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Combining the FtsZ-Targeting Prodrug TXA709 and the Cephalosporin Cefdinir Confers Synergy and Reduces the Frequency of Resistance in Methicillin-Resistant *Staphylococcus aureus*

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Combination therapy of bacterial infections with synergistic drug partners offers distinct advantages over monotherapy. Among these advantages are (i) a reduction of the drug dose required for efficacy, (ii) a reduced potential for drug-induced toxicity, and (iii) a reduced potential for the emergence of resistance. Here, we describe the synergistic actions of the third-generation oral cephalosporin cefdinir and TXA709, a new, FtsZ-targeting prodrug that we have developed with improved pharmacokinetics and enhanced *in vivo* efficacy against methicillin-resistant *Staphylococcus aureus* (MRSA) relative to earlier agents. We show that the active product of TXA709 (TXA707) acts synergistically with cefdinir *in vitro* against clinical isolates of MRSA, vancomycin-intermediate *S. aureus* (VISA), vancomycin-resistant *S. aureus* (VRSA), and linezolid-resistant *S. aureus* (LRSA). In addition, relative to TXA707 alone, the combination of TXA707 and cefdinir significantly reduces or eliminates the detectable emergence of resistance. We also demonstrate synergy *in vivo* with oral administration of the prodrug TXA709 and cefdinir in mouse models of both systemic and tissue (thigh) infections with MRSA. This synergy reduces the dose of TXA709 required for efficacy 3-fold. Viewed as a whole, our results highlight the potential of TXA709 and cefdinir as a promising combination for the treatment of drug-resistant staphylococcal infections.

The clinical utility of an antibiotic against its target bacterial pathogens can become compromised over time by the emergence of resistant bacteria upon exposure to the antibiotic (1, 2). One strategy to restrict the emergence of bacterial resistance is to treat infections with combinations of synergistic antibiotics rather than individual agents (3, 4). Synergistic combination therapies not only limit the potential for the emergence of resistance but also reduce the dose of each drug required for efficacy, thereby providing an added benefit of reduced potential for toxicity (5, 6). The benefits of synergistic combination therapies provided us with the impetus to develop a combination therapeutic approach for treatment with TXA709, a new, orally bioavailable prodrug that we recently reported to be associated with improved pharmacokinetics and enhanced *in vivo* efficacy against methicillin-resistant *Staphylococcus aureus* (MRSA) in mouse models of both systemic and tissue infections relative to those of earlier agents (7). The prodrug TXA709 can be easily formulated in aqueous acidic vehicles and is rapidly converted to the active benzamide product TXA707 in the presence of serum (7) (see Fig. S1 in the supplemental material for the structures of both compounds).

The biological target of TXA707 is the bacterial protein FtsZ (7), which forms a ring-like structure (termed the Z-ring) at midcell during cell division (8–10). The Z-ring plays a key role in constriction of the bacterial cell membrane prior to cytokinesis and serves as a scaffold for the recruitment of other critical components of the cell division machinery (the divisome) (11–13). Included in these important cell division components are the penicillin binding proteins (PBPs), which catalyze peptidoglycan biosynthesis at the septum (8–10, 12, 14, 15). Given the involvement of FtsZ and the PBPs in a common pathway leading to septum formation and cell division, we hypothesized that the FtsZ-targeting compound TXA707 may act synergistically with drugs that target the PBPs. In this connection, Tan and coworkers have shown that the carbapenem imipenem acts synergistically with an early FtsZ-targeting benzamide PC190723 (15). Here, we investigate the third-generation oral cephalosporin cefdinir as a potential synergistic partner for TXA707. We present results that demonstrate *in vitro* synergy between TXA707 and cefdinir against clinical isolates of MRSA, vancomycin-intermediate *S. aureus* (VISA), vancomycin-resistant *S. aureus* (VRSA), and linezolid-resistant *S. aureus* (LRSA). We also demonstrate *in vivo* synergy between the prodrug TXA709 and cefdinir in mouse models of both systemic and tissue (thigh) infections with MRSA. The observed synergy translates to a 3-fold reduction in the oral dose of TXA709 required for efficacy. In addition, relative to TXA707 alone, the combination of TXA707 and cefdinir significantly reduces or eliminates the detectable frequency of resistance (FOR) in MRSA, VISA, VRSA, and LRSA isolates. Thus, combination with cefdinir not only confers the benefits of synergy but also addresses any issues relating to a suboptimal FOR that may be associated with FtsZ-targeting benzamides.

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TABLE 1 Synergistic activities of TXA707 and cefdinir against clinical isolates of MRSA, VISA, VRSA, and LRSA

