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ORIGINAL ARTICLE

Correlation of the antimicrobial activity of ME1036 with its binding affinities to the penicillin-binding proteins from *Streptococcus pneumoniae* strains

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We have correlated the binding affinities of ME1036, a carbapenem, to the penicillin-binding proteins (PBPs) from *Streptococcus pneumoniae* strains, with its bactericidal potency against those same strains. Certain mutations in the PBPs from *S. pneumonaie* strains decrease the binding affinities of β-lactams for PBPs, which gives rise to clinical resistance to those β-lactams. ME1036 has been shown to be strongly active against genotypic penicillin-intermediate *S. pneumoniae* (gPISP) strains and genotypic penicillin-resistant *S. pneumoniae* (gPRSP) strains that contain more than one mutation in their PBPs, owing to its strong affinity for those PBPs.
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Keywords: carbapenem; ME1036; penicillin-binding proteins; penicillin-resistant *Streptococcus pneumoniae*

INTRODUCTION

The emergence of penicillin-intermediate and penicillin-resistant *Streptococcus pneumoniae* strains (PISP and PRSP strains, respectively) in Europe was first reported in the 1970s. The strains then spread throughout South America and Asia in the 1980s. In general, between 30 and 50% of clinically isolated *S. pneumoniae* strains are PISP or PRSP strains. In Japan, the prevalence of PISP and PRSP strains is ~60%.[1] PRSP strains are also resistant to tetracycline and macrolides. Furthermore, fluoroquinolone-resistant strains with mutations in DNA gyrase are being found.[2] The increase in these *S. pneumoniae* strains that resist the activities of penicillins, oral cephems, tetracyclines, macrolides and quinolones is a major concern because of decrease of antibiotics effective against these strains.

ME1036 (Figure 1) is a new broad-spectrum parenteral carbapenem that is active against multi-drug resistant Gram-positive and Gram-negative bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum β-lactamase-producing *Enterobacteriaceae*.[3] The study reported herein was designed to evaluate the relationship between the binding affinities of ME1036 for wild-type and mutant penicillin-binding proteins (PBPs) and its efficacy against *S. pneumoniae* strains containing those PBPs.

RESULTS

ME1036 antibacterial activity against *S. pneumoniae*

The number of strains that carried a given mutation(s) was correlated with their associated MIC values (Figure 2). The antibacterial activities against the *S. pneumoniae* strains for ME1036, panipenem and ceftriaxone were affected by mutations in *pbp1a*, *pbp2x* and *pbp2b*. The MIC_{100} value found for ME1036 was 0.03 μg ml^{-1}, which is substantially smaller than the largest values found for the other antibiotics (Table 1). The activity of ceftriaxone was also influenced by mutations in *pbp2x*, and its MIC_{100} was 4 μg ml^{-1}.

Affinity for PBPs

The fluorograms used to determine the IC_{50} values of ME1036 and the other antibiotics for the PBPs from *S. pneumoniae* R6 and 197 are shown in Figure 3 and 4, and the corresponding IC_{50} values are shown in Tables 2 and 3, respectively. The binding affinities of ME1036, imipenem and panipenem for PBP2A/2X and PBP2B from penicillin-susceptible *S. pneumoniae* (PSSP) strains are stronger than those of ceftriaxone. The strong affinities of the carbapenems for PBP2A/2X from the PRSP strain correlate with their low MICs. The IC_{50} values of the carbapenems against PBP1A, PBP1B and PBP2B from the PRSP strain were smaller than were those of ceftriaxone.

Time-kill study

The bactericidal activities of ME1036 and the other carbapenems against *S. pneumoniae* 197 over a 6-h period are shown in Figure 5. ME1036 killed >99.9% of the bacteria within 4 h when the dosage was its MIC value or greater. Conversely, imipenem and panipenem needed four times their MIC value to have the same effect within 4 h, and ceftriaxone was not bactericidal even at four times its MIC value at 6 h.

