to 50 $\mu$g/ml. Twenty-five-microliter aliquots of sample were placed in 1.5-ml microcentrifuge tubes containing 200 $\mu$l of 4.0 $\mu$g/ml doripenem in 10%, 45%, 45% of water-methanol-acetonitrile (vol/vol/vol). QPX7728 standard curves were prepared in 50-50 plasma-phosphate buffer at concentrations of 0.05 to 50.0 $\mu$g/ml. Thirty-microliter aliquots of sample were placed in 1.5-ml microcentrifuge tubes containing 30 $\mu$l of 2 $\mu$g/ml QPX7701 in 50-50 water-acetonitrile and 30 $\mu$l of 20 mM phosphate buffer (pH 7.4).

For all antibiotics, the samples were mixed using a vortex mixer and then centrifuged for 10 min at $15{,}000 \times g$ using a tabletop centrifuge. The supernatant was removed and placed in a 96-well plate, and then 10 $\mu$l of each sample was injected onto a high-performance liquid chromatography-mass spectrometer (HPLC-MS) for quantification.

Plasma concentrations were fitted using a one-compartment model with first-order input and first-order elimination (Phoenix WinNonlin version 8.1; Certara USA, Inc., Princeton, NJ).

**Treatment regimens.** Treatment regimens in mice were selected to approximate the %24h $fT_{>MIC}$ of each beta-lactam produced by their respective human dosage regimens (29–34). The total drug plasma profiles of each beta-lactam in humans were simulated using a one-compartment intravenous (i.v.) infusion model and first-order elimination (Phoenix WinNonlin version 8.1; Certara USA, Inc., Princeton, NJ) based on the literature. The free plasma profiles were calculated by correcting the total drug profiles for protein binding. For mice, the total drug plasma profiles of each beta-lactam were simulated using a one-compartment model with first-order input and first-order elimination (Phoenix WinNonlin version 8.1) based on single-dose studies from this work or from the literature. The plasma profiles were then corrected for protein binding and the dosage regimen selected that approximated the

%24h $fT_{>MIC}$ of the human dosage regimen. Ceftazidime and meropenem were administered at 300 mg/kg every 2 hours. Aztreonam, cefepime, and ceftolozane were administered at 150 mg/kg every 2 hours. Biapenem was administered at 100 mg/kg every 2 hours. Each beta-lactam was administered alone and in combination with QPX7728 at doses of 12.5, 25, and 50 mg/kg starting 2 hours postinfection and were continued every 2 hours over 24 hours by the i.p. route.

**CFU determination.** Untreated control animals were euthanized at the start of treatment to establish the baseline bacterial burden (2 hours postinfection). All treated and untreated control groups were euthanized by carbon dioxide asphyxiation. The thighs ($n = 4$/group) were removed aseptically and homogenized (Pro200 homogenizer; Pro Scientific, Monroe, CT) in ice-cold sterile saline. Serial 10-fold dilutions of the homogenized thighs were plated onto Mueller-Hinton agar, and the colonies were counted.

**Statistical analysis.** Bacterial counts were analyzed using an unpaired *t* test (GraphPad Prism version 6.03). A *P* value of $<0.05$ was considered statistically significant.

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