Chemotherapeutic Evaluation of 5-Episisomicin (Sch 22591), a New Semisynthetic Aminoglycoside

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5-episisomicin (Sch 22591) is a novel semisynthetic aminoglycoside with a spectrum and potency similar to gentamicin in its activity against susceptible bacterial strains, but with increased potency against *Pseudomonas*, *Providencia*, and *Proteus rettgeri*. It is also more active than tobramycin and amikacin against these last-mentioned species.

Against resistant strains, Sch 22591 is significantly more active than gentamicin or tobramycin. Against resistant gram-negative bacteria other than *Pseudomonas*, Sch 22591 has activity similar to that of amikacin, but Sch 22591 is more potent. Against *Pseudomonas* strains, it is active against most gentamicin- and tobramycin-resistant strains and is more active than the other three antibiotics. Some *Pseudomonas* strains are resistant to Sch 22591, but susceptible to amikacin. Against a selection of aminoglycoside-resistant staphylococci, Sch 22591 has very good activity against strains resistant to tobramycin, amikacin, and gentamicin. The superior in vitro potency of Sch 22591 against *Pseudomonas* has been confirmed in vivo in experimental infections in mice. Absorption in dogs is similar to that of other aminoglycoside antibiotics. The acute toxicity of Sch 22591 in mice is greater than that of gentamicin; its vestibular toxicity potential and nephrotoxicity potential in cats and rats appear to be similar to those of gentamicin.

In an extensive program of chemical and microbiological modifications of aminocyclitol-aminoglycoside antibiotics conducted in our laboratories, a large number of novel antibacterials have been prepared. These include a number of new antibiotics related to sisomicin prepared by mutational biosynthesis, as well as netilmicin, which was described earlier (4, 7). The purpose of the present report is to describe a new semisynthetic compound, Sch 22591, which was one of a series of aminoglycoside-antibiotic derivatives modified at the 5-position of the deoxystreptamine ring.

Sch 22591 is the 5-epimer of sisomicin. Similar derivatives have also been prepared from other antibiotic starting materials (D. F. Rane and P. J. L. Daniels, in preparation). Sch 22591 has also been isolated by mutational biosynthesis using 5-epi-2-deoxystreptamine as a precursor by the method described by Testa and co-workers (5).

MATERIALS AND METHODS

Sch 22591, gentamicin, sisomicin, and tobramycin (Eli Lilly & Co., Indianapolis, Ind.) were used as sulfate salts; results are reported in terms of base content from bioassay results. Amikacin (Bristol

Laboratories, Syracuse, N.Y.) was used as the base.

In vitro agar dilution tests were performed in the conventional manner by using 30 ml of Mueller-Hinton agar (pH 7.2 to 7.4) per plate. Plates were inoculated with a Steers-Foltz replicating device adjusted to deliver approximately 10^3 to 10^4 viable units of each organism to the agar surface. The minimal inhibitory concentration (MIC) values were determined after 24 and 48 h of incubation. The MIC was defined as the lowest concentration allowing growth of less than six isolated discrete colonies.

In vitro broth dilution tests were performed in the conventional manner by using 3 to 5 ml of Mueller-Hinton broth (pH 7.4). The inoculum was approximately 5×10^4 to 1×10^5 cells, obtained by dilution of overnight cultures. In one experiment, the pH of the Mueller-Hinton broth was varied by adding 1 N H_2SO_4 to achieve the desired initial pH value of 6.6. In experiments designed to study the effect of serum on antibacterial activity, sterile horse serum (Grand Island Biological Co., Grand Island, N.Y.) was added directly to Mueller-Hinton broth to achieve the desired concentrations. Similarly, in experiments designed to study the effect of calcium and magnesium on antibacterial activity, sterile filtered solutions of $MgSO_4$ and $CaCl_2 \cdot 2H_2O$ were added directly to the broth. Sufficient quantities of the solutions were added so that 58.9 mg of calcium per liter and 12.2 mg of magnesium per

liter were added to the Mueller-Hinton broth. Thus, the final broth calcium and magnesium concentrations were similar to physiological concentrations of calcium and magnesium.

