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In Vivo Antibacterial Activity of Vertilmicin, a New Aminoglycoside Antibiotic▽

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Vertilmicin is a novel aminoglycoside antibiotic with potent activity against gram-negative and -positive bacteria in vitro. In this study, we further evaluated the efficacy of vertilmicin in vivo in systemic and local infection animal models. We demonstrated that vertilmicin had relatively high and broad-spectrum activities against mouse systemic infections caused by *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Enterococcus faecalis*. The 50% effective doses of subcutaneously administered vertilmicin were 0.63 to 0.82 mg/kg, 0.18 to 0.29 mg/kg, 0.25 to 0.99 mg/kg, and 4.35 to 7.11 mg/kg against *E. coli*, *K. pneumoniae*, *S. aureus*, and *E. faecalis* infections, respectively. The therapeutic efficacy of vertilmicin was generally similar to that of netimicin, better than that of gentamicin in all the isolates tested, and better than that of verdamicin against *E. coli* 9612 and *E. faecalis* HH22 infections. The therapeutic efficacy of vertilmicin was further confirmed in local infection models of rabbit skin burn infection and mouse ascending urinary tract infection.

Aminoglycosides are a group of highly potent, broad-spectrum bactericidal antibiotics (8). Their history began with the discovery of streptomycin (12), followed by kanamycin, gentamicin, tobramycin, and a series of semisynthetic aminoglycosides (dibekacin, amikacin, and netilmicin) for the treatment of resistant organisms (8). The mechanisms of aminoglycoside resistance involved (i) modifying enzymes (the most common mechanism), (ii) mutations of the ribosomal binding site (causes resistance to streptomycin), and (iii) reduced drug uptake (mostly seen in *Pseudomonas* spp.) (2, 13). The semisynthetic aminoglycosides are mainly designed for the treatment of organisms that have developed resistance by producing aminoglycoside-modifying enzymes, i.e., *N*-acetyltransferase, *O*-nucleotidyltransferase, and *O*-phosphotransferases (8).

Vertilmicin is a novel semisynthetic aminoglycoside derived from verdamicin. Our earlier study showed that it had broad in vitro antimicrobial activity which is similar to that of netilmicin and has the advantage of lower susceptibility to *N*-acetyltransferase 6′-Ie modification (5). In this study, we further investigated the in vivo antibacterial activities of this agent in a systemic infection model, as well as local infection models, to fill the gap between in vitro characterization and clinical evaluation. All of our animals studies were approved by the Animal Research Committee of the Institute of Medicinal Biotechnology.

Mouse systemic infection model. The in vivo efficacy of vertilmicin against mouse systemic infections versus those of netilmicin, verdamicin, and gentamicin was determined with three strains of *Escherichia coli*, two strains of *Klebsiella pneumoniae*, three strains of *Staphylococcus aureus*, and two strains of *Enterococcus faecalis* (Table 1). The experiment was carried out by a method modified from the literature (11). CD-1 ICR mice (18 to 21 g) were randomly distributed into 21 groups with 5 groups for each compound and 1 control group (10 mice per group, 5 males and 5 females). The mice were intraperitoneally infected with 0.5 ml of a bacterial suspension in 5% mucin (100 times the median lethal dose). Different doses of vertilmicin and the reference compounds (saline for the control group) were administered subcutaneously 15 min and 6 h after infection, respectively. The dose ranges of vertilmicin were 0.32 to 1.59 mg/kg for *E. coli* infections, 0.1 to 0.5 mg/kg for *K. pneumoniae* infections, 0.16 to 2.5 mg/kg for *S. aureus* infections, and 2.5 to 12.6 mg/kg for *E. faecalis* infections, respectively. Deaths in each group were recorded daily for 7 days, and the 50% effective dose (ED_{50}) and 95% confidence limits were determined by Probit analysis (6).

