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Original Article

In vitro activity of isepamicin and other aminoglycosides against clinical isolates of Gram-negative bacteria causing nosocomial bloodstream infections

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Background and Purpose: Isepamicin is a newly introduced aminoglycoside in Taiwan. Since in vitro data for isepamicin against nosocomial Gram-negative bloodstream infection from Taiwan are limited, we compared the activity of isepamicin, amikacin, gentamicin and tobramycin against nosocomial Gram-negative blood isolates.
Methods: A total of 247 non-duplicate nosocomial blood isolates of Gram-negative bacteria collected between January 2003 and December 2003 in a major teaching hospital in Taiwan were tested for their in vitro susceptibilities to gentamicin, tobramycin, amikacin, and isepamicin using the agar dilution method. The isolates included *Escherichia coli* (31 isolates), *Klebsiella pneumoniae* (31), *Enterobacter cloacae* (30), *Serratia marcescens* (31), *Morganella morganii* (21), *Citrobacter freundii* (10), *Pseudomonas aeruginosa* (31), *Acinetobacter baumannii* (31), and *Stenotrophomonas maltophilia* (31).
Results: Overall, isepamicin had high antibacterial activity against the tested Gram-negative bacteria. For the 154 *Enterobacteriaceae* isolates, isepamicin had the lowest minimum concentration inhibiting 90% of isolates (MIC_{90}) among the tested drugs, while its resistance rate (3.9%) was equal to that of amikacin (3.9%) and lower than those of tobramycin (18.2%) and gentamicin (21.4%). For the 93 of non-fermentative Gram-negative bacilli isolates, isepamicin had the lowest MIC_{90}, and a resistance rate (23.7%) lower than those of amikacin (27.9%), tobramycin (38.7%) and gentamicin (40.9%).
Conclusions: The in vitro activity of isepamicin against Gram-negative bacteria isolates was equal or similar to amikacin and superior to other tested aminoglycosides. In view of its potential for less nephrotoxicity and ototoxicity than other aminoglycosides, isepamicin is a drug of choice for the empirical treatment of nosocomial infections caused by Gram-negative bacteria.

Key words: Aminoglycosides; Antimicrobial sensitivity tests; Drug resistance, bacterial; Gram-negative bacteria; Isepamicin

Introduction

Aminoglycosides are used extensively in clinical practice, and have broad activity against aerobic Gram-negative bacteria, including *Enterobacteriaceae* and non-fermentative Gram-negative bacilli. However, strains resistant to these agents, either by enzyme production or reduced cell wall permeability, have spread over the last 20 years [1-5] and led to a need for more potential drugs.

Isepamicin is a new semisynthetic aminoglycoside derived from gentamicin B [6], which is expected to have comparable efficacy, spectrum and pharmacokinetics to amikacin, but less toxicity [7-9] and greater resistance to aminoglycoside-inactivating enzymes [4,8,9]. However, studies of the in vitro activity of isepamicin against nosocomial Gram-negative bacteria bacteremia in Taiwan remain limited [10,11].

The purpose of this study was to compare the in vitro activities of isepamicin and other currently

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available aminoglycosides in Taiwan, against clinical isolates of Gram-negative bacteria causing nosocomial bloodstream infections collected in a university hospital in Taiwan.

Methods

Bacterial isolates

The bacteria used in this study were nosocomial Gram-negative bacteria isolated from blood cultures of patients treated at the National Taiwan University Hospital, a major teaching hospital with a capacity of 2200 beds located in northern Taiwan. A total of 247 isolates of Gram-negative bacteria causing nosocomial bloodstream infections, including *Escherichia coli* (31 isolates), *Klebsiella pneumoniae* (31 isolates), *Enterobacter cloacae* (31 isolates), *Serratia marcescens* (31 isolates), *Morganella morganii* (21 isolates), *Citrobacter freundii* (10 isolates), *Pseudomonas aeruginosa* (31 isolates), *Acinetobacter baumannii* (31 isolates), and *Stenotrophomonas maltophilia* (31 isolates), were selected from available blood isolates isolated from January 2003 to December 2003 in our stock. In 2003, there were 190, 164, 127, 122, 98, 69, 31, 21, and 10 episodes of nosocomial bloodstream infections caused by *A. baumannii*, *K. pneumoniae*, *E. cloacae*, *E. coli*, *P. aeruginosa*, *S. maltophilia*, *S. marcescens*, *M. morganii*, and *C. freundii*, respectively. Because of limitation of facility, only 31 isolates from each of *A. baumannii*, *K. pneumoniae*, *E. cloacae*, *E. coli*, *P. aeruginosa*, and *S. maltophilia* were randomly selected using a computer-generated random digital table. No duplicate isolates from a single patient were used. All isolates were routinely identified by standard conventional microbiological methods and suspensions were stored at –70°C in trypticase soy broth (Difco Laboratories, Detroit, MI, USA) supplemented with 15% glycerol. When required, the suspensions were thawed and subcultured on to Mueller-Hinton agar (Merck Belgium, Overijse, Belgium).

