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Antibacterial Activities, Nephrotoxicity, and Ototoxicity of a New Aminoglycoside, Win 42122-2

PAUL E. CAME,* JOHN R. O'CONNOR, RICHARD A. DOBSON, ROLAND B. WAGNER, AND RAYMOND J. FABIAN

Sterling-Winthrop Research Institute, Rensselaer, New York 12144

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Win 42122-2 is a new aminoglycoside antibiotic obtained from a mutant strain of *Micromonospora purpurea*. In vitro and in vivo comparisons of Win 42122-2 with gentamicin and amikacin revealed that Win 42122-2 generally was less active than gentamicin against *Pseudomonas* and many *Enterobacteriacae*, especially *Klebsiella* and indole-negative *Proteus*. Against most gentamicin-susceptible isolates, Win 42122-2 was more active than amikacin. Gentamicin-resistant clinical isolates were usually resistant to Win 42122-2, although it was active against certain gentamicin-resistant organisms, depending upon the aminoglycoside-modifying enzymes harbored by the organism. However, Win 42122-2 was markedly less toxic than gentamicin in subacute nephrotoxicity studies in rats, ototoxicity experiments in guinea pigs, and ataxia determinations in cats. This series of antibacterial determinations and toxicity evaluations indicated that the reduced toxicity of the antibiotic may be sufficient to provide an improved therapeutic ratio over gentamicin and other aminoglycosides, even though Win 42122-2 is less potent than gentamicin against some bacteria.

Aminoglycosides such as gentamicin, amikacin, and kanamycin are frequently employed in the treatment of severe infections with gram-negative organisms. Their potency and spectrum make them appropriate for use in hospitalized patients suffering from serious infections with gram-negative organisms. The use of amikacin, kanamycin, gentamicin, or tobramycin is limited primarily by ototoxicity or nephrotoxicity. A less toxic aminoglycoside would provide an agent that could be used with reduced risk, especially in patients with impaired kidney function. We now report on studies with such an aminoglycoside antibiotic that was prepared by mutational biosynthesis and designated Win 42122-2 (11). This report presents results of in vitro and in vivo microbiological evaluations of Win 42122-2 and the results of toxicity studies in rats, guinea pigs, and cats.

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MATERIALS AND METHODS

Test compounds. Amikacin, gentamicin, and Win 42122-2 were used as sulfate salts and were assayed microbiologically; all results are expressed in terms of base activity. Amikacin was purchased. Gentamicin was a gift from the Schering Corp., Bloomfield, N.J.

Bacteria. Between 1976 and 1978, 188 clinical isolates of gram-negative organisms were obtained from six hospitals in five states. Sixty-three gentamicin-resistant (minimal inhibitory concentration [MIC] $\geq$ 8.0 μg/ml) *Pseudomonas* isolates were also obtained from these institutions. In addition to the clinical isolates described above, a number of organisms from the culture collection of the Sterling-Winthrop Research Institute were included in the in vitro studies. Organisms known to contain aminoglycoside-inactivating enzymes were obtained from J. Davies, University of Wisconsin, Madison, and K. Price, Bristol Laboratories, Syracuse, N.Y.

In vitro testing. Tube dilution susceptibility testing was performed by adding 0.5 ml of a dilution of drug to an equal volume of Mueller-Hinton broth containing bacteria so that the final concentration approximated 1.0×10^5 colony-forming units per ml. After incubation overnight, the lowest concentration preventing visible growth was considered the MIC.

In vivo studies. Two isolates each of *Escherichia coli*, *Serratia marcescens*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* and one each of *Providencia alcalifaciens*, *Providencia stuartii*, and *Klebsiella pneumoniae* were used in mouse protection tests. Inocula were prepared from the surface of brain heart infusion agar, except for the *E. coli* inoculum which was derived from broth, and were diluted to contain 1.5×10^5 to 4.4×10^7 colony-forming units per mouse, depending upon the organism. The inocula killed control mice within 24 h. The *E. coli* and *P. aeruginosa* isolates were suspended in saline, and the *Klebsiella*, *Proteus*, *Providencia*, and *Serratia* cultures were sus-

pended in 5% hog gastric mucin. Female ICR mice, weighing 18 to 20 g, in groups of 10 were infected intraperitoneally with 0.5-ml volumes, and after 0.5 h, the drugs were administered subcutaneously in 0.5 ml of saline. Five medication levels were used. Two groups of 10 mice each served as controls. Deaths were recorded at 7 days, and the 50% protective dose values were calculated by probit analysis (6).