Isolate	TXA707			Cefdinir			
	MIC alone (μg/ml)	MIC in combination (μg/ml)	FIC[a]	MIC alone (μg/ml)	MIC in combination (μg/ml)	FIC[a]	FICI[b]
MRSA ($n = 13$)							
MPW011	1	0.25	0.25	8	1	0.125	0.375
MPW012	1	0.125	0.125	8	2	0.25	0.375
MPW013	1	0.25	0.25	8	1	0.125	0.375
MPW014	1	0.125	0.125	512	16	0.031	0.156
MPW015	0.5	0.125	0.25	256	8	0.031	0.281
MPW016	1	0.25	0.25	256	4	0.016	0.266
MPW017	1	0.125	0.125	128	16	0.125	0.25
MPW018	1	0.125	0.125	128	16	0.125	0.25
MPW019	1	0.125	0.125	128	16	0.125	0.25
MPW020	1	0.25	0.25	512	8	0.016	0.266
R2527	1	0.25	0.25	8	1	0.125	0.375
COL	1	0.25	0.25	256	8	0.031	0.281
ATCC 33591	1	0.25	0.25	512	16	0.031	0.281
VISA ($n = 4$)							
NRS4	1	0.25	0.25	8	0.5	0.063	0.313
NRS27	1	0.25	0.25	32	1	0.031	0.281
NRS54	1	0.25	0.25	128	8	0.063	0.313
Mu3	1	0.25	0.25	1,024	8	0.008	0.258
VRSA ($n = 4$)							
VRS1	1	0.25	0.25	512	32	0.063	0.313
VRS2	1	0.25	0.25	512	16	0.063	0.313
VRS4	1	0.25	0.25	512	16	0.031	0.281
VRS5	1	0.25	0.25	512	4	0.031	0.281
LRSA ($n = 4$)							
NRS119	1	0.25	0.25	256	16	0.031	0.281
NRS121	1	0.25	0.25	1,024	4	0.004	0.254
NRS127	1	0.25	0.25	64	8	0.125	0.375
NRS271	1	0.25	0.25	256	8	0.063	0.313

[a] An FIC of ≤0.25 for each agent indicates synergy.
[b] An FICI of ≤0.5 (which corresponds to the sum of the FICs for the two agents) indicates synergy.

MATERIALS AND METHODS

Bacterial strains. MRSA clinical isolates MPW011 to MPW020 and methicillin-sensitive *S. aureus* (MSSA) isolates MPW001 and MPW004 were provided by Melvin P. Weinstein (Rutgers Robert Wood Johnson Medical School, New Brunswick, NJ). These strains were isolated from the blood of patients admitted to the Robert Wood Johnson University Hospital in New Brunswick, NJ. MRSA strains R2527 and COL were provided by David R. Andes (University of Wisconsin School of Medicine and Public Health, Madison, WI) and Alex G. Therien (Merck & Co., Rahway, NJ), respectively. MRSA strain ATCC 33591 and MSSA strain ATCC 29213 were obtained from the American Type Culture Collection (ATCC, Manassas, VA). VISA isolate Mu3 was provided by George M. Eliopoulos (Beth Israel Deaconess Medical Center, Boston, MA). All of the other VISA isolates, as well as all of the VRSA and LRSA isolates, were provided by the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) for distribution by BEI Resources, NIAID, NIH. The NARSA website (http://www.narsa.net) describes the location of origin of each isolate.

Compounds and comparator antibiotics. TXA707 and TXA709 were synthesized as previously described (7). Vancomycin HCl and oxacillin (sodium salt) were obtained from Sigma-Aldrich (St. Louis, MO). Cefdinir and linezolid were obtained from TOKU-E (Bellingham, WA) and LKT Laboratories (St. Paul, MN), respectively.

MIC assays. MIC assays were conducted in duplicate according to Clinical and Laboratory Standards Institute (CLSI) guidelines (16). Microdilution assays with cation-adjusted Mueller-Hinton (CAMH) broth (Becton-Dickinson, Franklin Lakes, NJ) were used to determine the MICs of all agents, with the MIC being defined as the lowest compound or drug concentration at which no growth is visible after 16 to 24 h of incubation at 37°C.

Checkerboard titration assay for the detection of synergy *in vitro*. The checkerboard titration method was used to evaluate synergy between TXA707 and cefdinir against MRSA, VISA, VRSA, LRSA, and MSSA isolates. In this assay (performed with 96-well microtiter plates), TXA707 was serially diluted 2-fold in CAMH broth along the rows of the microtiter plate, while cefdinir was diluted along the columns. The final volume in each well was 0.1 ml. After the compounds were added, each well was inoculated with 5×10^5 CFU of log-phase bacteria. The microtiter plates were incubated aerobically for 16 to 24 h at 37°C, at which point bacterial growth was visualized. Fractional inhibitory concentrations (FICs) of the agents in each well that did not exhibit visible growth compared to agent-free control wells were calculated with the following two relationships: (i) FIC_{TXA707} = MIC of TXA707 in combination with cefdinir/MIC of TXA707 alone; (ii) $FIC_{cefdinir}$ = MIC of cefdinir in combination with TXA707/MIC of cefdinir alone. These FICs allow the determination of the FIC index (FICI) with the following relationship: $FICI = FIC_{TXA707} + FIC_{cefdinir}$. An FICI of ≤0.5 indicates synergy between the two agents, while an FICI between 0.5 and 2.0 indicates additivity.