DISCUSSION

We previously found ME1036 to be a broad spectrum antibiotic that is active against MRSA PRSP strains and extended-spectrum

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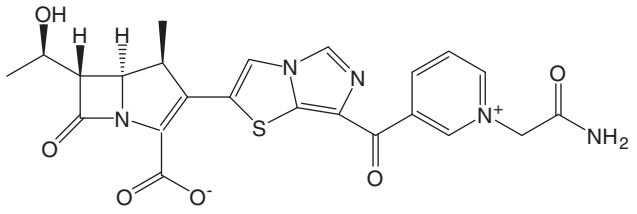

Figure 1 ME1036.

β-lactamase-producing *Enterobacteriaceae*.[3] For the study reported herein, ME1036 was found to have potent anitbacterial activity against genotypic PRSPs (gPRSPs) that are resistant to β-lactams due to mutated *pbp*s. In particular, the strong binding affinities of antibiotics for PBP1A, PBP2X and PBP2B from gPRSP is thought to be basis for its strong antibacterial activities.[4] Compared with other antimicrobial drugs in terms of binding affinity for each PBPs of genotypic PSSP (gPSSP) and gPRSP, the affinity for the PBPs of ME1036 was correlated with its MIC. Therefore, the potent antibacterial activity of ME1036 is likely the result of its strong affinity for the PBPs.