Toxicity studies. Studies were conducted in rats, guinea pigs, and cats to compare the toxicity of Win 42122-2 with that of gentamicin. Nephrotoxicity evaluations in rats were patterned after those of Gilbert et al. (7) and Luft et al. (8) and consisted of monitoring urine volume, protein excretion, osmolality, and microscopic characteristics of the urine. In addition, histopathological examination of the kidneys was done. Ototoxicity studies were done with guinea pigs by the procedures described previously (1, 4) in an acoustical chamber with the aid of an audio oscillator and measured diminution or loss of the Preyer reflex to pure tone frequencies. An ataxia study in cats similar to that described by Waitz et al. (13) was conducted for a period of 73 days, using parameters of impaired righting reflex, ataxia, and mortality. As the above toxicological studies were done according to published methods, only salient features of the studies will appear in this report.

RESULTS

In vitro studies. The results of in vitro tests comparing gentamicin, Win 42122-2, and amikacin against *E. coli* (44 strains), *K. pneumoniae* (25 strains), *P. mirabilis* (22 strains), indole-positive *Proteus* spp. (8 strains), *Pseudomonas* spp. (67 strains), *S. marcescens* (15 strains), and *Staphylococcus aureus* (15 strains) are shown in Table 1. All of the above organisms were selected as gentamicin-susceptible organisms (MIC $\leq$8.0 μg/ml). The ranges of the MICs, the MIC_{50}s, and the MIC_{90}s are provided to enable a comparison of relative potency. In addition, the ratios of the MIC_{90}s of Win 42122-2 to gentamicin and Win 42122-2 to amikacin are shown. It is recognized that organisms from other sources could provide different ratios. The ratios of the MIC_{90}s of Win 42122-2 to gentamicin and Win 42122-2 to amikacin reveal that gentamicin was generally a more potent and amikacin a less potent antibiotic. A similar relationship exists if the MIC_{50}s are compared. Against *E. coli*, indole-positive *Proteus* spp., and *Serratia*, the difference in in vitro potency as evidenced by comparing the

TABLE 1. *Comparative in vitro activity of Win 42122-2, gentamicin, and amikacin against gentamicin-susceptible strains*

Organism (no. of isolates)	Antimicrobial agent	Inhibitory concn (μg/ml)			MIC_{90} ratio	
		Range	MIC_{50}[a]	MIC_{90}[a]	Win 42122-2/ gentamicin	Win 42122-2/ amikacin
E. coli (44)	Win 42122-2	1.56–6.25	3.13	6.25	2.0	0.5
	Gentamicin	0.78–6.25	1.56	3.13		
	Amikacin	3.13–12.5	6.25	12.5		
K. pneumoniae (25)	Win 42122-2	1.56–12.5	3.13	3.13	2.6	1.0
	Gentamicin	0.39–1.56	0.78	1.2		
	Amikacin	1.56–25	3.13	3.13		
P. mirabilis (22)	Win 42122-2	1.56–25	3.13	12.5	4.0	0.6
	Gentamicin	0.39–3.13	1.56	3.13		
	Amikacin	1.56–25	6.25	22.5		
Indole-positive *Proteus* spp. (8)	Win 42122-2	1.56–12.5	3.13	7.5	2.0	0.8
	Gentamicin	1.56–6.25	3.13	3.8		
	Amikacin	3.13–25	3.13	10.0		
Pseudomonas spp. (67)	Win 42122-2	1.0–31.3	1.95	7.5	3.8	1.9
	Gentamicin	0.125–7.8	0.5	1.95		
	Amikacin	0.5–7.8	1.0	3.9		
S. marcescens (15)	Win 42122-2	0.5–3.9	1.5	3.9	2.0	0.5
	Gentamicin	0.5–1.95	1.0	1.95		
	Amikacin	1.0–7.8	1.95	7.8		
S. aureus (15)	Win 42122-2	0.05–1.0	0.5	1.0	4.0	0.3
	Gentamicin	0.025–0.25	0.25	0.25		
	Amikacin	0.1–3.9	0.6	2.9		

[a] MIC_{50}, Concentration required for inhibition of 50% of strains; MIC_{90}, concentration required for inhibition of 90% of strains.

MIC_{90}s was not great, with gentamicin being 2.0 times more potent than Win 42122-2. However, against *P. mirabilis* and *Pseudomonas* spp. (at least 16 were *P. aeruginosa*), gentamicin was 4.0 times more active. Amikacin was less potent than Win 4122-2 against *E. coli*, *P. mirabilis*, indole-positive *Proteus* spp., *S. marcescens*, and *S. aureus*. Against *Pseudomonas* spp., amikacin was more potent than Win 42122-2.