Antimicrobial agents

All of the antimicrobial agents tested in this study were provided by individual pharmaceutical companies as standard reference powder for laboratory use. The tested drugs included amikacin supplied by Bristol-Myers Squibb (Princeton, NJ, USA), gentamicin (Schering Plough, Bloomfield, NJ, USA), tobramycin (Eli Lilly, Indianapolis, IN, USA), and isepamicin (TTY Biopharm, Taipei, Taiwan).

Antimicrobial susceptibility testing

The susceptibilities to the tested drugs for all of the enrolled bacterial isolates were determined using the agar dilution method as described by the National Committee for Clinical Laboratory Standards (NCCLS) [12]. Briefly, the isolates were grown overnight on trypticase soy agar plates supplemented with 5% sheep blood (BBL Microbiology Systems, Cockeysville, MD, USA) at 37°C. Bacterial inocula were prepared by suspending the freshly grown bacteria in sterile normal saline and were adjusted to a 0.5 McFarland standard. Using a Steers' replicator, an organism density of 10^4 colony-forming units per spot was inoculated on to the appropriate plate of unsupplemented Mueller-Hinton agar (BBL Microbiology Systems) which contained a series of two-fold dilutions of tested antimicrobial agents (128-0.03 mg/L). The agar plates were incubated at 35°C in ambient air for 18-20 h before reading. The minimal inhibitory concentration (MIC) was identified according to the lowest concentration of the antimicrobial agent that completely inhibited the growth of bacteria on the agar plate. For gentamicin, tobramycin, and amikacin, the interpretation of susceptibility was according to the criteria suggested by NCCLS [13]. The breakpoint for susceptibility of isepamicin was as follows: susceptible, ≤16 mg/L; intermediate, 32 mg/L; resistant, ≥64 mg/L [12,13].

Reference strains

E. coli American Type Culture Collection (ATCC; Rockville, MD, USA) 25922, *K. pneumoniae* ATCC 700603 and *P. aeruginosa* ATCC 27853 obtained from the ATCC were used along with the antimicrobials in accordance with NCCLS recommendation as internal control in each test run.

Results

The MIC ranges, minimum concentration inhibiting 50% of isolates (MIC_{50}), minimum concentration inhibiting 90% of isolates (MIC_{90}) and the susceptibility rate of the 247 blood isolates to the tested drugs are listed in Table 1. In brief, isepamicin and amikacin had good activity (effective against more than 90% of the isolates) against all of the tested isolates of *Enterobacteriaceae* except *C. freundii* and *P. aeruginosa*. However, isepamicin and amikacin had limited activity against *C. freundii* (80% of isolates were susceptible; MIC_{50} of isepamicin and amikacin, 0.5 mg/L and 1 mg/L, respectively; MIC_{90}, >128 mg/L for isepamicin and amikacin), and poor activity against *A. baumannii*

Table 1. In vitro susceptibility of blood isolates of Gram-negative bacteria recovered from patients treated between January 2003 and December 2003 at National Taiwan University Hospital