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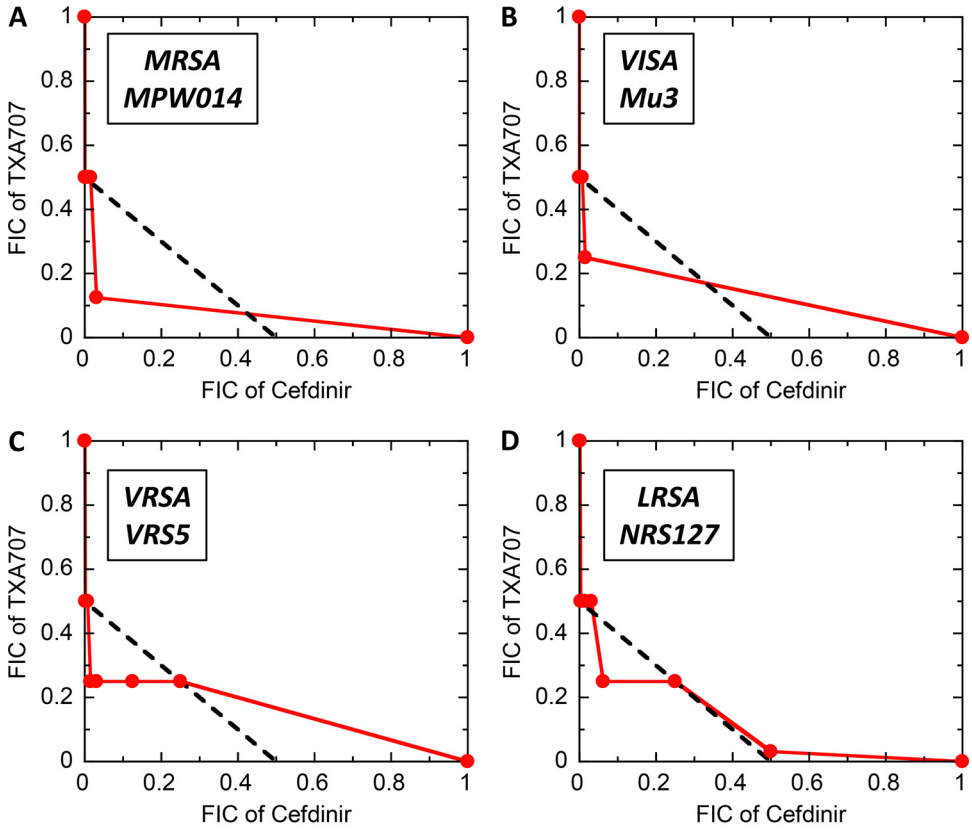

FIG 1 Isobolograms for the combination of TXA707 and cefdinir against clinical isolates MRSA MPW014 (A), VISA Mu3 (B), VRSA VRS5 (C), and LRSA NRS127 (D). Data points to the left of the dashed lines indicate synergy.

Time-kill assays. Exponentially growing MRSA MPW014, VISA Mu3, VRSA VRS5, and LRSA NRS127 isolates were diluted in CAMH broth to a final count of ~10^6 CFU/ml. The colony count at time zero was verified by plating serial dilutions of the culture in duplicate on tryptic soy agar (TSA; Becton-Dickinson) plates. The initial culture was aliquoted into tubes containing either 0.5 μg/ml TXA707 (0.5× MIC), 4 μg/ml cefdinir (≤0.06× MIC), or both agents combined. An equivalent volume of dimethyl sulfoxide (DMSO) was added to the vehicle control tube. The cultures were then incubated at 37°C with shaking. The number of CFU per milliliter in each culture was determined over time by withdrawing samples at time points ranging from 3 to 24 h and plating appropriate serial dilutions onto TSA plates. All TSA plates were incubated at 37°C, and the number of CFU per milliliter at each time point was determined by counting colonies after 24 h.

Assay for FOR and identification of resistant *ftsZ* mutations. The FOR of MRSA, VISA, VRSA, and LRSA isolates to TXA707 alone or in combination with cefdinir was assayed by using a large-inoculum approach described elsewhere (17). In these studies, TSA plates were prepared containing 4 μg/ml TXA707 (4× to 8× MIC) alone or in combination with cefdinir at concentrations in the range of 1 to 8 μg/ml (≤0.5× MIC). All plates were incubated at 37°C and examined after 48 h. The *ftsZ* genes of selected resistant mutants were sequenced as previously described (18).

***In vivo* synergy studies.** Synergy between TXA709 and cefdinir against MRSA was assessed *in vivo* by using both a mouse peritonitis model of systemic infection and a mouse tissue (thigh) model of infection. For experimental details associated with the *in vivo* synergy studies, see the supplemental material.

RESULTS AND DISCUSSION

TXA707 acts synergistically with cefdinir against clinical isolates of MRSA, VISA, VRSA, and LRSA. We sought to evaluate the combination of TXA707 and cefdinir for synergistic activity against a panel of clinical *S. aureus* isolates that included MRSA, VISA, VRSA, and LRSA strains. To this end, we selected 13 MRSA, 4 VISA, 4 VRSA, and 4 LRSA isolates. Note that vancomycin and linezolid are considered first-line antibiotics for the treatment of MRSA infections, and thus, VRSA and LRSA infections are of serious concern in the clinic because of limited therapeutic options. Significantly, TXA707 exhibits potent activity against all four types of isolates, with MICs ranging from 0.5 to 1 μg/ml (Table 1). In contrast, cefdinir is associated with poor activity against the four isolate types, with MICs ranging from 8 to 1,024 μg/ml (Table 1). Oxacillin, vancomycin, and linezolid (included as comparator antibiotics) exhibited the expected MIC ranges versus the four types of isolates tested (see Table S1 in the supplemental material).

