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In Vitro Activity of Tomopenem (CS-023/RO4908463) against Anaerobic Bacteria▽

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The antianaerobic activity of tomopenem, a new longer-half-life parenteral carbapenem, was compared with other carbapenems. Tomopenem showed broad activity against 63 reference species. The activity of tomopenem against 293 clinical isolates was potent (MIC_{90}, 0.06 to 4 μg/ml) and comparable to those of meropenem and doripenem and more potent than that of panipenem.

Tomopenem (CS-023/RO4908463) is a new parenteral carbapenem with a long half-life. It is a 2-substituted 1-β-methyl carbapenem with a unique guanidine-pyrrolidine side chain. Pharmacokinetic studies indicate that tomopenem has a longer half-life (about 2 h) than those of launched carbapenem (about an hour), such as imipenem-cilastatin and meropenem (6, 9, 11). Ertapenem, one of the new parenteral carbapenems, also has a prolonged plasma half-life of about 5 h, largely due to its high protein binding of >95% (13). As for tomopenem, it is reported that its low affinity to renal transporters is one of the reasons for its long plasma half-life in humans (10). Tomopenem has a broad spectrum of activity against gram-positive and gram-negative aerobic organisms, including methicillin-resistant *Staphylococcus aureus*, methicillin-resistant *Staphylococcus epidermidis*, penicillin-resistant *Streptococcus pneumoniae*, and *Pseudomonas aeruginosa* (5, 6, 14). Tomopenem was also reported to be active against *Bacteroides fragilis*, but its activity against other anaerobic bacteria is unknown. We evaluated the in vitro activity of tomopenem against anaerobic gram-positive and gram-negative species.

For the investigation of the anaerobic antibacterial spectrum, a total of 69 gram-positive and gram-negative reference strains (63 species in 24 genera) of anaerobic bacteria and some fastidious microaerophilic anaerobes were examined. Those reference strains include strains obtained from ATCC, DSM, JCM (Japan Collection of Microorganisms), NCTC, and VPI (Virginia Polytechnic Institute and State University, Blacksburg), and some characteristic clinical strains belong to GAI, the culture collection of our laboratory. A total of 293 clinical strains isolated from various sources (including intra-abdominal infection, head and neck space infection, pleuropulmonary infection, and skin and soft tissue infection) between 2000 and 2006 were also studied. Isolates were identified by standard criteria (3, 4, 12).

The antimicrobial agents used in this study were obtained as powders of known potency from their respective manufacturers and are as follows: tomopenem and panipenem (Daiichi Sankyo Co., Ltd., Tokyo, Japan), meropenem (Dainippon Sumitomo Pharma Co., Ltd., Osaka, Japan), and doripenem and metronidazole (Shionogi & Co., Ltd., Osaka, Japan).

The MICs were determined by an agar dilution method in accordance with NCCLS document M11-A6 (8). Brucella HK agar (Kyokuto Pharmaceutical Industrial Co., Ltd., Tokyo, Japan) supplemented with 5% laked sheep blood was used as the test medium. Brucella HK agar contains hemin (10 μg/ml) and vitamin K_1 (10 μg/ml) in its formula to support growth of fastidious anaerobes. A total of 10^5 CFU/spot of test strains was inoculated and incubated at 35°C in an anaerobic chamber (82% N_2, 10% CO_2, 8% H_2). *B. fragilis* ATCC 25285, *Bacteroides thetaiotaomicron* ATCC 29741, and *Eggerthella lenta* ATCC 43055 were used as quality control strains.

The results of the susceptibility test on the reference strains are listed in Table 1. Overall, tomopenem showed broad and potent activities against various reference species, comparable to those of other carbapenems, and was more potent than metronidazole. Tomopenem inhibited most of the reference strains at or below the concentration of 1 μg/ml, while it was not active against carbapenemase-producing *B. fragilis* (strains GAI 30079 and GAI 30144).