The MICs, ED_{50}s, and 95% confidence limits of vertilmicin and the reference compounds are listed in Table 1. Vertilmicin showed relatively potent and broad-spectrum in vivo activity against both gram-negative (*E. coli* and *K. pneumoniae*) and gram-positive (*S. aureus* and *E. faecalis*) bacteria. The ED_{50}s of vertilmicin against *E. coli* ATCC 25922, *E. coli* 9612, and *E. coli* 1515 systemic infections were 0.82, 0.67, and 0.63 mg/kg, respectively, which were similar to those of netilmicin but significantly lower than those of gentamicin ($P < 0.01$). Vertilmicin showed therapeutic efficacy similar to that of verdamicin against *E. coli* ATCC 25922 and *E. coli* 1515 systemic infections but significantly better efficacy against *E. coli* 9612 infec-

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TABLE 1. In vivo antibacterial activities in mouse systemic infection model

Strain (challenge dose [CFU/mouse]) and antibiotic	MIC[a] (μg/ml)	ED_{50} (mg/kg) (95% confidence limit)	*P* value vs vertilmicin
E. coli ATCC 25922 (1.0×10^5)			
Vertilmicin	0.5	0.82 (0.69–0.95)	
Verdamicin	0.5	1.09 (0.82–1.46)	>0.05
Netilmicin	0.5	0.77 (0.64–0.93)	>0.05
Gentamicin	0.5	1.50 (1.28–1.76)	<0.01
E. coli 9612 (3.7×10^4)			
Vertilmicin	0.5	0.67 (0.58–0.77)	
Verdamicin	4	2.66 (1.87–3.80)	<0.01
Netilmicin	0.5	0.63 (0.56–0.72)	>0.05
Gentamicin	8	7.88 (6.31–9.83)	<0.01
E. coli 1515 (2.4×10^6)			
Vertilmicin	0.5	0.63 (0.50–0.78)	
Verdamicin	0.5	0.49 (0.43–0.57)	>0.05
Netilmicin	0.5	0.46 (0.39–0.55)	>0.05
Gentamicin	0.5	1.11 (0.89–1.37)	<0.01
K. pneumoniae 935 (3.0×10^5)			
Vertilmicin	0.5	0.29 (0.20–0.41)	
Verdamicin	0.5	0.29 (0.23–0.38)	>0.05
Netilmicin	0.5	0.33 (0.27–0.40)	>0.05
Gentamicin	0.5	0.68 (0.56–0.82)	<0.01
K. pneumoniae 967 (2.6×10^5)			
Vertilmicin	0.25	0.18 (0.14–0.23)	
Verdamicin	0.25	0.16 (0.14–0.19)	>0.05
Netilmicin	0.5	0.17 (0.10–0.29)	>0.05
Gentamicin	0.5	0.32 (0.24–0.42)	<0.05
S. aureus ATCC 29213[b] (1.5×10^5)			
Vertilmicin	0.125	0.25 (0.21–0.30)	
Verdamicin	0.125	0.23 (0.17–0.31)	>0.05
Netilmicin	0.125	0.26 (0.22–0.32)	>0.05
Gentamicin	0.125	0.60 (0.48–0.75)	<0.01
S. aureus 9344[c] (2.5×10^5)			
Vertilmicin	0.25	0.99 (0.65–1.50)	
Verdamicin	0.25	1.33 (0.86–2.06)	>0.05
Netilmicin	0.25	0.88 (0.43–1.80)	>0.05
Gentamicin	0.25	2.84 (2.17–3.74)	<0.01
S. aureus 15[b] (2.5×10^5)			
Vertilmicin	0.125	0.70 (0.57–0.86)	
Verdamicin	0.125	0.66 (0.51–0.86)	>0.05
Netilmicin	0.125	0.49 (0.39–0.61)	>0.05
Gentamicin	0.125	0.61 (0.45–0.81)	>0.05
E. faecalis ATCC 29212 (2.8×10^7)			
Vertilmicin	8	4.35 (3.64–5.21)	
Verdamicin	8	5.24 (4.41–6.24)	>0.05
Netilmicin	8	3.44 (3.03–3.91)	>0.05
Gentamicin	8	17.52 (14.92–20.57)	<0.01
E. faecalis HH22 (1.8×10^8)			
Vertilmicin	8	7.11 (5.55–9.12)	
Verdamicin	>128	>100	<0.01
Netilmicin	8	6.56 (5.27–8.17)	>0.05
Gentamicin	>128	>100	<0.01