We assayed for synergy between TXA707 and cefdinir by using a checkerboard titration approach in which bacterial growth inhibition is monitored at differing concentration ratios of the two agents. These checkerboard assays enabled us to determine FICs at each combination of agents (as described in Materials and Methods). Synergy is indicated when the FIC of each agent is ≤0.25. Figure 1 shows isobolograms plotting the FICs of TXA707 as a function of the FICs of cefdinir for four representative isolates,

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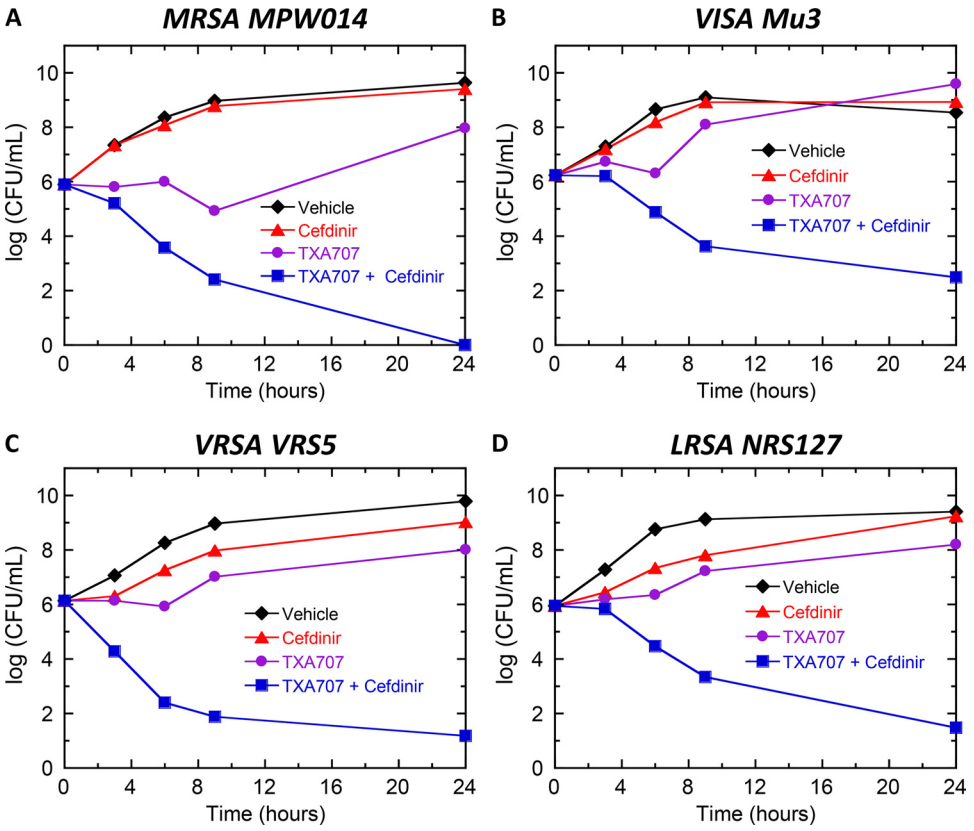

FIG 2 Time-kill curves for clinical isolates MRSA MPW014 (A), VISA Mu3 (B), VRSA VRS5 (C), and LRSA NRS127 (D). Bacteria were treated with DMSO vehicle (black diamonds), 4 μg/ml (≤0.06× MIC) cefdinir alone (red triangles), 0.5 μg/ml (0.5× MIC) TXA707 alone (purple circles), or a combination of 0.5 μg/ml TXA707 and 4 μg/ml cefdinir (blue squares).

one each of MRSA (MPW014), VISA (Mu3), VRSA (VRS5), and LRSA (NRS127). Note the presence of combination FICs in the lower left quadrant of each isobologram (to the left of the dashed line), an observation demonstrating synergy between TXA707 and cefdinir against each of the four isolates. We obtained similar results with the other 21 clinical isolates examined (data not shown).

Another indication of synergy is provided by the FICI, which is defined as the sum of the FICs of the two agents. An FICI of ≤0.5 therefore indicates synergy. Significantly, all of the FICIs determined from the results of our checkerboard analyses were ≤0.375 (Table 1), confirming synergy between TXA707 and cefdinir against all 25 isolates examined. Comparative studies with three different MSSA isolates (MPW001, MPW004, and ATCC 29213) revealed additivity between TXA707 and cefdinir, with the FICI for each MSSA strain being 0.75. Thus, the manifestation of synergy between TXA707 and cefdinir appears to be associated only with the β-lactam-resistant *S. aureus* (MRSA, VISA, VRSA, LRSA) isolates. Tan and coworkers observed similar behavior in their studies combining imipenem and PC190723 (15).

The synergy between TXA707 and cefdinir against MRSA, VISA, VRSA, and LRSA is bactericidal in nature. While the checkerboard titration method assesses inhibitory antibacterial effects, it does not examine bactericidal activity. We therefore further characterized the synergistic interaction between TXA707 and cefdinir by using a time-kill approach. In these assays, bacterial death was monitored in the presence of TXA707 alone at 0.5 μg/ml (0.5× MIC), cefdinir alone at 4 μg/ml (0.004× to 0.06× MIC, depending on the isolate), or TXA707 in combination with cefdinir. Figure 2 shows the resulting kill curves for MRSA MPW014, VISA Mu3, VRSA VRS5, and LRSA NRS127, the same representative isolates whose isobolograms are depicted in Fig. 1. In the presence of subinhibitory concentrations of TXA707 or cefdinir alone, little or no killing is evident, with various degrees of bacterial growth being observed instead. In striking contrast, the combination of TXA707 and cefdinir results in ≥4-log killing over 24 h, with at least 75% of that killing occurring within 9 h. These results not only corroborate the synergistic interaction between TXA707 and cefdinir but also indicate that the synergistic interaction is bactericidal in nature.