Table 2 shows the in vitro activities of tomopenem and reference agents against clinical strains frequently isolated in anaerobic infections. These results are expressed as MIC range, MIC_{50}s, and MIC_{90}s. Among the clinical isolates, tomopenem showed potent activity against anaerobic gram-negative species. Tomopenem showed potent activity against species of the *Bacteroides fragilis* group that are often found in surgical infections. The MIC_{50}s and MIC_{90}s of tomopenem against *B. fragilis* and other *B. fragilis* group strains were 0.25 to 0.5 μg/ml and 1 to 4 μg/ml, respectively. Tomopenem inhibited all other investigated gram-negative strains at or below 1 μg/ml. Its activity against gram-negative species was comparable to those of meropenem and doripenem and more potent than that of panipenem. The investigated agents showed similar activities against anaerobic gram-positive cocci. MIC_{50} and MIC_{90} against *Finegoldia magna*, *Parvimonas micra*, and *Pep-*

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TABLE 1. Antimicrobial activities of tomopenem and other reference compounds against anaerobic bacteria and facultative anaerobic bacteria

Strain	MIC (μg/ml)					
	Tomopenem	Panipenem	Meropenem	Doripenem	Clindamycin	Metronidazole
Gram-positive bacteria						
Anaerococcus prevotii ATCC 9321	0.125	0.125	0.25	0.25	0.125	2
Atopobium parvulum VPI 0546	0.5	0.25	0.5	1	1	0.5
Finegoldia magna ATCC 29328	0.125	0.25	0.125	0.25	2	1
Parvimonas micra VPI 5464–1	0.125	0.125	0.125	0.125	0.25	1
Peptoniphilus asaccharolyticus WAL 3218	0.03	0.25	≤0.015	0.03	>128	1
Peptoniphilus indolicus GAI 0915	≤0.015	0.125	≤0.015	≤0.015	32	0.5
Peptostreptococcus anaerobius ATCC 27337	0.5	0.125	0.5	0.5	0.25	0.125
Gemella morbillorum ATCC 27824	0.03	≤0.015	≤0.015	≤0.015	0.06	>128
Staphylococcus saccharolyticus ATCC 14953	0.06	0.03	0.125	0.06	0.125	>128
Streptococcus constellatus ATCC 27923	0.25	0.25	0.05	0.5	0.25	>128
Streptococcus intermedius ATCC 27335	0.125	0.25	0.25	0.125	0.25	>128
Clostridium clostridioforme NCTC 11224	0.03	0.25	0.25	0.25	0.25	≤0.015
Clostridium difficile GAI 10029	2	4	4	4	>128	0.5
Clostridium perfringens ATCC 13124	0.03	0.03	≤0.015	0.03	0.06	1
Clostridium septicum ATCC 12464	0.03	≤0.015	0.06	0.06	≤0.03	1
Clostridium sordellii ATCC 9714	≤0.015	0.03	0.03	≤0.015	1	1
Clostridium ramosum ATCC 25582	0.5	0.25	1	1	8	2
Actinomyces odontolyticus GAI 91002	0.5	0.5	0.5	0.25	0.25	16
Bifidobacterium adolescentis ATCC 15703	0.125	0.125	0.125	0.25	≤0.03	64
Bifidobacterium bifidum JCM 1255	0.06	0.06	0.06	0.125	≤0.03	4
Bifidobacterium breve ATCC 15700	1	0.5	1	1	≤0.03	16
Bifidobacterium longum subsp. *longum* ATCC 15707	0.5	1	0.5	1	≤0.03	8
Bifidobacterium pseudolongum ATCC 25526	0.125	0.25	0.25	0.5	≤0.03	>128
Eggerthella lenta ATCC 25559	0.5	2	1	0.25	0.25	0.5