[a] MICs were determined by the agar dilution method according to CLSI recommendations (10).
[b] MSSA.
[c] MRSA.

tion ($P < 0.01$). The ED_{50}s of vertilmicin against *K. pneumoniae* 935 and *K. pneumoniae* 967 systemic infections were 0.29 and 0.18 mg/kg, respectively, which were significantly lower than those of gentamicin ($P < 0.01$ or 0.05). However, vertilmicin was not significantly different from verdamicin and netilmicin. Vertilmicin showed ED_{50}s against *S. aureus* ATCC 29213 (methicillin [meticillin]-susceptible *S. aureus* [MSSA] strain), *S. aureus* 9344 (methicillin-resistant *S. aureus* [MRSA] strain), and *S. aureus* 15 (MSSA) infections (0.25, 0.99, and 0.70 mg/kg, respectively) similar to those of verdamicin and netilmicin. However, vertilmicin had significantly lower ED_{50}s against *S. aureus* ATCC 29213 and *S. aureus* 9344 infections than did gentamicin ($P < 0.01$). In *S. aureus* 9344 (MRSA) infections, the ED_{50}s of the semisynthetic aminoglycosides (vertilmicin and netilmicin) were lower than those of the natural compounds (verdamicin and gentamicin), suggesting the possible superiority of the semisynthetic aminoglycosides against MRSA infections. The ED_{50}s of vertilmicin against *E. faecalis* ATCC 29212 and *E. faecalis* HH22 infections were 4.35 and 7.11 mg/kg, respectively, which were higher than the ED_{50}s of vertilmicin against other isolates, suggesting that aminoglycosides have relatively lower efficacy against *E. faecalis* infections. The ED_{50}s of vertilmicin against both of the strains were significantly lower ($P < 0.01$) than those of gentamicin. In addition, the ED_{50} of vertilmicin was also significantly lower ($P < 0.01$) than that of verdamicin against infection caused by *E. faecalis* HH22, a clinical isolate highly resistant (MIC, >2,000 μg/ml) to gentamicin, tobramycin, amikacin, streptomycin, and kanamycin (7, 9).

Interestingly, there is a correlation between gentamicin MICs and ED_{50}s. For example, all of the agents tested had lower ED_{50}s against strains with lower gentamicin MICs (0.125 to 0.5 μg/ml). On the other hand, for the isolates with higher gentamicin MICs (≥8 μg/ml; e.g., *E. coli* 9612, *E. faecalis* HH22, and *E. faecalis* ATCC 29212), the semisynthetic antibiotics demonstrated superior efficacies against the first two isolates, but not against *E. faecalis* ATCC 29212. These findings may be related to the fact that *E. coli* 9612 and *E. faecalis* HH22 produce aminoglycoside-modifying enzymes. We recently demonstrated that the *ant*(2″)-*Ia*, *ant*(3″)-*Ia*, *aph*(4)-*Ia*, and *aph*(3′)-*IIIa* genes were detected in *E. coli* 9612 and the *aac*(6′)-*aph*(2″) gene was carried on pBEM10 in *E. faecalis* HH22 (3). In comparison, *E. faecalis* ATCC 29212 did not contain any aminoglycoside-modifying enzyme; the high MIC of gentamicin was due to the intrinsic resistance of enterococci to aminoglycosides (1). Therefore, no superiority of the semisynthetic compounds against this strain could be demonstrated.