Table 2 shows the comparative activity of gentamicin, Win 42122-2, and amikacin against a variety of additional bacterial species not in-

TABLE 2. *Comparative in vitro activity of Win 42122-2, gentamicin, and amikacin against a variety of gentamicin-susceptible bacterial strains*

Organism (no. of isolates)	Antimicrobial agent	MIC (μg/ml) range	Avg MIC ratios	
			Win 42122-2/ gentamicin	Win 42122-2/ amikacin
Bacillus spp. (2)	Win 42122-2	0.1–0.2	2.0	1.0
	Gentamicin	0.05–0.1		
	Amikacin	0.1–0.2		
Streptococcus spp. (5)	Win 42122-2	3.9–15.6	1.8	0.3
	Gentamicin	3.9–7.8		
	Amikacin	15.6–31.3		
Citrobacter spp. (4)	Win 42122-2	0.78–1.56	1.1	0.6
	Gentamicin	0.5–1.56		
	Amikacin	1.56–3.13		
Enterobacter spp. (6)	Win 42122-2	0.2–3.9	1.5	0.6
	Gentamicin	0.1–3.9		
	Amikacin	0.8–7.8		
Salmonella spp. (5)	Win 42122-2	0.2–0.8	1.6	0.4
	Gentamicin	0.1–0.8		
	Amikacin	0.8–3.2		
Vibrio parahaemolyticus (3)	Win 42122-2	1.6–12.5	8.6	2.2
	Gentamicin	0.4–0.8		
	Amikacin	1.6–3.2		
Gaffkya tetragena (1)	Win 42122-2	0.125	1.0	0.5
	Gentamicin	0.125		
	Amikacin	0.25		
Sarcina lutea (1)	Win 42122-2	1.0	4.0	1.0
	Gentamicin	0.25		
	Amikacin	1.0		
Edwardsiella tarda (1)	Win 42122-2	1.0	1.0	0.5
	Gentamicin	1.0		
	Amikacin	1.95		
Herellea vaginicola (1)	Win 42122-2	1.0	4.0	2.0
	Gentamicin	0.25		
	Amikacin	0.5		
Klebsiella oxytoca (1)	Win 42122-2	1.56	2.0	2.0
	Gentamicin	0.78		
	Amikacin	0.78		
Providencia spp. (2)	Win 42122-2	1.56–6.25	2.5	0.4
	Gentamicin	1.56		
	Amikacin	6.25–12.5		
Shigella dysenteriae (1)	Win 42122-2	0.8	2.0	0.5
	Gentamicin	0.4		
	Amikacin	1.6		

cluded in Table 1. These data reveal again that gentamicin was uniformly more potent than Win 42122-2 and show a trend similar to that seen in Table 1. *Citrobacter* and *Enterobacter* organisms were only slightly more susceptible (1.1 and 1.5 times) to gentamicin than to Win 42122-2. It can also be seen that Win 42122-2 was generally more active than amikacin against these gentamicin-susceptible strains.

In addition, 63 gentamicin-resistant recent clinical isolates were obtained from four hospitals. These consisted of *K. pneumoniae* (18 strains), *Pseudomonas* spp. (38 strains), and *S. marcescens* (7 strains). Table 3 shows the results of MIC determinations. Although results with individual organisms are not shown, only 7 of the 63 were susceptible to Win 42122-2 (MIC ≤15.6 μg/ml); 59 were susceptible to amikacin (MIC ≤20.0 μg/ml). These findings are in agreement with those of Price et al. (10) and Briedis and Robson (3), demonstrating that a large proportion of gentamicin-resistant isolates are susceptible to amikacin.

Table 4 shows the results obtained with selected organisms containing a variety of aminoglycoside-modifying enzymes (2). It can be seen that some of the enzymes that inactivate gentamicin also use Win 42122-2 as a substrate. However, the 2″-adenylylating enzymes from *Enterobacter cloacae* A20960, *K. pneumoniae* A20636 (GAdT), and *E. coli* JR76.2 did not inactivate Win 42122-2. These findings indicate that Win 42122-2 can be expected to inhibit organisms that are resistant to gentamicin due to certain of these plasmid-mediated enzymes.

In vivo studies. The results of mouse protection tests using gentamicin, Win 42122-2, and amikacin against two strains each of gentamicin-susceptible *E. coli*, *P. mirabilis*, *P. aeruginosa*, and *S. marcescens* and one strain each of *P. alcalifaciens*, *P. stuartii*, and *K. pneumoniae* are shown in Table 5. These in vivo results from single determinations are similar to the in vitro findings; i.e., gentamicin was approximately 1.4- to 4.1-fold more potent than Win 42122-2, depending on the organism. *P. mirabilis*, *S. mar-*

TABLE 3. *Comparative in vitro activity of Win 42122-2, gentamicin, and amikacin against gentamicin-resistant strains*

Organism (no. of isolates)	Antimicrobial agent	Inhibitory concn (μg/ml)		
		Range	MIC_{50}[a]	MIC_{90}[a]
K. pneumoniae (18)	Win 42122-2	7.8–>125	>125	>125
	Gentamicin	31.3–>125	62.5	>125
	Amikacin	0.5–3.9	1.95	3.9
Pseudomonas spp. (38)	Win 42122-2	3.9–>125	31.3	>125
	Gentamicin	15.6–>125	>125	>125
	Amikacin	1.0–>125	3.9	9.4
S. marcescens (7)	Win 42122-2	>125	>125	>125
	Gentamicin	>125	>125	>125
	Amikacin	1.95–3.9	1.95	3.9

[a] See Table 1, footnote *a*.