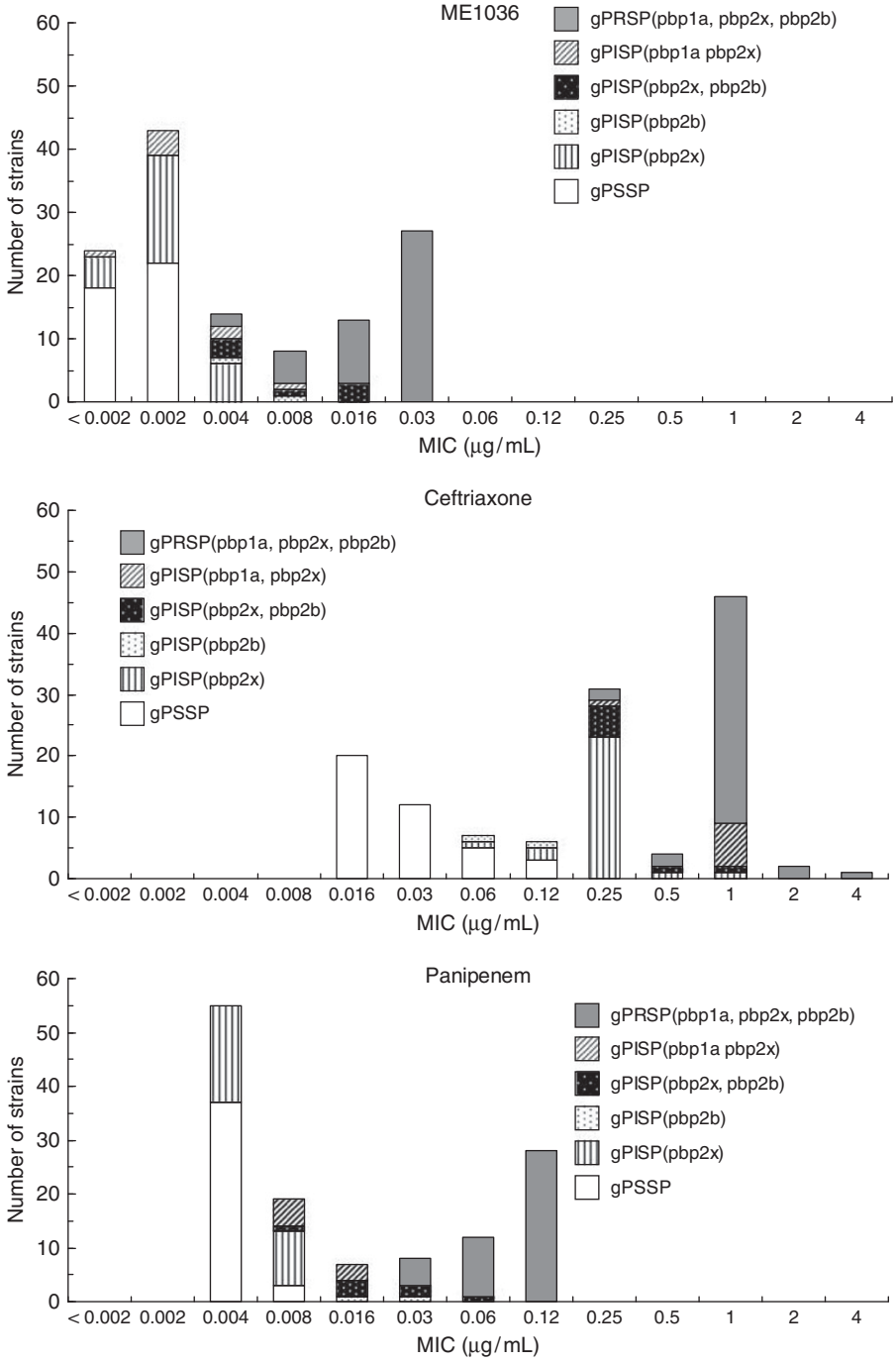

Figure 2 Distribution of ME1036, ceftriaxone and panipenem MIC values for clinical isolates of *S. pneumoniae*.

The interactions between the C-2 side chains of carbapenems and the conserved Trp and Thr residues in the PBPs have important roles in binding of carbapenems to the PBPs.[5] Crystallographic structures revealed that the carbapenem C-2 side chains form non-polar interactions with the side chains of Trp374 and Thr526 of PBP2X and with those of Trp411 and Thr543 of PBP1A. Because ME1036 possesses an imidazo[5,1-*b*]thiazole moiety at the C-2 position, it may also interact with the respective tryptophan and threonine residues of PBP1A and PBP2X in a similar manner, which would result in strong binding affinities. Considering that the corresponding positions in PBP2B also contain a Trp and Thr,[6] the strong binding affinities of ME1036 for all three PBPs may be a consequence of identical or similar non-polar interactions involving the C-2 side chains of ME1036. The binding affinities of antibiotics for PBP1A, PBP2X and PBP2B is thought to be important for antibacterial activity against *S. pneumoniae*.[7] Although the relationship between the binding affinities of antibiotics for PBPs and their bactericidal activities has yet to be definitively proven, it is possible that the potent bactericidal activity of ME1036 against the *S. pneumoniae* strains, including the gPRSPs, is caused by its strong affinities for PBPs.[8]

Table 1 Susceptibilities of *S. pneumoniae* isolates (n=129) to various antibiotics

	MIC ($\mu g\,ml^{-1}$)		
Antibiotic	MIC_{50}	MIC_{90}	*Range*
ME1036	0.002	0.03	<0.002–0.03
Meropenem	0.03	0.5	0.008–0.5
Panipenem	0.008	0.12	0.004–0.12
Cefotaxime	0.25	1	0.016–4
Ceftriaxone	0.25	1	0.016–4
Penicillin	0.06	2	0.016–4
Amoxicillin	0.06	2	0.008–2
Vancomycin	0.5	0.5	0.25–0.5
Levofloxacin	1	1	0.25–2

Drug-resistant *S. pneumoniae* strains are usually isolated from the sputum, pharynx, nasal cavity or otorrhea of patients, and from the otitis media and tissues affected by adenoiditis in children. The major serotypes of gPRSPs are 6, 9, 14, 19 and 23, and these may cause meningitis in young and children, although the evidence is not definitive. Although, a vaccine against the major PRSP serotypes is widely available, PRSP infection is still of concern and requires medical intervention.[9–11]

In conclusion, we have shown that ME1036 is potent against MRSA and also *S. pneumoniae*, including PRSP, and that it has a strong affinity for PBPs. The medical need for a parenteral carbapenem with broad-spectrum and potent bactericidal activity is still great, such that further development of antimicrobial agents is expected to be necessary.