In general, vertilmicin showed broad-spectrum in vivo activity in the mouse systemic infection model; it had greater efficacy than gentamicin against most of the study isolates and better activity than verdamicin against isolates producing aminoglycoside-modifying enzymes (*E. coli* 9612 and *E. faecalis* HH22). However, vertilmicin showed ED_{50}s similar to those of netilmicin against all of the isolates tested, which reflected their similar in vitro activities (5).

The ED_{50}s of vertilmicin, netilmicin, and verdamicin track their MICs, but that of gentamicin does not (Table 1). The most likely reason for this is that the pharmacokinetic (PK) profile of gentamicin is slightly different from those of the other three compounds, considering the relatively similar structures of

TABLE 2. In vivo antibacterial activities of vertilmicin and reference compounds in rabbit skin burn infections caused by *E. coli* 9612

Antibiotic, dose (mg/ml)	Log CFU/g ($\bar{x} \pm$ SD, $n = 6$)	*P* value vs:	
		Control	1-mg/ml vertilmicin group
Vertilmicin			
0.5	6.33 ± 1.65	<0.05	
1	5.09 ± 1.65	<0.01	
2	5.00 ± 1.62	<0.01	
Netilmicin, 1	6.06 ± 1.97	<0.05	>0.05
Verdamicin, 1	8.07 ± 0.63	>0.05	<0.01
Gentamicin, 1	8.31 ± 0.54	>0.05	<0.01
Control	8.57 ± 0.35		

TABLE 3. In vivo antibacterial activities of vertilmicin and reference compound in mouse ascending urinary tract infections caused by *E. coli* 9612

Antibiotic and dose (mg/kg)	Log CFU/g ($\bar{x} \pm$ SD, $n = 10$)	*P* value vs control
Vertilmicin		
20	2.36 ± 0.41	<0.01
12	3.11 ± 0.34	<0.01
7.2	3.07 ± 0.37	<0.01
4.32	3.22 ± 0.48	<0.01
2.59	3.32 ± 0.33	<0.01
Gentamicin		
40	2.78 ± 0.80	<0.01
24	3.24 ± 0.48	<0.01
14.4	3.34 ± 0.57	<0.01
8.64	3.44 ± 0.63	<0.01
5.18	3.71 ± 0.23	>0.05
Control	4.45 ± 0.37	

vertilmicin, netilmicin, and verdamicin in comparison to that of gentamicin, which is a mixture of gentamicin C1, C1a, C2, etc.

Rabbit skin burn infection model. The skin burn infection study was carried out with six male New Zealand White rabbits (body weight, 2.5 to 3.0 kg) infected with *E. coli* 9612. Three doses of vertilmicin (0.5, 1, and 2 mg/ml) and one dose of netilmicin, verdamicin, and gentamicin (1 mg/ml) were tested in this model.

One day before infection, the back hair of the animals was clipped and then removed by applying a paste of barium sulfide-zinc oxide-starch (2:3:3, wt/wt/wt). On the day of infection, the rabbits were anesthetized by intravenous injection of pentobarbital sodium (30 mg/kg of body weight) and the back skin of the animals was sterilized with 75% ethanol. Seven deep second-degree burn wounds (six for administering compounds and one for a control) were then created on the back of each animal by applying a brass probe (diameter of 1.8 cm, heated to 100°C) for 10 s (14, 16). Fifteen minutes later, 0.1 ml (challenge dose, 1×10^7 CFU/burn wound) of an *E. coli* 9612 suspension in saline was intracutaneously injected into each wound and a homemade cap was used to protect each wound. One hour after the burning, 0.4 ml of different compound solutions or saline (for the control) was administered to the corresponding wounds by loading it onto a sterile gauze patch of the same size as the burn wound. Sterile petrolatum-containing gauze was used on top of the compound-containing gauze to keep the wounds humid. The rabbits were sacrificed 24 h after administration of the compounds. Full-thickness skin biopsy samples were then taken from the center of the burns sterilely and homogenized in saline. The homogenates were serially 10-fold diluted, and 0.1-ml aliquots were spread onto nutrient agar plates. The plates were incubated at 35°C for 48 h, and the numbers of viable organisms in the burn wounds (CFU per gram) were determined.