TABLE 4. *In vitro activity of three aminoglycosides against selected bacterial strains containing aminoglycoside-modifying enzyymes*

Microorganism	Enzyme[a]	MIC (μg/ml)		
		Gentamicin	Win 42122-2	Amikacin
S. marcescens ROS167	GAT_1	>125	>125	15.6
E. coli JR88	GAT_1	31.3	31.3	1.95
E. coli JR225	GAT_4	125	125	1.0
E. coli JR228	GAT_4	0.5	0.5	1.0
E. coli JR76.2	GAdT	50	6.25	6.25
E. coli CH15	KAT	1.0	1.0	62.5
E. coli R5/W677	KAT	1.0	3.9	15.6
E. coli JR214	GAdT, NPT	0.5	1.0	1.0
E. cloacae A20960	GAdT, NPt	25	3.13	3.13
K. pneumoniae A20636	GAdT, NPT	25	6.25	3.13
S. aureus BH2	GPT-2″, KAT	31.3	62.5	7.8
S. aureus Rivet	$TadT_4$	0.5	1.95	31.3

[a] For a definition of the enzyme code and nomenclature, see reference 11.

TABLE 5. *Protective effect of Win 42122-2, gentamicin, and amikacin against experimental infections in mice with gentamicin-susceptible gram-negative organisms*

Microorganism	MIC (μg/ml)			PD_{50}[a] (mg/kg) (95% confidence limits)			PD_{50} ratio	
	Win 42122-2	Gentamicin	Amikacin	Win 42122-2	Gentamicin	Amikacin	Win 42122-2/ gentamicin	Win 42122-2/ amikacin
E. coli Mc	3.9	1.9	7.8	0.82 (0.60–1.1)	0.48 (0.38–0.58)	1.7 (1.3–2.3)	1.7	0.5
E. coli Re	3.9	1.9	3.9	0.96 (0.63–1.2)	0.54 (0.33–0.73)	2.0 (1.3–3.0)	1.8	0.5
K. pneumoniae Wi. 1	3.9	1.9	7.8	6.9 (4.7–10)	5.0 (3.3–7.2)	13 (6.6–20)	1.4	0.5
P. mirabilis no. 33	15.6	7.8	31.3	2.8[b]	0.87 (0.57–1.2)	1.7 (1.0–2.6)	3.2	1.6
P. mirabilis no. 36	7.8	7.8	15.6	2.3[b]	1.4[b]	2.0[b]	1.6	1.2
P. aeruginosa 7700	1.5	0.4	0.78	25[b]	6.0 (4.0–8.7)	17[b]	4.1	1.5
P. aeruginosa VMS	1.0	0.25	1.0	35 (19–82)	19 (4.2–31)	27 (17–43)	1.8	1.3
P. stuartii no. 1	1.56	1.56	12.5	2.9 (1.6–5.2)	1.2 (0.65–1.8)	6.9 (4.8–9.9)	2.4	0.4
P. alcalifaciens no. 1	6.25	1.56	6.25	1.3 (0.62–1.9)	0.71 (0.42–1.0)	1.9[b]	1.8	0.7
S. marcescens Wi. 8	0.5	0.5	1.95	4.0[b]	2.6 (1.5–4.2)	4.5 (3.1–6.2)	1.5	0.9
S. marcescens Wi. 9	1.0	0.5	1.95	6.8 (3.5–14)	2.1 (1.3–3.0)	11 (6.9–22)	3.2	0.6

[a] PD_{50}, 50% protective dose.
[b] Estimate $P \cong 0.01$ to 0.02.

cescens Wi.9, and *P. aeruginosa* 7700 were the least susceptible to Win 42122-2. *K. pneumoniae* Wi.1, *S. marcescens* Wi.9, and *P. mirabilis* no. 36 were only slightly more susceptible to gentamicin than to Win 42122-2. In general, Win 42122-2 was more active than amikacin, and the 50% protective dose values usually reflected the potency differences seen in the in vitro determinations.

Toxicology studies. (i) Nephrotoxicity study in rats. Win 42122-2 and gentamicin were administered subcutaneously to groups of five Charles River CDF (Fisher 344) rats in daily doses of 40, 80, and 160 mg of base per kg for 14 consecutive days or longer. The parameters used to assess the nephrotoxicity of the two compounds were urine volume, protein excretion, osmolality, microscopic characteristics, blood chemistry, and histopathology. Urinalyses and body weights were determined on 7 days of the study, and blood urea nitrogen and creatinine determinations were done on day 15. Table 6 shows the results of the body weights and urinalyses on days 1, 8, 10, and 15 of this study. The body weights of animals receiving all three dose levels of Win 42122-2 were not significantly different from those of the controls. However, 80 mg of gentamicin per kg significantly affected weight gain by day 10, and there was a net loss of weight compared with the premedication value by day 15. At 160 mg/kg, gentamicin caused a significant weight loss by day 8.