In addition to exhibiting synergistic bactericidal activity, the combination of TXA707 and cefdinir also reduces the FOR in MRSA, VISA, VRSA, and LRSA isolates. We also explored how the combination of TXA707 and cefdinir impacts the potential for the emergence of resistance in MRSA, VISA, VRSA, and LRSA isolates. In this connection, we used a large-inoculum approach to assess the FOR associated with 4 μg/ml TXA707 (4× to 8× MIC) alone and in combination with cefdinir at concentrations in the range of 1 to 8 μg/ml (≤0.5× MIC) in 20 of the 25 clinical isolates investigated above (13 MRSA, 3 VISA, 2 VRSA, and 2 LRSA isolates). The FOR of TXA707 alone was on the order of 10^{-8} in all 20 isolates exam-

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TABLE 2 FOR to TXA707 alone and in combination with cefdinir in clinical isolates of MRSA, VISA, VRSA, and LRSA

Isolate	FOR to:			
	4 μg/ml TXA707 alone	4 μg/ml TXA707 + 1 μg/ml cefdinir	4 μg/ml TXA707 + 4 μg/ml cefdinir	4 μg/ml TXA707 + 8 μg/ml cefdinir
MRSA ($n = 13$)				
MPW011	6.31×10^{-8}	1.89×10^{-8}	$<9.01 \times 10^{-10}$	
MPW012	3.16×10^{-8}	9.52×10^{-9}	$<7.94 \times 10^{-10}$	
MPW013	2.84×10^{-8}	2.79×10^{-9}	$<5.68 \times 10^{-10}$	
MPW014	4.85×10^{-8}	2.02×10^{-9}	$<1.01 \times 10^{-9}$	
MPW015	1.10×10^{-8}	2.20×10^{-9}	1.10×10^{-9}	$<1.10 \times 10^{-9}$
MPW016	1.05×10^{-8}	4.65×10^{-9}	$<5.81 \times 10^{-9}$	
MPW017	5.26×10^{-8}	7.55×10^{-9}	$<3.77 \times 10^{-9}$	
MPW018	6.05×10^{-8}	2.04×10^{-8}	$<6.17 \times 10^{-10}$	
MPW019	2.89×10^{-8}	1.02×10^{-8}	$<8.51 \times 10^{-10}$	
MPW020	4.29×10^{-8}	2.52×10^{-8}	$<6.29 \times 10^{-10}$	
R2527	1.24×10^{-8}	$<1.55 \times 10^{-9}$	$<1.55 \times 10^{-9}$	
COL	4.14×10^{-8}	8.39×10^{-9}	4.52×10^{-9}	$<6.45 \times 10^{-10}$
ATCC 33591	4.64×10^{-8}	8.47×10^{-9}	7.91×10^{-9}	6.78×10^{-9}
VISA ($n = 3$)				
NRS4	9.14×10^{-8}	8.62×10^{-10}	$<4.31 \times 10^{-10}$	
NRS27	6.32×10^{-8}	$<4.37 \times 10^{-10}$	$<4.37 \times 10^{-10}$	
Mu3	9.87×10^{-8}	3.44×10^{-8}	$<2.15 \times 10^{-9}$	
VRSA ($n = 2$)				
VRS2	5.46×10^{-8}	1.04×10^{-8}	$<8.70 \times 10^{-10}$	
VRS5	2.53×10^{-8}	$<1.27 \times 10^{-9}$	$<1.27 \times 10^{-9}$	
LRSA ($n = 2$)				
NRS127	1.78×10^{-8}	3.55×10^{-9}	$<5.92 \times 10^{-10}$	
NRS271	8.03×10^{-8}	3.01×10^{-8}	1.92×10^{-8}	$<9.13 \times 10^{-10}$

ined (Table 2). Sequencing analysis demonstrates that this FOR is the result of specific mutations in the *ftsZ* gene (see Table S2 in the supplemental material) (7). Significantly, the combination of TXA707 and cefdinir reduced the FOR below detectable levels ($<10^{-9}$ to $<10^{-10}$) in 19 of the 20 isolates tested, while reducing the FOR by an order of magnitude in the other isolate (Table 2). Thus, combining TXA707 and cefdinir not only enhances antibacterial potency through synergistic interaction but also reduces the FOR.

The bactericidal synergy observed *in vitro* is also observed *in vivo* in a mouse systemic (peritonitis) model of infection with MRSA or VISA. We next sought to determine whether the synergy between TXA707 and cefdinir demonstrated *in vitro* is also observable *in vivo* by using a mouse systemic (peritonitis) model of *S. aureus* infection. In all of our *in vivo* studies, TXA707 was administered in the form of its readily formulatable prodrug (TXA709), which we have previously shown rapidly converts to TXA707 in the presence of mouse serum (with a conversion half-life of approximately 3 min) (7). Prior to combination studies with TXA709 and cefdinir, we first determined the minimal efficacious dose of orally administered TXA709 alone against a lethal infection with either MRSA MPW014 or VISA Mu3. A dose of TXA709 that results in >50% survival (i.e., ≥4 out of 6 mice) is viewed as an efficacious dose. As revealed in Fig. 3A, the minimal efficacious oral dose of TXA709 against MRSA MPW014 was 96 mg/kg, which yielded 67% survival (4 out of 6 mice). The corresponding minimal efficacious oral dose of TXA709 against VISA Mu3 was 144 mg/kg (Fig. 3B), which resulted in 100% survival (6 out of 6 mice).