Propionibacterium acnes ATCC 11828	1	0.125	2	1	0.125	>128
Propionibacterium granulosum ATCC 25564	0.5	0.125	1	0.5	≤0.03	>128
Lactobacillus acidophilus JCM 1132	0.25	0.25	0.25	0.125	1	>128
Lactobacillus brevis subsp. *brevis* JCM 1059	0.25	0.03	0.5	0.125	≤0.03	>128
Lactobacillus casei subsp. *casei* JCM 1134	1	0.5	2	1	2	>128
Lactobacillus fermentum JCM 1173	0.125	0.03	0.25	0.06	≤0.03	>128
Lactobacillus plantarum JCM 1149	0.06	0.03	0.25	0.125	0.25	>128
Lactobacillus reuteri JCM 1112	0.125	0.03	0.5	0.125	≤0.03	>128
Lactobacillus salivarius subsp. *salivarius* JCM 1231	0.5	0.25	1	0.5	0.06	>128
Gram-negative bacteria						
Bacteroides fragilis GAI 5562	0.25	0.25	0.125	0.5	0.5	1
Bacteroides fragilis ATCC 25285	0.25	0.25	0.25	0.5	1	0.5
Bacteroides fragilis NCTC 10581	0.125	0.25	0.25	0.5	≤0.03	1
Bacteroides fragilis GAI 0558	1	0.5	0.05	0.5	0.5	0.5
Bacteroides fragilis GAI 7955	4	4	2	2	1	0.25
Bacteroides fragilis GAI 10150	4	8	2	2	0.5	1
Bacteroides fragilis GAI 30079	>128	>128	>128	>128	>128	1
Bacteroides fragilis GAI 30144	>128	>128	>128	>128	2	1
Bacteroides vulgatus GAI 0673	0.25	0.125	0.125	0.25	0.06	0.25
Parabacteroides distasonis ATCC 8503	0.25	1	0.125	0.5	0.06	1
Bacteroides ovatus ATCC 8483	0.5	0.5	0.25	0.5	0.5	2
Bacteroides thetaiotaomicron ATCC 29741	0.5	0.5	0.25	0.5	4	1
Bacteroides uniformis ATCC 8492	0.25	0.25	0.125	0.25	≤0.03	0.5
Bacteroides eggerthii ATCC 27754	0.125	0.25	0.125	0.25	≤0.03	0.5
Bacteroides ureolyticus NCTC 10941	0.03	0.25	≤0.015	0.06	0.25	2
Campylobacter gracilis JCM 8538	0.06	0.25	0.06	0.125	0.125	0.5
Sutterella wadsworthensis ATCC 51579	0.5	0.5	0.06	0.25	16	1
Prevotella bivia ATCC 29303	0.5	0.5	0.25	0.5	≤0.03	2
Prevotella buccae ATCC 33574	0.25	0.25	0.25	0.25	≤0.03	0.5
Prevotella corporis GAI 91000	0.06	0.125	0.03	0.125	≤0.03	0.125
Prevotella heparinolytica ATCC 35895	0.125	0.125	0.06	0.06	≤0.03	0.06
Prevotella intermedia ATCC 25611	0.06	0.06	0.06	0.06	≤0.03	1
Prevotella melaninogenica JCM 6325	0.125	0.06	0.125	0.125	ND	1
Prevotella denticola GAI 5490	0.06	0.06	0.06	0.06	≤0.03	0.25
Prevotella oralis ATCC 33269	0.06	0.06	0.03	0.06	≤0.03	0.125
Prevotella oris ATCC 33573	0.125	0.06	0.06	0.125	≤0.03	0.25
Porphyromonas asaccharolytica ATCC 25260	≤0.015	0.03	≤0.015	0.03	≤0.03	0.25
Porphyromonas gingivalis ATCC 33277	0.03	0.03	≤0.015	≤0.015	≤0.03	0.03
Fusobacterium nucleatum ATCC 25586	2	1	8	4	0.06	≤0.015
Fusobacterium varium ATCC 8501	0.5	4	0.25	0.5	4	0.25
Fusobacterium necrophorum ATCC 25286	≤0.015	0.125	≤0.015	≤0.015	≤0.03	0.125
Bilophila wadsworthia WAL 7959	0.125	0.5	0.03	0.06	0.5	0.06
Desulfovibrio desulfuricans ATCC 29577	0.25	0.5	0.125	0.125	0.5	0.06
Desulfovibrio piger DSM 749	0.03	0.125	0.03	0.06	0.06	0.25
Capnocytophaga ochracea GAI 5586	0.06	0.125	0.03	0.125	≤0.03	1
Veillonella parvula ATCC 10790	0.03	0.125	0.03	0.06	≤0.03	2
Veillonella dispar ATCC 17748	0.03	0.06	0.03	0.06	≤0.03	2