The parameters of urine osmolality, volume, and total protein were normal throughout the study in specimens collected from the rats given 40 mg of Win 42122-2 per kg. At 80 mg/kg, all parameters were normal throughout, except for a significant decrease in osmolality by day 15. At 160 mg/kg, total protein was significantly affected on days 8 and 15, osmolality was significantly affected on days 10 and 15, and volume was significantly affected on day 15.

In rats receiving 40 mg of gentamicin per kg, significant differences were first observed in total protein and osmolality by days 8 and 10, respectively. Volume was not affected significantly until day 15. At 80 mg/kg, all three parameters were significantly affected by day 10, and at 160 mg/kg, osmolality was significantly decreased by day 8. The other two parameters (urine volume and mean body weight) at the high dose of gentamicin were increased to biologically, but not statistically, significant levels (three of the four survivors were moribund and died within a day after the 24-h urine collection on day 8).

When urine sediments were examined, granular casts were found in urines from all rats medicated with gentamicin, except for two ad-

TABLE 6. *Nephrotoxicity study in rats*

Anti-microbial agent	Dose mg/kg	No. of animals	Mean body wt ± SE[a] (g)				Urine osmolality ± SE (mosmol/kg of water)				Urine vol/24 h ± SE (ml)				Urine protein/24 h ± SE (mg of total protein)			
			Day 1[b]	Day 8	Day 10	Day 15	Day 1	Day 8	Day 10	Day 15	Day 1	Day 8	Day 10	Day 15	Day 1	Day 8	Day 10	Day 15
Control	0	5	196 ±4.4	220 ±3.6	228 ±3.7	241 ±4.6	2,150 ±117	2,194 ±234	2,235 ±66	2,217 ±90	6.4 ±0.2	5.5 ±1.4	7.0 ±0.5	8.0 ±0.8	37.3 ±3.1	28.6 ±4.3	38.1 ±4.0	40.0 ±3.8
Win 42122-2	40	5	195 ±4.0	215 ±4.9	220 ±4.9	232 ±3.5	2,197 ±167	2,282 ±67	2,274 ±48	2,174 ±97	6.4 ±0.5	6.0 ±0.4	7.5 ±0.7	7.4 ±0.8	37.7 ±1.7	35.2 ±1.7	40.5 ±2.9	36.9 ±2.3
	80	5	195 ±3.4	214 ±4.5	219 ±4.3	230 ±4.5	2,572 ±193	2,212 ±54	1,971 ±47	1,455 ±33[c]	4.6 ±0.8	5.8 ±0.7	8.1 ±0.7	11.0 ±0.7	28.2 ±4.0	33.8 ±3.0	37.8 ±2.7	42.1 ±2.0
	160	5	196 ±2.1	210 ±4.5	212 ±5.0	215 ±10.1	1,991 ±235	1,520 ±157	1,242 ±216[d]	1,021 ±133[c]	7.2 ±1.2	10.4 ±1.8	15.7 ±2.2	16.5 ±1.6[d]	36.9 ±3.7	55.9 ±5.7[d]	76.7 ±19.1	65.0 ±3.8[d]
Gentamicin	40	5	199 ±5.7	217 ±5.9	220 ±5.7	222 ±3.8	2,263 ±271	1,598 ±77	1,300 ±40[c]	981 ±58[c]	7.4 ±1.8	9.6 ±0.7	13.2 ±1.6	16.8 ±1.3[c]	39.4 ±4.5	47.7 ±1.7[d]	54.1 ±5.5	64.9 ±8.3
	80	5	199 ±4.6	211 ±5.7	207 ±3.2[c]	158 ±4.3[c]	2,273 ±87	1,271 ±134	744 ±93[c]	608 ±52[c]	6.4 ±0.7	11.1 ±1.1	18.8 ±1.7[d]	14.2 ±2.4	38.1 ±2.4	59.9 ±8.0	105.9 ±8.0[c]	45.3 ±7.4
	160	5[e]	195 ±2.9	176 ±5.9[d]	180[f]		2,094 ±89	458 ±40[d]	292[f]		6.0 ± 0.8	19.0 ±3.4	31.0[f]		33.7 ±2.7	86.0 ±16.2	68.2[f]	

[a] SE, Standard error.
[b] Premedication value.
[c] $P = 0.001$.
[d] $P = 0.01$.
[e] One rat died on day 7, three rats died after urine collection on day 8, and one rat died on day 14.
[f] One rat only.

ministered 40 mg/kg on day 8, and in all but one survivor on day 15. In contrast, no casts were seen at any dose level of Win 42122-2 on day 8; however, casts were observed in one of five rats in the low-dose group and in four of five rats at 80 and 160 mg/kg on day 15.