Bacterium (no. of isolates tested)	Antimicrobial agent	MIC (mg/L)			Susceptibility (no. of isolates tested)		
		Range	MIC_{50}	MIC_{90}	S	I	R
Escherichia coli (31)	Gentamicin	0.5-64	0.5	64	81 (25)	3 (1)	16 (5)
	Amikacin	0.5-4	1	1	100 (31)	0 (0)	0 (0)
	Tobramycin	0.125-32	0.5	4	90 (28)	3 (1)	7 (2)
	Isepamicin	0.25-1	0.5	0.5	100	0 (0)	0 (0)
Klebsiella pneumoniae (31)	Gentamicin	0.25->128	0.25	8	87 (27)	3 (1)	10 (3)
	Amikacin	0.25->128	0.50	1	97 (30)	0 (0)	3 (1)
	Tobramycin	0.125->128	0.25	2	90 (28)	7 (2)	3 (1)
	Isepamicin	0.125->128	0.50	0.5	97 (30)	0 (0)	3 (1)
Enterobacter cloacae (30)	Gentamicin	0.25->128	1	128	60 (18)	0 (0)	40 (12)
	Amikacin	0.5-16	2	8	100 (30)	0 (0)	0 (0)
	Tobramycin	0.25-128	0.5	64	63 (19)	3 (1)	34 (10)
	Isepamicin	0.25-2	0.5	1	100 (30)	0 (0)	0 (0)
Serratia marcescens (31)	Gentamicin	0.5->128	0.5	8	58 (18)	32 (10)	10 (3)
	Amikacin	1->128	2	4	94 (29)	0 (0)	6 (2)
	Tobramycin	1->128	1	16	65 (20)	0 (0)	35 (11)
	Isepamicin	0.5->128	1	1	94 (29)	0 (0)	6 (2)
Morganella morganii (21)	Gentamicin	0.25->128	0.5	128	67 (14)	0 (0)	33 (7)
	Amikacin	0.5->128	1	2	95 (20)	0 (0)	5 (1)
	Tobramycin	0.25->128	0.5	2	95 (20)	0 (0)	5 (1)
	Isepamicin	0.25->128	1	2	95 (20)	0 (0)	5 (1)
Citrobacter freundii (10)	Gentamicin	0.5->128	0.5	>128	60 (6)	10 (1)	30 (3)
	Amikacin	0.5->128	1	>128	80 (8)	0 (0)	20 (2)
	Tobramycin	0.25->128	4	>128	60 (6)	10 (1)	30 (3)
	Isepamicin	0.25->128	0.5	>128	80 (8)	0 (0)	20 (2)
Pseudomonas aeruginosa (31)	Gentamicin	1->128	2	>128	81 (25)	0 (0)	19 (6)
	Amikacin	1-32	2	8	97 (30)	3 (1)	0 (0)
	Tobramycin	0.25->128	0.5	128	81 (25)	3 (1)	16 (5)
	Isepamicin	1-128	4	8	94 (29)	3 (1)	3 (1)
Acinetobacter baumannii (31)	Gentamicin	0.125->128	2	>128	55 (17)	0 (0)	45 (14)
	Amikacin	1->128	2	>128	58 (18)	0 (0)	42 (13)
	Tobramycin	0.125->128	2	>128	52 (16)	3 (1)	45 (14)
	Isepamicin	0.25->128	2	>128	58 (18)	0 (0)	42 (13)
Stenotrophomonas maltophilia (31)	Gentamicin	1-128	16	64	26 (8)	16 (5)	58 (18)
	Amikacin	2-128	32	64	42 (13)	16 (5)	42 (13)
	Tobramycin	1->128	16	128	35 (11)	10 (3)	55 (17)
	Isepamicin	2-64	32	64	45 (14)	29 (9)	26 (8)

Abbreviations: MIC = minimal inhibitory concentration; MIC_{50} = minimum concentration inhibiting 50% of isolates; MIC_{90} = minimum concentration inhibiting 90% of isolates; S = susceptible; I = intermediate; R = resistant

(58% of isolates were susceptible; MIC_{50} and MIC_{90} of isepamicin and amikacin, 2 mg/L and >128 mg/L, respectively) and *S. maltophilia* (45% of isolates were susceptible to isepamicin and 42% of isolates were susceptible to amikacin; MIC_{50} and MIC_{90} of isepamicin and amikacin, 32 mg/L and 64 mg/L, respectively).

Susceptibilities of the 247 isolates to gentamicin varied. Over 80% of *E. coli*, *K. pneumoniae*, and *P. aeruginosa* were susceptible to gentamicin (susceptibility, 81%, 87% and 81%, respectively), whereas this agent had limited activity against other bacteria, particularly *M. morganii* (susceptibility, 67%; MIC_{90}, 128 mg/L), *E. cloacae* (susceptibility, 60%; MIC_{90}, 128 mg/L), *C. freundii* (susceptibility, 60%; MIC_{90}, >128 mg/L), *S. marcescens* (susceptibility, 58%; MIC_{90}, 8 mg/L), *A. baumannii* (susceptibility, 55%; MIC_{90}, >128 mg/L), and *S. maltophilia* (susceptibility, 26%; MIC_{90}, 64 mg/L).

