ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 2007, p. 1849–1851 Vol. 51, No. 5
0066-4804/07/$08.00+0 doi:10.1128/AAC.01551-06

In Vitro Activities of Isepamicin, Other Aminoglycosides, and Capreomycin against Clinical Isolates of Rapidly Growing Mycobacteria in Taiwan▽

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Received 14 December 2006/Returned for modification 29 December 2006/Accepted 1 March 2007

The in vitro activities of isepamicin against 117 *Mycobacteria abscessus*, 48 *Mycobacterium fortuitum*, and 20 *Mycobacterium chelonae* isolates were evaluated by a microdilution test. Isepamicin MIC_{90}s were ≤16 μg/ml for the three species. Isepamicin was as active as amikacin and kanamycin and more active than tobramycin, capreomycin, gentamicin, and streptomycin.

Rapidly growing mycobacteria (RGM) can cause a wide spectrum of disseminated or localized diseases, especially pulmonary, skin, or soft tissue infections (6). *Mycobacterium abscessus*, *Mycobacterium chelonae*, and *Mycobacterium fortuitum* are the three major pathogenic RGM species. The management of RGM remains very difficult, especially for the problems associated with infection caused by *M. abscessus* (12).

Aminoglycoside agents have the potential to be extremely active against RGM (1, 5, 15). Amikacin has shown excellent activities against RGM in several studies and currently is the most widely used aminoglycoside in the treatment of RGM (1, 5, 15, 18). Amikacin and isepamicin, an aminoglycoside used in Asia, were developed by introducing the (*S*)-4-amino-2-hydroxybutyryl and (*S*)-3-amino-2-hydroxypropionyl side chains into the 1-amino groups of kanamycin and gentamicin, respectively (8). Isepamicin has shown excellent activities against a wide range of bacteria (4). The cyclic peptide capreomycin is sometimes considered an aminoglycoside because of its actions on bacterial ribosomes (7). This study compared the activities of isepamicin with those of five other aminoglycosides (amikacin, gentamicin, kanamycin, tobramycin, and streptomycin) and capreomycin against RGM.

RGM isolates were collected between November 2005 and July 2006 and identified by the conventional biochemical methods (10). Some of these (136 isolates) were confirmed by PCR restriction enzyme analysis of the 65-kDa *hsp* gene (13). Totals of 117 *M. abscessus*, 48 *M. fortuitum*, and 20 *M. chelonae* nonduplicate clinical isolates were collected. Of them, 71 (61%), 12 (25%), and 7 (35%), respectively, were recovered from patients with probable RGM infections (in which cases identical RGM species were recovered from three or more specimens from the same patient).

Broth microdilution MIC testing was performed according to CLSI guidelines (11, 16–18). The isolates were subcultured on Trypticase soy agar plates with 5% sheep blood (BBL Microbiology Systems) and incubated at 30°C for 72 h. Bacteria on the agar plates were collected and adjusted to a final inoculum (5×10^5 CFU/ml) in cation-supplemented Mueller-Hinton broth (Difco, Detroit, MI). Serial double dilutions of the tested antimicrobial agents were prepared with the same broth, and the concentrations in the wells ranged from 0.25 to 128 μg/ml. The inoculated trays were incubated at 30°C, and MICs were recorded after 3 to 5 days.

RGM isolates with amikacin MICs of ≥64 μg/ml are interpreted as resistant to amikacin and those with amikacin MICs of ≤16 μg/ml as susceptible to amikacin according to the CLSI cutoff criteria (11). No interpretive criteria have been approved for the susceptibilities of RGM to the other six agents except for that of *M. chelonae* to tobramycin. Quality control strain *Staphylococcus aureus* ATCC 29213 was included, and the results were in the acceptable range (MICs of 1 to 4 μg/ml).