METHODS

Bacterial strains

A total of 129 strains of *S. pneumoniae* clinically isolated in Japan in 2001 were identified as gPSSP strains (with no mutations in any *pbps*), genotypic PISP (gPISP) (with mutations in *pbp2x* or *pbp2b*, in *pbp1a* and *pbp2x*, in *pbp2x* and

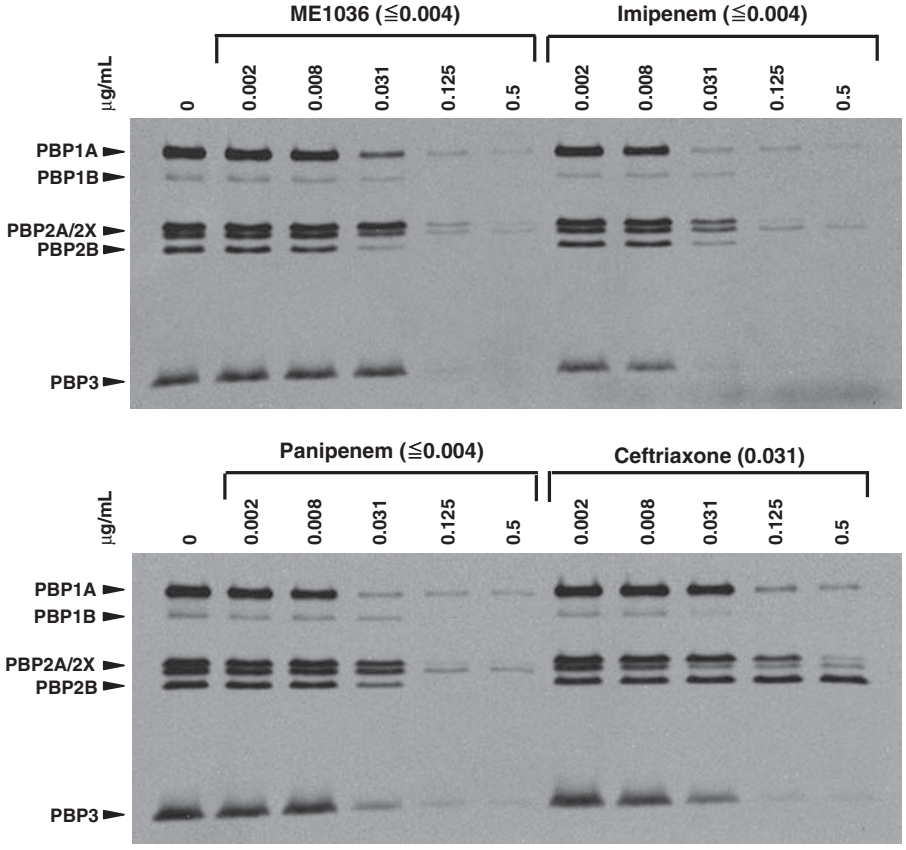

Figure 3 Fluorograms of complexes of [^{3}H]-benzylpenicillin and PBPs from *S. pneumoniae* R6, a PSSP, that had been pretreated with various β-lactams. The MIC values ($\mu g\,ml^{-1}$) are shown in parentheses.