toniphilus asaccharolyticus were ≤0.015 to 0.25 and 0.06 to 0.5 μg/ml, respectively. Although carbapenems are not used against *Clostridium difficile* infections, tomopenem showed the most potent activity among the carbapenems tested, with MIC_{50}s and MIC_{90}s of 1 and 2 μg/ml. The most potent agent against *C. difficile* was metronidazole, with MIC_{50}s and MIC_{90}s of 0.5 and 1 μg/ml, respectively.

The reported antianaerobic activities of ertapenem (1, 2, 7,

TABLE 2. In vitro activity of tomopenem and other reference compounds against clinical isolates of anaerobic bacteria

Organism (no. of isolates)	Antimicrobial agent	MIC (μg/ml)		
		Range	MIC_{50}	MIC_{90}
Bacteroides fragilis (25)	Tomopenem	0.125–16	0.25	1
	Meropenem	0.125–8	0.25	0.5
	Doripenem	0.25–8	0.25	0.5
	Panipenem	0.125–16	0.25	8
	Metronidazole	0.25–1	0.5	1
Bacteroides thetaiotaomicron (25)	Tomopenem	0.25–8	0.5	2
	Meropenem	0.25–4	0.5	2
	Doripenem	0.25–2	0.5	1
	Panipenem	0.125–32	0.5	2
	Metronidazole	0.25–2	0.5	2
Other *B. fragilis* group (16)[a]	Tomopenem	0.125–8	0.5	4
	Meropenem	0.06–4	0.25	4
	Doripenem	0.125–4	0.5	4
	Panipenem	0.125–16	1	4
	Metronidazole	0.06–1	0.5	1
Prevotella intermedia (25)	Tomopenem	0.06–0.25	0.06	0.125
	Meropenem	0.03–0.125	0.06	0.125
	Doripenem	0.03–0.125	0.06	0.125
	Panipenem	0.03–0.25	0.06	0.25
	Metronidazole	0.25–1	0.5	0.5
Prevotella spp. (13)[b]	Tomopenem	0.06–0.5	0.125	0.5
	Meropenem	0.03–0.25	0.125	0.25
	Doripenem	0.03–0.25	0.06	0.125
	Panipenem	0.03–0.25	0.125	0.25
	Metronidazole	0.5–4	1	2
Porphyromonas spp. (25)[c]	Tomopenem	≤0.015–0.06	0.03	0.06
	Meropenem	≤0.015–0.03	≤0.015	0.03
	Doripenem	≤0.015–0.06	0.03	0.06
	Panipenem	≤0.015–0.125	0.06	0.06
	Metronidazole	≤0.015–0.25	0.06	0.25
Fusobacterium spp. (24)[d]	Tomopenem	≤0.015–0.5	≤0.015	0.5
	Meropenem	≤0.015–0.25	0.03	0.125
	Doripenem	≤0.015–0.5	0.03	0.25
	Panipenem	≤0.015–2	0.125	2
	Metronidazole	≤0.015–0.5	≤0.015	0.5
Desulfovibrio desulfuricans (13)	Tomopenem	0.125–1	0.125	0.5
	Meropenem	0.03–0.25	0.06	0.125
	Doripenem	0.125–0.25	0.125	0.25
	Panipenem	0.25–1	0.5	1
	Metronidazole	0.03–0.25	0.125	0.25
Finegoldia magna (19)	Tomopenem	0.06–0.125	0.06	0.125
	Meropenem	0.06–0.125	0.125	0.125
	Doripenem	0.06–0.25	0.125	0.25
	Panipenem	0.06–0.5	0.25	0.5
	Metronidazole	0.25–1	0.5	1
Parvimonas micra (25)	Tomopenem	0.06–1	0.125	0.125
	Meropenem	0.03–1	0.06	0.125
	Doripenem	0.03–1	0.06	0.125
	Panipenem	0.06–0.25	0.125	0.125
	Metronidazole	0.25–2	0.5	1
Peptostreptococcus anaerobius (10)	Tomopenem	0.25–8	0.5	4
	Meropenem	0.125–4	0.25	2
	Doripenem	0.125–4	0.25	2
	Panipenem	0.06–2	0.125	1
	Metronidazole	0.06–0.5	0.125	0.5