On day 15, the blood urea nitrogen levels for all doses of Win 42122-2 and 40 mg of gentamicin per kg were normal, except for one rat given 160 mg of Win 42122-2 per kg which had a reading of 224 mg/dl. Animals receiving gentamicin at 80 mg/kg had biologically significant levels ranging from 53 to 392 mg/dl (controls, 21 to 22 mg/dl). All rats receiving 160 mg of gentamicin per kg died. A similar pattern was seen with the blood creatinine levels.

Histopathological changes in the kidneys of rats given 40 mg of gentamicin per kg were similar to those seen in rats given 160 mg of Win 42122-2 per kg. These changes consisted of focal swelling and vacuolation of the epithelium of the proximal tubules, focal areas of interstitial inflammation, and dilation of tubules, many of which contained casts (colloid, granular, or cellular).

An overview of the urinalyses, microscopic characteristics of urine, blood chemistry, and histopathological changes indicates that 160 mg of Win 42122-2 per kg produced approximately the same changes as did 40 mg of gentamicin per kg.

(ii) Ototoxicity study in guinea pigs. Win 42122-2 was administered subcutaneously once a day for 77 days at 80 or 160 mg/kg to groups of six guinea pigs. Gentamicin at a dose of 80 mg/kg, kanamycin at a dose of 240 mg/kg, and saline were administered to three additional groups. Auditory loss was evaluated by using the Preyer reflex to pure tone frequencies. The guinea pigs were individually placed in an acoustical chamber and exposed to nine selected pure tone frequencies ranging from 2.5 to 20.0 kHz generated by an audio oscillator. During medication, the guinea pigs were tested twice weekly at approximately 18 h after medication. The initial sound intensity for each frequency was 90 ± 2 dB and was increased if necessary in 5-dB intervals until a positive Preyer reflex was observed. If no Preyer reflex was observed at the maximum intensity which could be produced for any given frequency (approximately 115 dB for frequencies in the 10- to 15-kHz range), a negative response was recorded.

The results of this study are shown in Table 7. All guinea pigs in the gentamicin and kanamycin groups lost or developed an extremely reduced Preyer reflex at all frequencies tested, the mean days of onset being 23 and 20 days, respectively. Response to high frequencies was lost first, followed by losses of response to the middle and low frequencies, and a complete loss of the reflex generally occurred over 4 to 11 days. In contrast, a loss of the reflex was observed in only one of the six animals given 80 mg of Win 42122-2 per kg and in none of six given 160 mg/kg. A reduction in Preyer reflex sensitivity occurred in one in the 80-mg/kg group and in two in the 160-mg/kg group, but this also occurred in two of six control guinea pigs. We have no explanation for the hearing impairment in the control guinea pigs, but none of the animals displayed clinical signs of ear infection and there was no evidence of gross infection at autopsy. Thus, Win 42122-2 given at 160 mg/kg was better tolerated than gentamicin at 80 mg/kg, and the projected mean day of onset occurred approximately three times later, i.e., day 23 for gentamicin and greater than day 69 for Win 42122-2. Although the total dose of drug required to induce auditory dysfunction may not be a reliable parameter for comparing toxicity, it is of interest to note that an average of only 1,840 mg of gentamicin was required to bring about hearing losses in all animals. At the 80-mg/kg dose of Win 42122-2, a total of 5,760 mg was administered, and at the 160-mg/kg dose, a total of 11,893 mg (6.5×) was given without resultant ototoxicity. Waitz et al. (13) have previously considered the total tolerated dose as an estimate of toxicity.

(iii) Ataxia study in cats. A comparative study in groups of five mongrel cats with 40 mg of gentamicin per kg and 40, 80, and 160 mg of Win 42122-2 per kg was conducted for a period of 73 days. The results are shown in Table 8. It can be seen that gentamicin at the 40-mg/kg dose impaired the righting reflex and induced ataxia in all five cats, with the average day of onset occurring on day 17, with the first cat showing evidence of ototoxicity on day 10 and the last cat showing these effects on day 22. At the highest dose level of Win 42122-2 used, 160 mg/kg, the results were similar to those obtained with gentamicin at the 40-mg/kg level; however, at 80 mg of Win 42122-2 per kg, the average day of onset of ataxia did not appear until day 31. At 40 mg of Win 42122-2 per kg, only one cat became ataxic on day 34. If one views the total dose required to induce ataxia, it can be seen that 680 mg of gentamicin given in 17 days was needed to cause ataxia in cats receiving 40 mg/kg, whereas cats receiving 80 mg of Win 42122-2 per kg withstood 2,480 mg in 31 days, or 3.65 times as much aminoglycoside. When the amount of drug needed to bring about death is considered, the data show that 1,000 mg of gen-