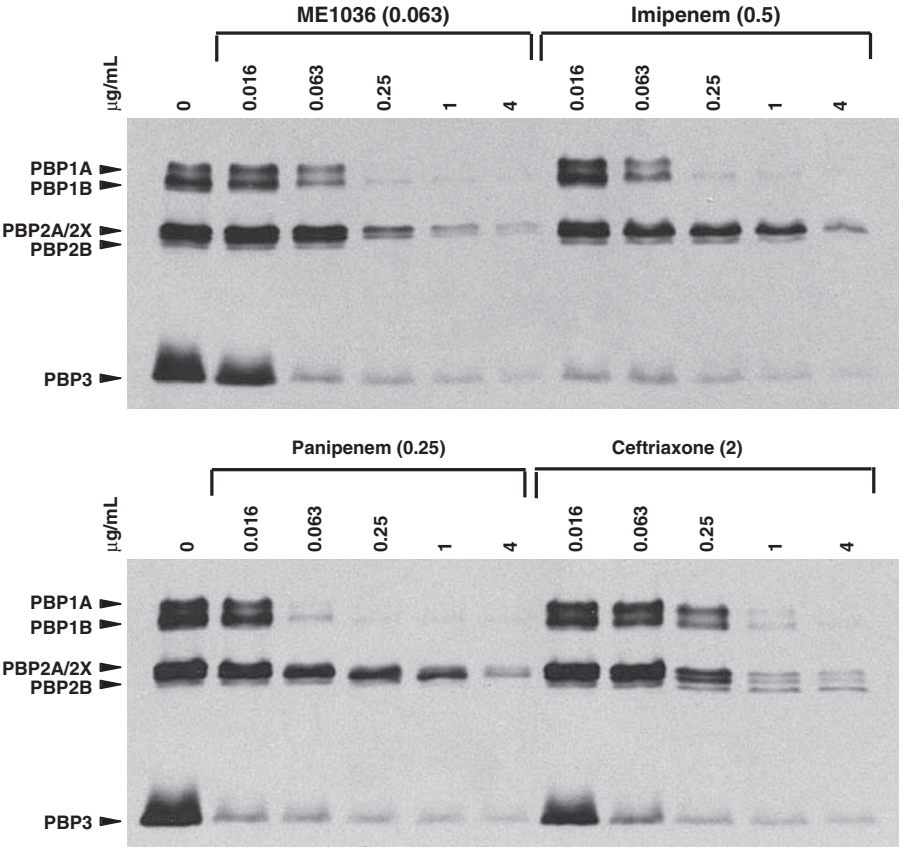

Figure 4 Fluorograms of complexes of [^{3}H]-benzylpenicillin and PBPs from *S. pneumoniae* 197, a PRSP, that had been pretreated with various β-lactams. The MIC values (μg ml^{-1}) are shown in parentheses.

Table 2 Antibiotic-binding affinities for PBPs from the *S. pneumoniae* R6, a PSSP

	IC_{50} (μg ml^{-1})					MIC
Antibiotic	*PBP1A*	*PBP1B*	*PBP2A/2X*	*PBP2B*	*PBP3*	(μg ml^{-1})
ME1036	0.033	0.023	0.073	0.022	0.061	0.001
Imipenem	0.016	0.024	0.050	0.017	0.012	0.004
Panipenem	0.017	0.033	0.064	0.033	0.021	0.002
Ceftriaxone	0.079	0.017	0.23	>0.5	0.035	0.031

Abbreviation: PBPs, penicillin-binding proteins.

Table 3 Antibiotic-binding affinities for PBPs from *S. pneumoniae* 197, a PRSP

	IC_{50} (μg ml^{-1})					*MIC*
Antibiotic	*PBP1A*	*PBP1B*	*PBP2A/2X*	*PBP2B*	*PBP3*	(μg ml^{-1})
ME1036	0.093	0.046	0.19	0.074	0.028	0.06
Imipenem	0.094	0.065	1.1	0.62	<0.016	0.5
Panipenem	0.028	0.030	0.62	0.52	<0.016	0.25
Ceftriaxone	0.41	0.12	0.32	>4	0.028	2

Abbreviations: PBPs, penicillin-binding proteins; PRSP, penicillin-resistant *S. pneumoniae*.

pbp2b) and gPRSP (with mutations in *pbp1a*, *pbp2x* and *pbp2b*),[12,13] and provided by Dr Kimiko Ubukata (Laboratory of Molecular Epidemiology for Infectious Agents, Kitasato Institute for Life Sciences, Kitasato University). *S. pnenumoniae* R6 (BAA-255) was purchased from the American Type Culture Collection and *S. pneumoniae* 197 had been clinically isolated. These strains were stored at Meiji Seika Pharma.