Armed with the efficacious doses of TXA709 alone versus the MRSA and VISA isolates, we then examined the impact of combining TXA709 and cefdinir on the oral doses required for efficacy against the same two isolates. In these studies, TXA709 was administered at a dose 3-fold lower than its efficacious dose. As expected, these low doses of TXA709 (32 mg/kg for MRSA MPW014 infection and 48 mg/kg for VISA Mu3 infection) were associated with no survival when the compound was administered by itself (Fig. 3C and D). However, when these low doses of TXA709 were administered in combination with cefdinir (at doses of 48 mg/kg for MRSA MPW014 infection and 80 mg/kg for VISA Mu3 infection), we observed an efficacious response against both infections, with 83% survival in each case (5 out of 6 mice) (Fig. 3C and D). Note that there were no survivors when cefdinir was administered alone at the doses used in the combination treatments (Fig. 3C and D). Thus, the oral combination of TXA709 and cefdinir is synergistic against both the MRSA and VISA infections. Importantly, this synergy lowers the dose of TXA709 required for efficacy 3-fold (from 96 to 32 mg/kg against the MRSA infection and from 144 to 48 mg/kg against the VISA infection).

TXA709 is also synergistic with cefdinir in a mouse tissue (thigh) model of infection with MRSA. As staphylococcal infections frequently occur in soft tissue, it is of interest to assess synergistic activity in a tissue model of infection. To this end, we probed for the synergistic actions of orally administered TXA709 and cefdinir in a mouse tissue (thigh) model of infection with MRSA ATCC 33591. An oral dose of TXA709 alone at 40 mg/kg was not associated with an efficacious response, and

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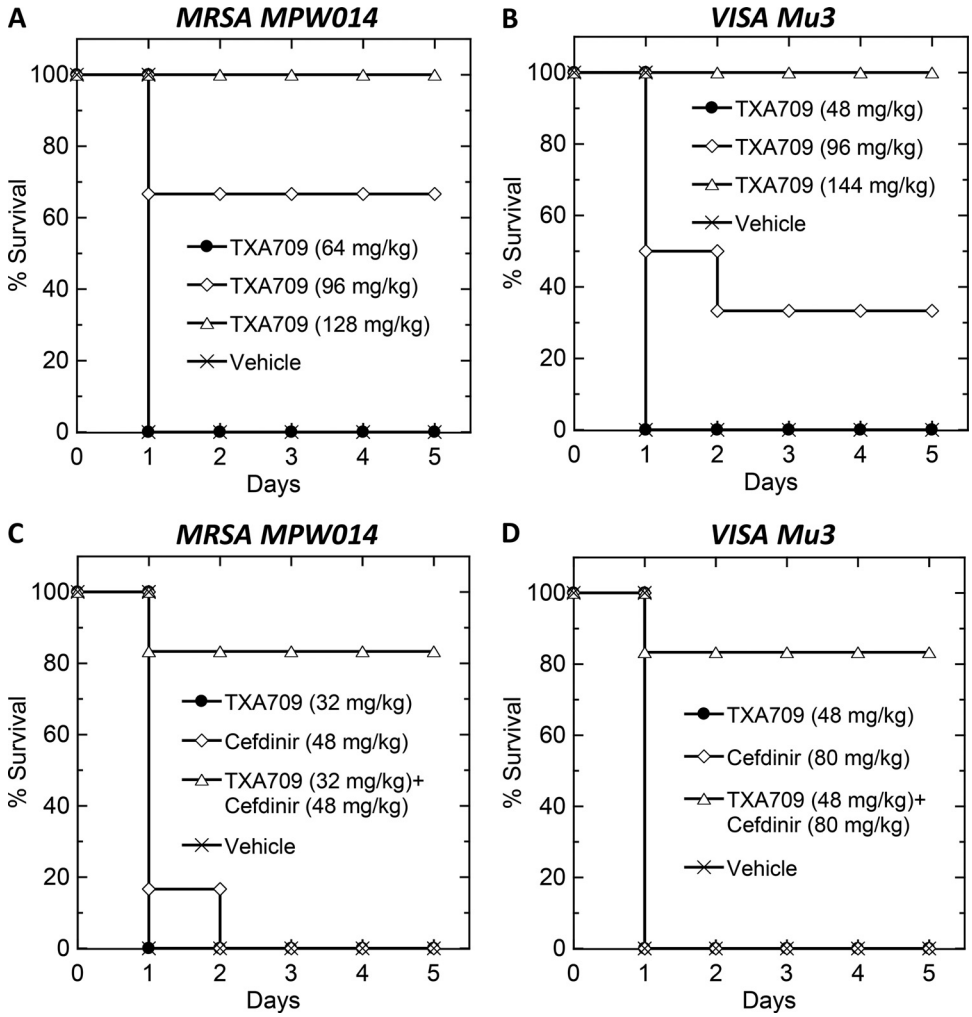

