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Affinity of Doripenem and Comparators to Penicillin-Binding Proteins in *Escherichia coli* and *Pseudomonas aeruginosa*▽

Todd A. Davies,* Wenchi Shang, Karen Bush, and Robert K. Flamm

Johnson and Johnson Pharmaceutical Research and Development LLC, Raritan, New Jersey

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Doripenem, a parenteral carbapenem, exhibited high affinity for penicillin-binding protein 2 (PBP2) and PBP3 in *Pseudomonas aeruginosa* and PBP2 in *Escherichia coli*, the primary PBPs whose inhibition leads to cell death. This PBP affinity profile correlates with the broad-spectrum gram-negative activity observed with doripenem.

Doripenem, a parenteral carbapenem, was recently approved in the United States for treatment of complicated intra-abdominal and complicated urinary tract infections including pyelonephritis. Doripenem exhibits a broad spectrum of activity against many clinically important gram-positive and gram-negative pathogens including *Enterobacteriaceae*, such nonfermenters as *Pseudomonas aeruginosa*, anaerobes, *Staphylococcus* spp., group A streptococci, and pneumococci (2, 5, 9).

Penicillin-binding proteins (PBPs), the targets of β-lactam antibiotics, are membrane-associated bacterial enzymes involved in the last steps of peptidoglycan (cell wall) biosynthesis. Carbapenems in general have high affinity for multiple PBPs in gram-negative bacteria (1). Among the PBPs of primary importance—the essential high-molecular-weight PBPs 1a, 1b, 2, and 3—the carbapenems show the greatest affinity for PBP2 in *Escherichia coli* and *Pseudomonas aeruginosa* (1, 11), with variations in PBP profiles dependent on the particular carbapenem. For example, in *E. coli* both imipenem and meropenem have high affinity for PBP2, but imipenem has low affinity for PBP3; in contrast, meropenem also has high affinity for PBP3 albeit to a lesser degree than for PBP2 (1, 11). The inhibition of PBP2 causes changes in cell morphology leading to the formation of spherical cells, whereas inhibition of PBP3 leads to filamentation (4, 8).

In this study, the affinity of doripenem and other β-lactam comparators for PBPs from *E. coli* and *P. aeruginosa* was examined, and the associated changes in cell morphology after incubation with doripenem were also studied. Since a key attribute of doripenem is its improved in vitro antipseudomonal activity (two- to fourfold more potent) compared to the other carbapenems, two pseudomonal strains were tested.

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* Corresponding author. Mailing address: Johnson and Johnson Pharmaceutical Research and Development, LLC, Room B225, 1000 Route 202, Raritan, NJ 08869. Phone: (908) 707-3465. Fax: (908) 707-3501. E-mail: tdavies@prdus.jnj.com.

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Membranes containing PBPs from *E. coli* MC4100, *P. aeruginosa* PAO1, and *P. aeruginosa* ATCC 27853 were isolated as described by Zhao et al. (12). PBPs were labeled according to methods based on Sifaoui et al. (10) except that Bocillin FL (Invitrogen, Carlsbad, CA) was used instead of [^{3}H]benzylpenicillin. Labeled PBPs were visualized using a LumiImager (Roche, Indianapolis, IN) at a 520-nm setting, and the 50% inhibition concentration (IC_{50}) values were determined using Quantity One software (Bio-Rad, Hercules, CA). All susceptibility testing by broth microdilution was done according to CLSI methods (3) using cation-adjusted Mueller-Hinton broth. A cell morphology assay was performed by growing cultures in nutrient broth to early log phase (optical density at 600 nm of ~0.2) and then adding drug at 1× the MIC. The morphology of the cells was monitored at 30-min intervals by microscopic examination at a magnification of ×1,000 using a Nikon Eclipse E800 microscope.