Table 1 shows the MIC ranges, the MIC_{50}s and MIC_{90}s, and the percentages of isolates with MICs of ≤16, 32, and ≥64 μg/ml for the seven antimicrobial agents against the 185 RGM isolates. It is clear that amikacin, isepamicin, and kanamycin had excellent activities against RGM (MIC_{50}s, 1 to 16 μg/ml; MIC_{90}s, 4 to 32 μg/ml). For these three agents, >87% of the isolates of each of the three RGM species had MICs of ≤16 μg/ml. When MIC_{50}s were compared, isepamicin was found to be onefold more active than amikacin against *M. abscessus* and *M. chelonae* and as active as kanamycin against the 185 RGM isolates but sevenfold less active than amikacin against *M. fortuitum*. When MIC_{90}s were compared, isepamicin was found to be onefold more active than amikacin against *M. abscessus*, onefold more active than kanamycin against *M. fortuitum*, and as active as amikacin and kanamycin against *M. chelonae* but onefold less active than kanamycin against *M. abscessus* and threefold less active than amikacin against *M. fortuitum*. Gentamicin exhibited limited activities (MIC_{50}s, 16 to 32 μg/ml; MIC_{90}s, 32 to 64 μg/ml) and streptomycin poor activities (MIC_{50}s, 64 to 128 μg/ml; MIC_{90}s, 128 to >128 μg/ml) against each of the three RGM species. Tobramycin showed excellent activity against *M. abscessus* and limited to good activities

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▽ Published ahead of print on 12 March 2007.

TABLE 1. In vitro inhibitory activities of amikacin, isepamicin, kanamycin, tobramycin, gentamicin, streptomycin, and capreomycin against 117 isolates of *M. abscessus*, 48 isolates of *M. fortuitum*, and 20 isolates of *M. chelonae*

Bacterium and antimicrobial agent	MIC (μg/ml)			% (no.) of isolates with indicated MIC[a] (μg/ml)		
	Range	50%	90%	≤16	32	≥64
M. abscessus (*n* = 117)						
Amikacin	4–>128	16	32	87.2 (102)	10.3 (12)	2.6 (3)
Isepamicin	2–>128	8	16	95.7 (112)	2.6 (3)	1.7 (2)
Kanamycin	1–>128	8	8	98.3 (115)	0 (0)	1.7 (2)
Tobramycin	4–>128	8	16	94.9 (111)	3.4 (4)	1.7 (2)
Gentamicin	8–>128	32	64	10.3 (12)	69.2 (81)	20.5 (24)
Streptomycin	4–>128	128	>128	1.7 (2)	11.1 (13)	87.2 (102)
Capreomycin	64–>128	128	>128	0 (0)	0 (0)	100 (117)
M. fortuitum (*n* = 48)						
Amikiacin	0.5–4	1	4	100 (48)	0 (48)	0 (48)
Isepamicin	2–64	8	16	93.8 (45)	4.2 (2)	2.1 (1)
Kanamycin	2–>128	8	32	89.6 (43)	6.3 (3)	4.2 (2)
Tobramycin	2–128	16	64	52.1 (25)	29.2 (14)	18.8 (9)
Gentamicin	1–32	16	32	64.6 (31)	35.4 (17)	0 (0)
Streptomycin	16–>128	64	128	4.2 (2)	14.6 (7)	81.3 (39)
Capreomycin	1–128	8	32	81.3 (39)	12.5 (6)	6.3 (3)
M. chelonae (*n* = 20)						
Amikacin	4–16	8	8	100 (20)	0 (0)	0 (0)
Isepamicin	4–8	4	8	100 (20)	0 (0)	0 (0)
Kanamycin	2–64	4	8	90 (18)	5 (1)	5 (1)
Tobramycin	4–128	16	32	80 (16)	15 (3)	5 (1)
Gentamicin	16–64	16	32	60 (12)	58.3 (7)	5 (1)
Streptomycin	32–128	64	128	0 (0)	15 (3)	85 (17)
Capreomycin	32–>128	128	128	0 (0)	5 (1)	95 (19)

[a] RGM isolates with amikacin MICs of ≤16, 32, and ≥ 64 μg/ml were within the susceptible, intermediate, and resistant ranges for amikacin, respectively, according to the CLSI guidelines.