Mouse protection tests were performed in male Carworth CF1 mice weighing 18 to 20 g each. Infection was with either 10^7 organisms per mouse or sufficient inocula to cause death of control groups within 18 h after infection. One hour after intraperitoneal infection, groups of 10 mice were given a single subcutaneous dose of antibiotic. Seven groups receiving doses ranging from 5.0 to 0.05 mg/kg of body weight were used. Mean protective dose (PD_{50}) values were estimated by probit-type procedures based on the number of survivors at 48 h after infection.

The acute toxicity determinations in mice were carried out with groups of 10 CF1 mice weighing 18 to 20 g each. The antibiotic was administered as an aqueous solution in a dosage equivalent to either 10 or 100 mg/kg. The dose required to kill 50% of the mice (LD_{50}) was estimated on the basis of the number of survivors at 18 and 48 h after injection.

The drug was also administered in multiple doses to male cats, and the length of time necessary to produce ataxia was measured as described previously (6). Groups of five cats were given daily doses seven days per week, subcutaneously, in a dosage equivalent to 40 mg of either Sch 22591 or gentamicin per kg of body weight and were observed daily for impairment of righting reflex and gait. Cats were bled 24 h after dose 9 and at the time of sacrifice for measurement of the concentrations of Sch 22591 or gentamicin in the serum.

For estimation of effects on renal function, groups of six Sprague-Dawley rats (150 to 200 g each, initially) were given a daily intramuscular (i.m.) dose of either 80 or 160 mg/kg. Twenty-four hours after the last dose, the rats were prepared for estimation of renal function. The animals were

anesthetized with 100 mg of Inactin per kg, intraperitoneally. The left jugular vein was cannulated for blood sampling, and the right femoral vein was cannulated for fluid infusion. Blood pressure was recorded from the right carotid artery. After urine samples were taken by puncture for urinalysis, the urinary bladder was catheterized for continuous urine collection. Inulin clearance was used to measure the glomerular filtration rate (GFR). The individual experiments consisted of three consecutive 30-min clearance periods. The detailed method used in this study was recently reported (2). Student's *t* test was used for statistical comparisons between each drug-treated group and the control group.

The drug concentrations in serum, wound fluid, and urine from a male beagle dog were determined after an i.m. dose of ca. 10 mg/kg. Wound fluid was recovered from stainless steel mesh cages surgically implanted under the skin of the dog (1, 3).

RESULTS

A number of studies were designed to evaluate the activity of Sch 22591 against susceptible microbial strains, as well as against strains resistant to aminoglycosides. These studies included: (i) direct comparative tests of Sch 22591 with gentamicin against a collection of susceptible strains; (ii) comparison of Sch 22591 with gentamicin, tobramycin, and amikacin against strains with known resistance mechanisms; and (iii) comparisons of Sch 22591 with gentamicin, tobramycin, and amikacin against recent resistant clinical isolates received in our laboratory over the past 2 years. Results of tests in comparison to gentamicin against a collection of aminoglycoside-susceptible strains are shown in Table 1. From these data, it can

TABLE 1. Comparative *in vitro* activity of Sch 22591 and gentamicin against aminoglycoside-susceptible strains