Susceptibilities of 247 isolates to tobramycin also varied. Tobramycin had good activity against the *E. coli*, *K. pneumoniae*, and *M. morganii* isolates tested (susceptibility, 90%, 90% and 95%, respectively) and

over four-fifths of *P. aeruginosa* isolates tested were susceptible to tobramycin (susceptibility, 81%). But tobramycin had limited activity against other bacteria tested, including *E. cloacae* (susceptibility, 63%; MIC_{90}, 64 mg/L), *S. marcescens* (susceptibility, 65%; MIC_{90}, 16 mg/L), *C. freundii* (susceptibility, 60%; MIC_{90}, >128 mg/L), *A. baumannii* (susceptibility, 52%; MIC_{90}, >128 mg/L), and *S. maltophilia* (susceptibility, 35%; MIC_{90}, 128 mg/L).

Comparing the susceptibility of four aminoglycoside agents against the 247 Gram-negative bacteria isolates, isepamicin had equal or better activity than amikacin against all the tested Gram-negative bacteria except *P. aeruginosa* (susceptibility of isepamicin and amikacin, 94% and 97%, respectively). Both isepamicin and amikacin had better susceptibility than tobramycin and gentamicin. Additionally, tobramycin had equal or better activity than gentamicin against all tested bacteria except *A. baumannii* (susceptibility of tobramycin and gentamicin, 52% and 55%, respectively).

Discussion

Gram-negative bacteria are the most common pathogens of sepsis and septic shock, and are significantly associated with high morbidity and mortality in hospitalized patients [14]. Hsueh et al reported that Gram-negative bacteria remained the predominant bacterial pathogens (66.1% in 1981, 51.3% in 1993, and 53.4% in 1999) among nosocomial infections, and that nosocomial pathogens have shifted away from easily treated bacteria toward more resistant bacteria with fewer options for therapy [15]. Previous studies concerning the activity of isepamicin usually stressed its effect on specific bacteria, but did not clarify its specific activity on nosocomial pathogens [10,11]. Therefore, we enrolled only nosocomial pathogens in this study, in order to address this issue. Early initiation of appropriate antimicrobial therapy is critically important for reducing complications and deaths resulting from infections due to these Gram-negative bacteria [16]. Such therapy is almost empiric initially and requires precise knowledge of the possible pathogens and their usual antimicrobial susceptibility patterns because multidrug-resistant microorganisms have become a serious threat to the management of infectious disease.

The aminoglycosides are useful antibiotics for the treatment of serious Gram-negative infectious, despite their narrow therapeutic index [17]. The uptake of aminoglycosides, which differs from most antibiotics in that it is energy-dependent and includes one step of rapid passive diffusion across the outer membrane protein and two steps of active irreversible drug binding to the bacterial 30S ribosomal subunit. This interaction affects bacterial protein synthesis and eventually leads to a major loss of permeability control and cell death [5].

However, bacterial resistance to the aminoglycosides through the acquisition of plasmid-mediated aminoglycoside-modifying enzymes is an increasing problem where aminoglycoside usage is prevalent [18]. As a result of these modifications, the binding affinity of the aminoglycosides to ribosomes is altered, resulting in resistance. In addition to these modifying enzymes, the presence of new aminoglycoside resistance mechanisms, including permeability resistance, was also demonstrated among Gram-negative bacteria, particularly in non-fermentative Gram-negative bacilli [3,4]. Therefore, it is necessary to monitor the in vitro activity of aminoglycosides against important Gram-negative bacteria and to search for more potential drugs.

Isepamicin, the 1-N-S-alpha-hydroxy-beta-aminoproprionyl derivative of gentamicin B, is a novel, semisynthetic aminoglycoside, with activity against many bacteria resistant to other aminoglycosides. The drug has been introduced for once-daily administration, in contrast to earlier aminoglycosides that were developed and approved on the basis of thrice- or twice-daily administration [19]. The spectrum of activity of isepamicin includes all species of *Enterobacteriaceae*, *Pseudomonas* spp., and many strains of *Acinetobacter* spp. [20]. Like other aminoglycosides, isepamicin acts by binding bacterial ribosomes, resulting in the misreading of mRNA and ultimately cellular death via inhibition of bacterial protein synthesis [21]. The reason why isepamicin was the most effective against against Gram-negative bacteria among all tested aminoglycosides might be that it had better stability to aminoglycoside-modifying enzymes [20]. This feature of isepamicin might extrapolate to its expanded clinical use in hospitals where bacterial resistance to other aminoglycosides had become endemic.