TABLE 7. *Incidence of Preyer reflex loss in guinea pigs*

Antimicrobial agent (dose [mg of base per kg])	Animal no.	Total no. of medications received	Day sacrificed	Day of onset of Preyer reflex loss	Total dose before onset of Preyer reflex loss (mg/kg)
Control	1	77	77	66[a]	
	2	77	77	N[b]	
	3	77	77	N	
	4	77	77	45[a]	
	6	77	77	N	
	7	77	77	52[c]	
Win 42122-2 (80.0)	14	77	77	N	6,160
	8	13	13	N (sacrificed)	
	9	77	77	N	6,160
	10	77	77	N	6,160
	11	77	77	59[d]	4,720
	12	77	77	70[c]	5,600
Mean					>5,760 (3.1×)
Win 42122-2 (160.0)	15	77	77	75[a]	12,000
	18	77	77	N	12,320
	19	77	77	N	12,320
	20	77	77	63[c]	10,080
	21	77	77	N	12,320
	23	77	77	N	12,320
Mean					>11,893 (6.5×)
Gentamicin (80.0)	24	31	56	24[d]	1,920
	26	45	56	31[d]	2,480
	27	21	56	21[d]	1,680
	28	26	56	17[d]	1,360
	30	31	56	21[d]	1,680
	31	31	56	24[d]	1,920
Mean		31		23	1,840 (1.0×)
Kanamycin (240.0)	35	18	37	14[d]	
	36	21	37	17[d]	
	37	21	37	17[d]	
	38	29	37	21[d]	
	39	29	37	24[d]	
	40	31	37	24[d]	
Mean		25		20	

[a] Slight reduction of auditory sensitivity beginning near the end of the study.
[b] N = Preyer reflex remained normal throughout the study.
[c] Moderate reduction of auditory sensitivity beginning near the end of the study.
[d] Extreme or complete loss of Preyer reflex at all frequencies.

tamicin was administered during 25 days. The corresponding figure for Win 42122-2 at the 80-mg/kg dose was 6,560 mg, or 6.5 times more.

The mortality data in the cats also support the trend seen when ataxia was used as the index parameter. At 40 mg of gentamicin per kg, all five cats died, with the average day of death at day 25. At the 80-mg/kg dose level of Win 42122-2, i.e., twice the dose of gentamicin, only two cats died, and the average day of death was on day 62. These data show that gentamicin is substantially more toxic than Win 42122-2. Cats tolerated twice as much Win 42122-2 for over twice as long (25 versus 62 days), which suggests that gentamicin may be ≥4 times more toxic than Win 42122-2.

DISCUSSION

Three important indices were studied to compare important characteristics of aminoglycosides: antimicrobial activities, nephrotoxicity, and ototoxicity. The in vitro comparison of Win 42122-2, gentamicin, and amikacin showed that gentamicin was the most active of the three antibiotics against gentamicin-susceptible bac-

TABLE 8. *Ataxia study in cats*

Antimicrobial agent (dose [mg/kg])	Ataxia and righting reflex impairment			Mortality		Blood chemistry			
	No. affected/ total	Avg day of onset	1st day in 1st cat–1st day in last cat	Deaths/ total	Avg day of death	Blood urea nitrogen (mean mg/dl)		Creatinine (mean mg/dl)	
						Day 16	Day 73[a]	Day 16	Day 73[a]
Control	0/5			0/5		23	27	1.7	1.7
Gentamicin (40.0)	5/5	17 (680)[b]	10–22	5/5	25 (1,000)[c]	88	All dead	5.7	All dead
Win 42122-2									
40.0	1/5		34 (ataxia only)	0/5		24	30	1.4	1.5
80.0	5/5	31 (2,480)[b]	28–45	2/5	62 (6,560)[c]	23	53[d]	1.4	4.9[d]
160.0[e]	5/5	11	7–20	3/5	29	22	35	1.5	1.7

[a] The day 73 value for 80.0 mg of Win 42122-2 per kg includes one cat bled and sacrificed moribund on day 72.

[b] Total dose (milligrams per kilogram) at average day of onset of ataxia and righting reflex impairment.

[c] Total dose (milligrams per kilogram) at average day of death.

[d] Three of four were normal for blood urea nitrogen and creatinine; one has a good urea nitrogen level of 126 mg/dl and a creatinine level of 14.8 mg/dl.

[e] In this 73-day study, the medication was suspended from day 23 to 35 at 160-mg/kg dose.

teria. Amikacin generally was the least active against the commonly encountered gram-negative gentamicin-susceptible strains, except for *Klebsiella* and *Pseudomonas*. In vitro determinations with gentamicin-resistant strains indicated that amikacin was superior to both gentamicin and Win 42122-2 and would support the rationale for the initial use of amikacin against most resistant strains.

Gentamicin-susceptible isolates representing six genera were tested in mouse protection studies in this comparative study and generally showed that the susceptibility patterns observed in vitro were also reflected in vivo. Collectively, the in vitro and in vivo studies revealed that Win 42122-2 had a spectrum similar to that of gentamicin; however, the in vivo activity of gentamicin ranged between 1.4 and 4.1 times more active than Win 42122-2, depending upon the species. A greater number of organisms will need to be evaluated to ascertain the relative potency with greater accuracy.