Antibacterial agents

ME1036 was synthesized at the Pharmaceutical Research Center of Meiji Seika Kaisha. Other antibiotics were purchased commercially: meropenem (Dainippon Sumitomo Pharma, Osaka, Japan), panipenem (Daiichi Sankyo, Tokyo, Japan), cefotaxime, (Sanofi-aventis KK, Tokyo, Japan), ceftriaxone (Chugai Pharmaceutical, Tokyo, Japan), penicillin G and amoxicillin (Sigma-Aldrich Chemical, St Louis, MO, USA), vancomycin (Shionogi, Osaka, Japan), levofloxacin (Daiichi Sankyo) and imipenem (MSD KK, Tokyo, Japan).

Susceptibility testing

The MICs for the *S. pneumoniae* were determined by the two-fold agar dilution method that used Mueller-Hinton agar (Difco, Becton Dickinson, Sparks, MD, USA), supplemented with 5% defibrinated sheep blood.[14] Each inoculum was prepared by making a direct Mueller-Hinton broth (Difco) suspension of isolated colonies selected from an agar plate. Each inoculum (10^4 CFU) was spotted onto a separate agar plate with a multiple inoculator (Microplanter, Sakuma Seisakusho, Tokyo, Japan). MIC values were defined as the lowest concentrations of the antibiotics that prevented visible bacterial growth after incubation at 35 °C for 20 h.

PCR for the identification of PBP genes

Fragments of PBP genes and *lytA*, which encodes the autolysin[15] specific to *S. pneumoniae* were PCR amplified as reported.[13] After PCR amplification, if the DNA for these gene fragments was not detected or was found to be a size different from that expected, the strains from which the gene fragments were acquired were considered to possess mutant PBPs, and the *S. pneumoniae* strains were classified as gPSSP, gPISP or gPRSP.