FIG 3 Intrinsic efficacy of orally administered TXA709 alone (A, B) and synergy associated with the combination of orally administered TXA709 and cefdinir (C, D) in a mouse peritonitis model of systemic infection with MRSA MPW014 (A, C) or VISA Mu3 (B, D).

an oral dose of cefdinir alone at 200 mg/kg yielded only a 1-log reduction in the bacterial CFU count relative to that associated with vehicle treatment (Fig. 4). In contrast, the combination of 40 mg/kg TXA709 and 200 mg/kg cefdinir was associated with a robust efficacious response, yielding a 4-log reduction in the bacterial CFU count relative to that associated with vehicle treatment. We have previously shown that orally administered TXA709 alone is efficacious in this infection model at a dose of 120 mg/kg (7). Thus, as observed in the mouse peritonitis model, synergistic combination with cefdinir lowers the oral dose of TXA709 required for efficacy in the thigh infection model 3-fold (from 120 to 40 mg/kg). Additional thigh infection model studies with VRSA VRS2 also revealed synergistic activity with the oral combination of TXA709 and cefdinir (see Fig. S2 in the supplemental material).

Concluding remarks. For two drugs to be useful in combination therapy, they must be efficacious at doses and dosage frequencies that are clinically achievable and safe. With regard to cefdinir, the maximal recommended daily dose in adults and children is 14 mg/kg (19). The recommended allometric scaling for conversion of a mouse dose to its human-equivalent dose is to divide the mouse dose by 12.3 (20). Applying this allometric scaling to the cefdinir doses used in our four mouse synergy studies yields human-equivalent cefdinir doses of 3.9, 6.5, 8.1, and 16.2 mg/kg. Three of these human-equivalent cefdinir doses fall well below the maximal recommended dose for both adults and children, the fourth being only slightly above the maximal recommended dose. Thus, the doses of cefdinir that synergize with TXA709 in our mouse infection models translate to human-equivalent doses that match recommended human dosage regimens well. With regard to TXA709, phase I toxicity and pharmacokinetic profiles in humans represent important assessments that remain to be made. Nevertheless, TXA709 and cefdinir are eliminated with similar pharmacokinetics in animals, with the oral bioavailability of TXA709 being greater than that of cefdinir (7, 21). TXA709 and cefdinir may therefore be administrable to humans at similar dosage frequencies. These collective considerations highlight the promise of the combination of TXA709 and cefdinir for the treatment of drug-resistant staphylococcal infections.

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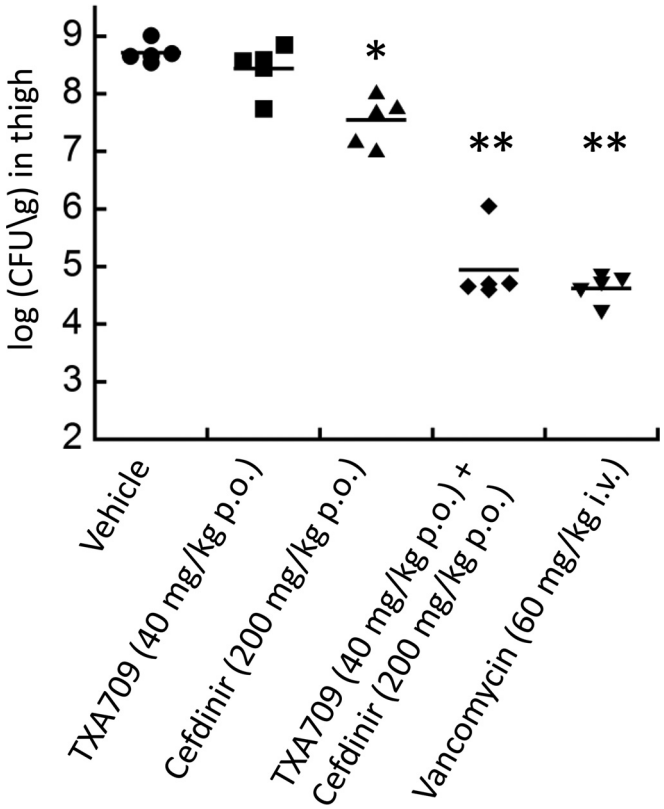

FIG 4 Synergy associated with the combination of orally (p.o.) administered TXA709 and cefdinir in a mouse tissue (thigh) model of infection with MRSA ATCC 33591. Intravenous (i.v.) vancomycin was included as a comparator control antibiotic. The numbers of CFU recovered from infected thighs after 26 h are shown. Statistically significant reductions in the mean CFU count relative to that obtained with the vehicle are denoted as follows: *, $P \leq 5 \times 10^{-4}$; **, $P \leq 2 \times 10^{-6}$.