The maximal antimicrobial effect is reached when isepamicin is given at a dosage of 15 mg/kg once daily, with the target concentrations higher than 48 mg/L for peak (i.e., 6 times the lower breakpoint) and lower than 5 mg/L for trough concentrations [22]. Isepamicin monitoring is useful only when patients are immunosuppressed or likely to be treated for more than 10 days, or the MIC for the causative strain is in the intermediate

range (8-16 mg/L) [22]. Although isepamicin can induce nephro-, vestibule- and ototoxicity from animal and clinical studies, it is one of the less toxic aminoglycosides [23].

In our study, isepamicin demonstrated in vitro activity against tested nosocomial bloodstream Gram-negative bacteria equal or similar to that of amikacin, and superior to gentamicin and tobramycin. For the 154 isolates belonging to the *Enterobacteriaceae*, the resistance rate among tested isolates for isepamicin (3.9%) was the same as amikacin (3.9%) and lower than tobramycin (18.2%) and gentamicin (21.4%). For the 93 isolates of non-fermentative Gram-negative bacilli, the resistance rate for isepamicin (23.7%) was lower than those of amikacin (27.9%), tobramycin (38.7%) and gentamicin (40.9%).

Against the *Enterobacteriaceae*, isepamicin also had equal or better range values for MIC_{50} (0.5-1 mg/L) and MIC_{90} (0.5->128 mg/L) than amikacin (0.5-2 mg/L and 1->128 mg/L, respectively); the MICs of isepamicin and amikacin against the non-fermentative Gram-negative bacteria were equivalent, except for a lower MIC_{50} of amikacin against *P. aeruginosa* (2 vs 4 mg/L).

In general, tobramycin had activity equal to or better than gentamicin as assessed by MIC_{50} range (0.25-16 mg/L for both) and MIC_{90} range (2->128 and 8->128 mg/L, respectively). However, gentamicin displayed lower MIC_{50} values for *S. marcescens* (0.5 vs 1 mg/L) and *C. freundii* (0.5 vs 4 mg/L) and lower MIC_{90} values for *S. marcescens* (8 vs 16 mg/L) and *S. maltophilia* (64 vs 128 mg/L).

Our results were similar to those of the previous studies of Cheng et al [10] and Liao et al [11], in which isepamicin showed susceptibility rates equivalent to amikacin against most Gram-negative bacteria in Taiwan, including isolates producing extended-spectrum beta-lactamase (ESBL). Our results were also similar to those reported from other countries [21,24-27]. Taken together, the results of studies thus far have shown that isepamicin has promising in vitro activities against most Gram-negative bacteria, and suggest that isepamicin and amikacin can be considered as alternative agents or be incorporated into combination therapy in more resistant nosocomial Gram-negative infections.

There were limitations to the present study. First, all tested bacterial isolates were nosocomial, and no community-acquired isolates were included. Second, as isolates of *Proteus* spp. were not available in our stock, *Proteus* spp. were not evaluated. However, based on the results of Cheng et al [10], isepamicin had similar activity to amikacin for *Proteus* spp. in both nosocomial and community-acquired isolates. Third, bacterial isolates producing ESBL, an important contributor to bacterial resistance [11], were not identified and specifically analyzed in this study. Liao et al reported that around 80% of ESBL-producing *E. coli* (ESBL-EC) and 70% of ESBL-producing *K. pneumoniae* (ESBL-KP) were susceptible to both isepamicin and amikacin and only around 10% were susceptible to gentamicin [11]. However, Wu et al reported that clinical isolates of ESBL-producing *P. mirabilis* expressing co-resistance to gentamicin, isepamicin, and amikacin [28]. The differences in susceptibility to isepamicin among these ESBL-producing pathogens needs further study.

In summary, isepamicin is highly active against blood isolates of *Enterobacteriaceae* and non-fermentative Gram-negative bacilli collected from our hospital. Taking into account potential safety advantages of isepamicin in terms of nephrotoxicity and ototoxicity compared with other aminoglycosides, isepamicin is a drug of choice for the empirical treatment of infections suspected to be caused by Gram-negative bacteria.

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