Vertilmicin significantly reduced the viable colony counts in comparison to that of the control group in a dose-dependent manner ($P < 0.05$; Table 2). The netilmicin (1 mg/ml) group also showed a significantly lower viable colony count than the untreated control ($P < 0.05$). However, the reductions of viable cell counts in the verdamicin (1 mg/ml) and gentamicin (1 mg/ml) groups were not significant in comparison to that of the control group. In addition, vertilmicin at 1 mg/ml produced significantly lower bacterial counts than did verdamicin (1 mg/ml) and gentamicin (1 mg/ml) ($P < 0.01$). The efficacies of the compounds tested were in the order vertilmicin ≥ netilmicin > verdamicin > gentamicin, which was similar to the results of the systemic infections (Table 1) and the MICs. We also studied the diffusion of vertilmicin, netilmicin, verdamicin, and gentamicin from the sites of placement by determining the concentrations of the compounds in skin tissues by liquid chromatography-tandem mass spectrometry. The results showed that these compounds diffused from the sites of placement similarly (data not shown), which supports the finding that the efficacies of the compounds in the rabbit skin burn infection model track the MICs.

Mouse ascending urinary tract infection model. The therapeutic efficacies of vertilmicin and gentamicin in mouse ascending urinary tract infections caused by *E. coli* 9612 was evaluated with female CD-1 ICR mice (body weight, 20 to 22 g, 10 mice/group) as previously described with modifications (4, 15). Five different doses of vertilmicin or gentamicin were used in these experiments. Gentamicin doses were twofold higher than the corresponding doses of vertilmicin, considering the relatively lower efficacy of gentamicin shown previously (Tables 1 and 2). The mice were subjected to water restriction for 24 h prior to and after infection, respectively. Under pentobarbital anesthesia, a round-point needle was inserted transurethrally for injection of 0.05 ml of the bacterial suspension in 5% mucin (challenge dose, 2.2×10^9 CFU/mouse) into the bladder. The urethral needle was removed immediately after inoculation, and the external urethral meatus was clamped for 1 h. The compounds (saline for the control group) were administered subcutaneously at 6 h, 24 h, 30 h, 48 h, and 54 h postinfection. The mice were sacrificed 72 h after infection. The kidneys were then removed and homogenized in saline, the homogenates were serially 10-fold diluted, and 0.1-ml aliquots were spread onto nutrient agar plates. The plates were incubated at 35°C for 48 h, and the numbers of viable organisms in the kidneys (CFU per gram) were determined.

Vertilmicin exhibited a high degree of dose-dependent efficacy against *E. coli* 9612; all groups showed a significant decrease in kidney viable colony counts ($P < 0.01$) in comparison to that of the control group (Table 3). Gentamicin also showed

dose-dependent antibacterial efficacy, with significantly decreased kidney viable colony counts ($P < 0.01$, except for the lowest-dose group). Vertilmicin had better efficacy than gentamicin at a similar dose. For example, vertilmicin at 20 mg/kg caused a decrease in the colony count of 2.09 $\log_{10}$ CFU/g, while a gentamicin dose of 40 mg/kg, which was twofold higher than that of vertilmicin, only caused a decrease of 1.67 $\log_{10}$ CFU/g. However, gentamicin seems to work better on a per-dose basis with respect to MICs (8 μg/ml of gentamicin versus 0.5 μg/ml of vertilmicin). One possible explanation is the difference of the PK profiles of the two compounds in the urinary tract.

In conclusion, our systemic and local infection studies demonstrated that vertilmicin had in vivo antimicrobial activity comparable to that of netilmicin and better efficacy than verdamicin and gentamicin (especially against isolates producing aminoglycoside-modifying enzymes). This in vivo antimicrobial activity study confirmed that vertilmicin has high activity and deserves further investigation.

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