E. coli MC4100 is a β-lactamase-negative isolate with MICs for doripenem and meropenem of 0.03 μg/ml and an imipenem MIC of 0.25 μg/ml (Table 1). All the carbapenems tested had high affinity for PBP2, the primary killing target, with IC_{50} values of 0.008 μg/ml (Table 1). Ceftazidime and aztreonam had the greatest affinity for PBP3, the primary killing target of monobactams and most cephalosporins in gram-negative bacteria (6, 7), with IC_{50} values of ≤0.07 μg/ml (Table 1). Meropenem also bound to PBP3 with an IC_{50} of 0.6 μg/ml, which was 10- to 20-fold less active than ceftazidime and aztreonam but bound approximately 3- to 13-fold more tightly than doripenem or imipenem (Table 1). Unlike ceftazidime and aztreonam, all the carbapenems had high affinity for PBP4, with IC_{50} values of ≤0.02 μg/ml (Table 1). Imipenem had two- to fourfold-greater affinity for PBPs 1a and 1b than ceftazidime, doripenem, and meropenem. Of all the β-lactams, imipenem had the highest affinity for the nonessential PBPs 5 and 6, with IC_{50} values of ≤0.4 μg/ml (Table 1). However, all the carbapenems had IC_{50} values of ≤4 μg/ml for these PBPs.

P. aeruginosa PAO1 and 27853 had doripenem MICs of 0.25 μg/ml while meropenem and imipenem MICs ranged from 0.25 to 2 μg/ml (Table 1). PBP binding profiles of the carbapenems were similar in both strains of *P. aeruginosa*. Imipenem had two- to eightfold-lower IC_{50} values for PBP1a and PBP1b

TABLE 1. IC_{50} values of β-lactam binding to PBPs from *E. coli* and *P. aeruginosa*

Organism and PBP	IC_{50} of the indicated drug (μg/ml)[a]				
	DOR	IPM	MEM	CAZ	ATM
E. coli MC4100 PBP					
1a	1.2	0.5	1.7	1.1	>8
1b	1.2	0.4	1.3	1.1	>8
2	0.008	0.008	0.008	4	>8
3	2	8	0.6	0.07	0.03
4	0.02	0.01	0.02	>4	>8
5	4	0.4	4	>4	>8
6	1	0.1	0.03	>4	>8
MIC of the strain (μg/ml)	0.03	0.25	0.03	0.12	0.12
P. aeruginosa PAO1 PBP					
1a	0.5	0.1	0.5	0.2	2
1b	0.6	0.2	0.5	3	2
2	0.06	0.1	0.05	>32	16
3	0.07	0.09	0.08	0.1	0.03
4	0.008	0.008	0.008	2	16
5/6	8	2	16	>32	>16
MIC of the strain (μg/ml)	0.25	1	0.5	1	4
P. aeruginosa 27853 PBP					
1a	0.8	0.1	0.6	0.3	2
1b	0.6	0.1	0.6	1.6	2
2	0.04	0.1	0.06	4	4
3	0.06	0.3	0.08	0.1	<0.02
4	<0.008	<0.008	0.02	2	>4
5/6	>4	1	>4	>4	>4
MIC of the strain (μg/ml)	0.25	2	0.25	1	4

[a] Concentration of β-lactam that inhibits 50% of Bocillin FL compared to a control containing no drug. DOR, doripenem; IPM, imipenem, MEM, meropenem; CAZ, ceftazidime; ATM, aztreonam. At least duplicate values were averaged from replicate experiments.

than doripenem and meropenem (Table 1). The lowest carbapenem IC_{50}, ≤0.3 μg/ml, was for PBPs 2, 3, and 4. Doripenem and meropenem had similar or slightly lower (two- to fivefold) IC_{50} values than imipenem for PBPs 2 and 3 (Table 1). Ceftazidime had the highest affinity for PBP3 (IC_{50} of 0.1 μg/ml), followed by its affinity for PBP1a (IC_{50} ranging from 0.2 to 0.3 μg/ml). Aztreonam bound tightly only to PBP3, with an IC_{50} of ≤0.03 μg/ml (Table 1).