The relative toxicity of aminoglycosides in animals has been used as an important consideration for the selection of agents for clinical studies. In this investigation we used published methods for determining aminoglycoside-induced ototoxicity and nephrotoxicity in guinea pigs, cats, and rats. The results of these tests reveal that Win 42122-2 is substantially better tolerated than gentamicin, although more studies will be required before the magnitude of the improvement can be determined precisely. Although it has not been possible to quantitate with great accuracy how much less toxic Win 42122-2 is than gentamicin, the data can be interpreted to indicate that Win 42122-2 may be four- to sixfold better tolerated than gentamicin. This interpretation relies on the parameters of dose level, time required to observe the onset of the dysfunction, and the total dose required to induce the toxic effect. Similar calculations and parameters were employed by other workers to evaluate the chronic toxicity of gentamicin (13).

In preliminary rat nephrotoxicity studies not reported here, Win 42122-2 was shown to be better tolerated than netilmicin. Others have reported that netilmicin is less toxic than gentamicin (8). However, animal toxicity studies may not be completely predictable for aminoglycoside toxicity in humans; for example, clinical trials with netilmicin at 6 mg/kg per day have revealed that the incidence of nephrotoxicity approximates that observed with gentamicin at 3 mg/kg per day (12) although animal studies had suggested that a greater separation in the toxic doses of gentamicin and netilmicin might have been expected (9). Cox (5) reported comparable tolerance in humans with 4 mg of netilmicin per kg per day or 3 mg of gentamicin per kg per day. Thus, clinical studies with newer aminoglycosides that display improved animal toxicity will be required before an assessment of the predictability of the animal studies can be realized. In the meantime, the present study shows that the improved therapeutic ratio (antibacterial potency to toxicity) of Win 42122-2 compared with gentamicin for many important

pathogens warrants continued interest and study.

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LITERATURE CITED

1. **Akiyoshi, M.** 1978. Evaluation of ototoxicity of tobramycin in guinea pigs. J. Antimicrob. Chemother. **4**(Suppl. A):69–72.
2. **Benveniste, R., and J. Davies.** 1973. Mechanisms of antibiotic resistance of bacteria. Annu. Rev. Biochem. **42**:471–506.
3. **Briedis, D. J., and H. G. Robson.** 1976. Comparative activity of netilmicin, gentamicin, amikacin, and tobramycin against *Pseudomonas aeruginosa* and *Enterobacteriacae*. Antimicrob. Agents Chemother. **10**:592–597.
4. **Brummett, R. E., K. E. Fox, T. W. Bendrick, and D. L. Himes.** 1978. Ototoxicity of tobramycin, gentamicin, amikacin, and sisomicin in the guinea pig. J. Antimicrob. Chemother. **4**(Suppl. A):73–78.
5. **Cox, C. E.** 1979. Comparison of netilmicin and gentamicin in the treatment of urinary tract infections. Curr. Ther. Res. Clin. Exp. **25**:603–608.
6. **Finney, D. J.** 1964. Probit analysis. Cambridge University Press, New York.
7. **Gilbert, D. N., C. Plamp, P. Starr, W. M. Bennett, D. C. Houghton, and G. Porter.** 1979. Comparative nephrotoxicity of gentamicin and tobramycin in rats. Antimicrob. Agents Chemother. **13**:34–40.
8. **Luft, F. C., M. N. Yum, and S. A. Kleit.** 1976. Comparative nephrotoxicities of netilmicin and gentamicin in rats. Antimicrob. Agents Chemother. **10**:845–849.
9. **Miller, G. H., G. Arcieri, M. J. Weinstein, and J. A. Waitz.** 1976. Biological activity of netilmicin, a broad-spectrum semisynthetic aminoglycoside antibiotic. Antimicrob. Agents Chemother. **10**:827–836.
10. **Price, K. E., T. A. Pursiano, M. D. DeFuria, and G. E. Wright.** 1974. Activity of BB-K8 (amikacin) against clinical isolates resistant to one or more aminoglycoside antibiotics. Antimicrob. Agents Chemother. **5**:143–152.
11. **Rosi, D., W. A. Goss, and S. J. Daum.** 1977. Mutational biosynthesis by idiotrophs of *Micromonospora purpurea*. 1. Conversion of aminocyclitols to new aminoglycoside antibiotics. J. Antibiot. **30**:88–97.
12. **Snydman, D. R., F. P. Tally, S. H. Landesman, M. Barza, and S. L. Gorbach.** 1979. Netilmicin in gram-negative bacterial infections. Antimicrob. Agents Chemother. **15**:50–54.
13. **Waitz, A. J., E. J. Moss, Jr., and M. J. Weinstein.** 1971. Aspects of chronic toxicity of gentamicin sulfate in cats. J. Infect. Dis. **124**(Suppl.):S125–S129.