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REFERENCES

1. **Blair JM, Webber MA, Baylay AJ, Ogbolu DO, Piddock LJ.** 2015. Molecular mechanisms of antibiotic resistance. Nat Rev Microbiol **13:**42–51. http://dx.doi.org/10.1038/nrmicro3380.
2. **Davies J, Davies D.** 2010. Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev **74:**417–433. http://dx.doi.org/10.1128/MMBR.00016-10.
3. **Fischbach MA.** 2011. Combination therapies for combating antimicrobial resistance. Curr Opin Microbiol **14:**519–523. http://dx.doi.org/10.1016/j.mib.2011.08.003.
4. **Tamma PD, Cosgrove SE, Maragakis LL.** 2012. Combination therapy for treatment of infections with Gram-negative bacteria. Clin Microbiol Rev **25:**450–470. http://dx.doi.org/10.1128/CMR.05041-11.
5. **Anantharaman A, Rizvi MS, Sahal D.** 2010. Synergy with rifampin and kanamycin enhances potency, kill kinetics, and selectivity of de novo-designed antimicrobial peptides. Antimicrob Agents Chemother **54:**1693–1699. http://dx.doi.org/10.1128/AAC.01231-09.
6. **Rahal JJ.** 2006. Novel antibiotic combinations against infections with almost completely resistant *Pseudomonas aeruginosa* and *Acinetobacter* species. Clin Infect Dis **43**(Suppl 2):S95–S99. http://dx.doi.org/10.1086/504486.
7. **Kaul M, Mark L, Zhang Y, Parhi AK, Lyu YL, Pawlak J, Saravolatz S, Saravolatz LD, Weinstein MP, LaVoie EJ, Pilch DS.** 2015. TXA709, an FtsZ-targeting benzamide prodrug with improved pharmacokinetics and enhanced in vivo efficacy against methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother **59:**4845–4855. http://dx.doi.org/10.1128/AAC.00708-15.
8. **Adams DW, Errington J.** 2009. Bacterial cell division: assembly, maintenance and disassembly of the Z ring. Nat Rev Microbiol **7:**642–653. http://dx.doi.org/10.1038/nrmicro2198.
9. **Bi EF, Lutkenhaus J.** 1991. FtsZ ring structure associated with division in *Escherichia coli*. Nature **354:**161–164. http://dx.doi.org/10.1038/354161a0.
10. **Erickson HP, Anderson DE, Osawa M.** 2010. FtsZ in bacterial cytokinesis: cytoskeleton and force generator all in one. Microbiol Mol Biol Rev **74:**504–528. http://dx.doi.org/10.1128/MMBR.00021-10.
11. **Egan AJ, Vollmer W.** 2013. The physiology of bacterial cell division. Ann N Y Acad Sci **1277:**8–28. http://dx.doi.org/10.1111/j.1749-6632.2012.06818.x.
12. **Kirkpatrick CL, Viollier PH.** 2011. New(s) to the (Z-)ring. Curr Opin Microbiol **14:**691–697. http://dx.doi.org/10.1016/j.mib.2011.09.011.
13. **Lutkenhaus J, Pichoff S, Du S.** 2012. Bacterial cytokinesis: from Z ring to divisome. Cytoskeleton **69:**778–790. http://dx.doi.org/10.1002/cm.21054.
14. **Pinho MG, Errington J.** 2003. Dispersed mode of *Staphylococcus aureus* cell wall synthesis in the absence of the division machinery. Mol Microbiol **50:**871–881. http://dx.doi.org/10.1046/j.1365-2958.2003.03719.x.
15. **Tan CM, Therien AG, Lu J, Lee SH, Caron A, Gill CJ, Lebeau-Jacob C, Benton-Perdomo L, Monteiro JM, Pereira PM, Elsen NL, Wu J, Deschamps K, Petcu M, Wong S, Daigneault E, Kramer S, Liang L, Maxwell E, Claveau D, Vaillancourt J, Skorey K, Tam J, Wang H, Meredith TC, Sillaots S, Wang-Jarantow L, Ramtohul Y, Langlois E, Landry F, Reid JC, Parthasarathy G, Sharma S, Baryshnikova A, Lumb KJ, Pinho MG, Soisson SM, Roemer T.** 2012. Restoring methicillin-resistant *Staphylococcus aureus* susceptibility to β-lactam antibiotics. Sci Transl Med **4:**126ra135. http://dx.doi.org/10.1126/scitranslmed.3003592.
16. **CLSI.** 2009. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard—eighth edition, CLSI document M07-A8. Clinical and Laboratory Standards Institute, Wayne, PA.
17. **Kaul M, Mark L, Zhang Y, Parhi AK, LaVoie EJ, Pilch DS.** 2013. Pharmacokinetics and *in vivo* antistaphylococcal efficacy of TXY541, a 1-methylpiperidine-4-carboxamide prodrug of PC190723. Biochem Pharmacol **86:**1699–1707. http://dx.doi.org/10.1016/j.bcp.2013.10.010.
18. **Kaul M, Parhi AK, Zhang Y, LaVoie EJ, Tuske S, Arnold E, Kerrigan JE, Pilch DS.** 2012. A bactericidal guanidinomethyl biaryl that alters the dynamics of bacterial FtsZ polymerization. J Med Chem **55:**10160–10176. http://dx.doi.org/10.1021/jm3012728.
19. **Abbott Laboratories.** 2007. Omnicef (Cefdinir) package insert. Abbott Laboratories, North Chicago, IL.
20. **Sharma V, McNeill JH.** 2009. To scale or not to scale: the principles of dose extrapolation. Br J Pharmacol **157:**907–921. http://dx.doi.org/10.1111/j.1476-5381.2009.00267.x.
21. **Sakamoto H, Hirose T, Nakamoto S, Hatano K, Shibayama F, Kikuchi H, Mine Y, Kuwahara S.** 1988. Pharmacokinetics of FK482, a new orally active cephalosporin, in animals. J Antibiot (Tokyo) **41:**1896–1905. http://dx.doi.org/10.7164/antibiotics.41.1896.

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