E. coli MC4100 grown in the presence of doripenem, imipenem, or meropenem at 1× the MIC changed from a rod-shaped cell to a sphere-shaped cell (Fig. 1B). No differences were observed among the carbapenems. Sphere formation was consistent with primary binding to PBP2.

P. aeruginosa 27853 was grown in the presence of each of the carbapenems. By 60 min doripenem-exposed cells became elongated and formed spheres in the center of the cell (data not shown). At 240 min doripenem-exposed cells were five times the length of control cells, and one or more spheres were present in most formations (Fig. 1D). Imipenem-exposed cells at 60 min formed spheres, with some cells demonstrating limited elongation (data not shown). By 240 min many imipenem-exposed cells had formed spheres or had lysed, perhaps due to high affinity for PBPs 1a and 1b, the PBPs associated with lysis (Fig. 1E). After 60 min meropenem-exposed cells were elongated, and very few, if any, spheres formed (data not shown). By 240 min meropenem-exposed cells were five times the length of control cells, and one or more spheres were present in most formations (Fig. 1F). The combination of filamentation and sphere formation for doripenem- and meropenem-exposed cells may reflect the high affinity for PBP2 (sphere formation) and slightly better affinity for PBP3 (filamentation) than imipenem. Different effects on the cell morphology show that the carbapenems do not act uniformly despite their similar *P. aeruginosa* PBP binding profiles.

Among the important high-molecular-weight PBPs, doripenem and meropenem had the highest binding affinity for PBPs 2 and 3 in *P. aeruginosa* and PBP2 in *E. coli*, the primary PBPs whose inhibition leads to cell death. The enhanced potency in doripenem binding to *P. aeruginosa* PBPs 2 and 3 compared to imipenem may contribute to its improved antipseudomonal activity. The overall carbapenem PBP profile, with potent binding to multiple essential PBPs in both *E. coli* and *P. aeruginosa*, is related to the broad-spectrum gram-negative activity observed with doripenem.

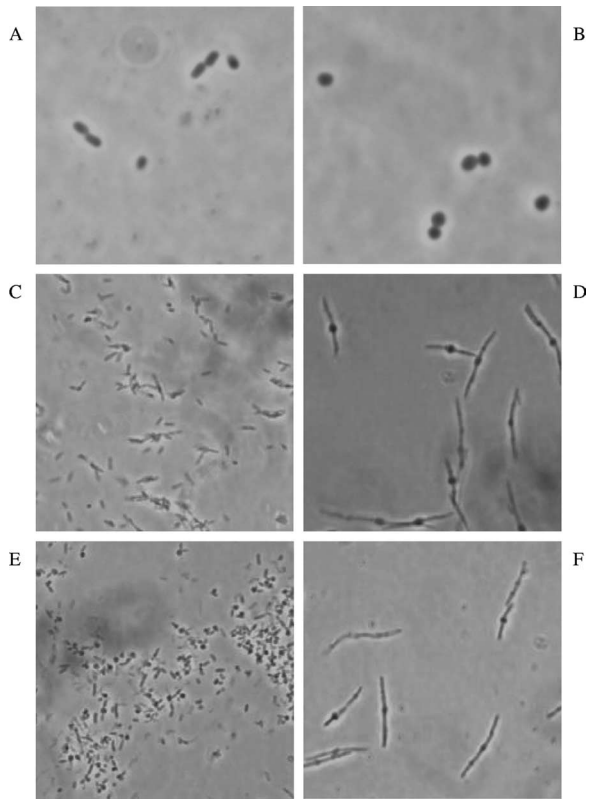

FIG. 1. *E. coli* MC4100 was grown for 4 h in nutrient broth (control) alone (A) or in broth containing 0.03 μg/ml doripenem (1× MIC) (B). *P. aeruginosa* 27853 was grown for 4 h in nutrient broth alone (control) (C) or in broth containing 0.25 μg/ml doripenem (1× MIC) (D), 2 μg/ml imipenem (1× MIC) (E), or 0.25 μg/ml meropenem (1× MIC) (F).

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