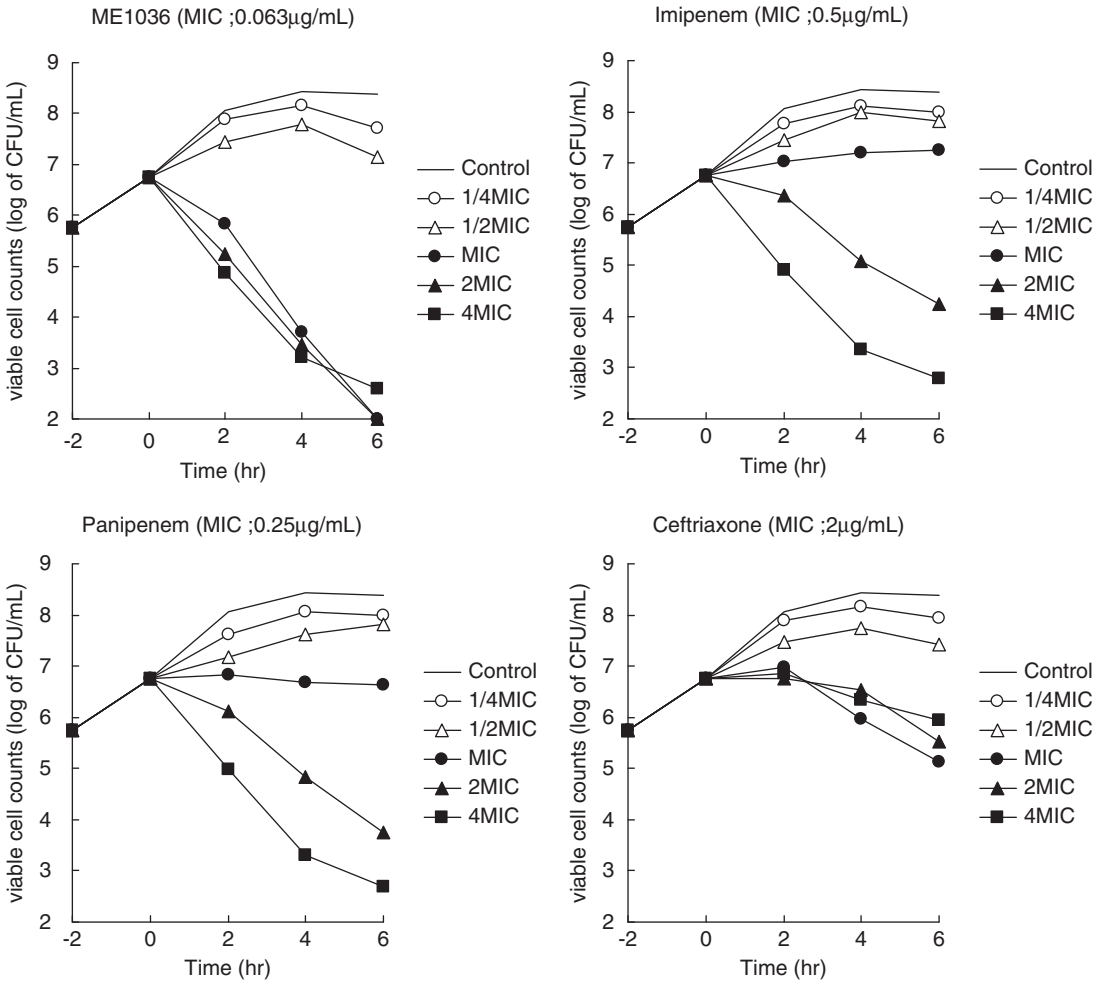

Figure 5 The degree of antibiotic bactericidal activity vs time for ME1036 and other β-lactams assessed with *S. pneumoniae* 197, a PRSP.

Antibiotic affinities for PBPs

The affinities of ME1036 and other β-lactams for PBPs were assessed using a [^{3}H]-benzylpenicillin-competition assay as reported.[16] Membrane proteins were isolated from *S. pneumoniae* strains R6 (PSSP) and 197 (PRSP). Antibiotics were individually incubated with the membrane proteins at 30 °C for 10 min, and then [^{3}H]-benzylpenicillin was added. After a 10-min incubation, excess unlabeled benzylpenicillin was added. Sarkosyl-solubilized membrane fractions were subjected to SDS-polyacrylamide gel electrophoresis and fluorography. Because PBP2X and PBP2A were not completely separable, their binding to the antibiotics were assessed together as PBP2A/2X. The binding affinities of antibiotics to PBPs were quantified by scanning densitometry from the fluorographs. The fluorograph images were analyzed with NIH image software, and IC_{50} was estimated from the logistic curve of the dose–response relationship.

Time-kill study

Overnight cultures of *S. pneumoniae* grown on Mueller-Hinton agar supplemented with 5% horse blood were individually inoculated into 5 ml of cation-adjusted Mueller-Hinton broth supplemented with 5% lysed horse blood. After a 2-h incubation at 35 °C, the antibiotics were each added at concentrations equivalent to 1/4, 1/2, 1, 2 or 4 times its MIC to a medium sample. Surviving bacteria were counted after 0, 2, 4 and 6 h of incubation at 35 °C by subculturing 50 μl of serial 10-fold dilutions on Mueller-Hinton agar supplemented with 5% horse blood. The number of colonies for each sample was counted after 24 h of incubation at 35 °C.

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