Continued on following page

TABLE 2. In vitro activity of tomopenem and other reference compounds against clinical isolates of anaerobic bacteria

Organism (no. of isolates)	Antimicrobial agent	MIC (μg/ml)		
		Range	MIC_{50}	MIC_{90}
Peptoniphilus asaccharolyticus (21)	Tomopenem	≤0.015–0.125	≤0.015	0.06
	Meropenem	≤0.015–0.25	≤0.015	0.125
	Doripenem	≤0.015–0.25	≤0.015	0.125
	Panipenem	≤0.015–0.125	0.03	0.06
	Metronidazole	0.125–2	1	2
Eubacterium/*Eggerthella* spp. (14)[e]	Tomopenem	0.03–0.5	0.5	0.5
	Meropenem	0.03–1	0.5	1
	Doripenem	0.03–0.25	0.25	0.25
	Panipenem	0.03–2	1	2
	Metronidazole	0.125–0.5	0.5	0.5
Clostridium difficile (19)	Tomopenem	0.25–4	1	2
	Meropenem	1–4	2	4
	Doripenem	0.5–4	2	4
	Panipenem	1–8	4	8
	Metronidazole	0.25–2	1	1
Clostridium perfringens (19)	Tomopenem	≤0.015–0.125	≤0.015	0.06
	Meropenem	≤0.015–0.03	≤0.015	0.03
	Doripenem	≤0.015–0.06	0.03	0.06
	Panipenem	≤0.015–0.125	0.03	0.06
	Metronidazole	0.25–2	1	2

[a] Includes six *Parabacteroides distasonis* strains, three *B. uniformis* strains, three *B. vulgatus* strains, two *Bacteroides caccae* strains, one *Bacteroides stercoris* strain, and one *Odoribacter splanchnicus* strain.
[b] Includes 11 *P. melaninogenica* strains and 2 other *Prevotella* sp. strains.
[c] Includes 18 *P. asaccharolytica* strains, 5 *P. gingivalis* strains, and 2 *Porphyromonas uenonis* strains.
[d] Includes 14 *F. nucleatum* strains, 2 *F. necrophorum* strains, 5 *F. varium* and *Fusobacterium mortiferum* strains, and 3 other *Fusobacterium* sp. strains.
[e] Includes 11 *Eggerthella lenta* strains and 3 *Eubacterium limosum* strains.

15) were comparable to those of tomopenem measured in this study. This study demonstrates that tomopenem has potent activity that is comparable to other carbapenems against clinically important gram-positive and gram-negative anaerobic bacteria. Most anaerobic infections are polymicrobial and involve both aerobes and anaerobes. It has been reported that tomopenem is the agent with broad and potent activities against aerobic bacteria (5, 6, 14) and with the longer half-life (9). These reports and our in vitro data indicate the potential role of tomopenem in those polymicrobial infections. Further in vivo studies are necessary to demonstrate this point.

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