TABLE 2. In vitro inhibitory activities of amikacin, isepamicin, kanamycin, tobramycin, gentamicin, streptomycin, and capreomycin against *M. abscessus* and *M. fortuitum* isolates that had amikacin MICs of ≥64 or 32 μg/ml and/or isepamicin MICs of ≥64 or 32 μg/ml

Isolate group	MIC (μg/ml) for indicated drug						
	Amikacin	Isepamicin	Kanamycin	Tobramycin	Gentamicin	Streptomycin	Capreomycin
M. abscessus isolates with amikacin and isepamicin MICs of ≥ 64 μg/ml							
CH10	>128	>128	>128	>128	>128	>128	128
R31	>128	>128	>128	>128	>128	>128	>128
M. abscessus isolates with amikacin MIC of ≥ 64 μg/ml and isepamicin MIC of 32 μg/ml							
R39	64	32	16	32	128	>128	>128
M. abscessus isolates with amikacin and isepamicin MICs of 32 μg/ml							
NTU445	32	32	16	16	64	>128	>128
NTU459	32	32	16	8	64	>128	>128
M. abscessus isolates with amikacin MIC of 32 μg/ml							
R51	32	16	16	32	64	>128	>128
NTU446	32	16	8	4	64	>128	>128
R47	32	16	8	16	64	>128	>128
R50	32	16	8	16	64	>128	>128
R54	32	16	8	32	64	>128	128
R61	32	16	8	16	64	>128	>128
R65	32	16	8	16	64	>128	>128
R53	32	16	4	8	64	128	>128
R49	32	8	4	16	64	128	>128
V120	32	8	8	16	32	128	128
M. fortuitum isolates with isepamicin MIC of ≥ 64 μg/ml							
V13	4	64	>128	128	32	128	64
M. fortuitum isolates with isepamicin MIC of 32 μg/ml							
V61	4	32	32	64	32	>128	64
V146	4	32	32	64	32	>128	32

against *M. fortuitum* and *M. chelonae*. Capreomycin showed good activity against *M. fortuitum* but poor activities against *M. abscessus* and *M. chelonae*, which is consistent with the results of Lévy-Frébault et al. (9).

While none of the *M. chelonae* isolates tested had amikacin or isepamicin MICs of ≥32 μg/ml, 15 (13%) *M. abscessus* and 3 (6%) *M. fortuitum* isolates had amikacin and/or isepamicin MICs of ≥32 μg/ml (Table 2). Two *M. abscessus* isolates (CH10 and R31) were essentially resistant to all of the seven agents tested (MICs, ≥128 μg/ml). For the remaining 13 *M. abscessus* isolates, isepamicin was either as active as (2 isolates) or one- to threefold more active than (11 isolates) amikacin. Similar phenomena were observed with kanamycin and tobramycin. For the three *M. fortuitum* isolates with isepamicin MICs of ≥32 μg/ml, amikacin was 7- or 15-fold more active than isepamicin (Table 2). Isepamicin may be a good therapeutic option for RGM isolates that are nonsusceptible to amikacin, and vice versa.

Because of the high prevalence of antimicrobial resistance in RGM in Taiwan (18), the use of a single agent for treatment is not recommended. Our study indicates that isepamicin, amikacin, and kanamycin exhibited excellent activities against RGM, and tobramycin exhibited excellent activity against *M. abscessus*. These antimicrobial agents can be used in the combination regimens for RGM. Isepamicin is particularly important since animal and clinical trials have shown that isepamicin is one of the less toxic aminoglycosides (3, 14). The activities of isepamicin against five *M. chelonae* and *M. fortuitum* strains were previously reported (2).

Ho et al. (7) found poor activities for amikacin, kanamycin, tobramycin, gentamicin, streptomycin, and capreomycin against *M. chelonae* and for kanamycin, tobramycin, streptomycin, and capreomycin against *M. fortuitum*. Only amikacin and gentamicin had good activities against *M. fortuitum*. Our results are largely different from theirs. The discrepancies may be due to differences in the methods of in vitro testing or the RGM strains used in their studies.

We thank Po-Ren Hsueh for providing *M. chelonae* isolates.

This study was supported by grants from the Center for Disease Control (DOH 95-DC-1106) and the National Science Foundation (NSC 91-2316-B-005-003-CC3) of Taiwan.

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