Organism	Median MIC (range) ^a (μg/ml)		
	N	Sch 22591	Gentamicin
<i>Bacillus subtilis</i>	1	<0.03	<0.03
<i>Staphylococcus aureus</i>	15	0.125 (0.03-0.5)	0.125 (0.03-0.5)
<i>Enterococcus</i>	9	4.0 (2.0-8.0)	8.0 (2.0-16.0)
<i>Acinetobacter calcoaceticus</i>	1	2.0	8.0
<i>Citrobacter</i>	2	1.0 (0.5-1.0)	1.0
<i>Enterobacter</i>	10	0.25 (0.06-1.0)	0.5 (0.25-1.0)
<i>Escherichia coli</i>	43	0.5 (0.125-1.0)	0.5 (0.125-1.0)
<i>Klebsiella pneumoniae</i>	23	0.25 (0.125-1.0)	0.25 (0.125-1.0)
<i>Proteus mirabilis</i>	16	1.0 (0.5-2.0)	1.0 (0.5-2.0)
<i>P. morganii</i>	6	0.5 (0.06-1.0)	1.0 (0.25-8.0)
<i>P. rettgeri</i>	15	0.5 (0.06-1.0)	2.0 (0.25-4.0)
<i>P. vulgaris</i>	3	1.0 (0.25-2.0)	2.0 (1.0-4.0)
<i>Providencia</i>	14	0.5 (0.25-1.0)	4.0 (2.0-8.0)
<i>Pseudomonas aeruginosa</i>	33	0.25 (0.06-1.0)	1.0 (0.125-4.0)
<i>Salmonella</i>	1	1.0	0.5
<i>Serratia</i>	21	0.5 (0.25-2.0)	1.0 (0.5-4.0)

^a MIC values in Mueller-Hinton agar.

TABLE 2. Comparative in vitro activity of Sch 22591, gentamicin, tobramycin, and amikacin against strains with known resistance mechanisms

Organism	Resistance mechanism	MIC ^a (µg/ml)			
		Sch 22591	Gentamicin	Tobramycin	Amikacin
<i>Citrobacter</i> 0412601	AAC (3)-I	0.5	32	1	2
<i>Escherichia coli</i> St. Michael 589	APH (3')-I	2	4	32	4
Baker 2	APH (3')-I	1	1	8	8
JR67	APH (3')-II	2	2	4	8
R55/W677	ANT (2')	1	64	64	2
JR66	ANT (2')	1	32	64	8
R55/LA290	ANT (2')	0.5	16	16	1
JR88	AAC (3)-I	0.5	32	2	1
JR90	AAC (3)-I	1	64	2	8
R5/W677	AAC (6')	16	4	>64	>64
HL97/W677	AAC (6')	32	2	64	64
Swidinsky 4195	Unknown	2	8	32	16
1205503	Permeability (?)	8	16	32	64
<i>Klebsiella pneumoniae</i> AD 17	APH (3')-II	0.5	0.5	0.5	4
AD 18	APH (3')-II	0.25	0.25	0.25	1
Georgetown 3694	ANT (2')	0.5	32	32	2
Georgetown 3020	ANT (2')	0.25	16	16	1
Oklahoma 6	ANT (2')	1	64	>64	1
<i>Proteus morganii</i> 0916515	ANT (2')	1	64	32	4
<i>Proteus rettgeri</i> Membel	Unknown	1	16	16	8
Anderson	AAC (3)-II	1	>64	64	8
0128608	AAC (3)-II	0.25	64	32	2
<i>Providencia</i> 164	AAC (2')	1	64	32	16
Palo Alto 2	AAC (2')	2	32	32	16
<i>Pseudomonas aeruginosa</i> D-2	APH (3')	1	2	1	4
Powe	ANT (2')	0.125	32	16	1
Stone 130	AAC (3)-I	0.125	16	0.125	1
Stone 138	AAC (3)-I	0.125	16	0.125	1
Capetown 18	AAC (3)-I	0.125	8	0.125	1
Travers 1	AAC (3)-II	64	>64	64	1
Shreveport 3796	AAC (6')-II	64	>64	32	2
GN 315	AAC (6')	16	0.5	16	16
Shriners 10099	Unknown	1	2	0.5	4
Shriners 10006	Unknown	2	8	1	16
0828520	Permeability (?)	4	8	8	64
1219501	Permeability (?)	4	16	16	32
<i>Serratia</i> sp. Dalton	APH (3')-I	0.5	0.5	1	2
0323601	ANT (2')	1.0	>64	>64	8
0323603	AAC (6')	8	2	64	16
0323605	AAC (3)-II	16	>64	>64	16
<i>Staphylococcus aureus</i> 5	ANT (4')	1	1	>64	32
7	ANT (4')	0.5	0.5	>64	32
9	ANT (4')	1	1	>64	>64
12	ANT (2')	1	32	16	2
14	APH (3')-II				
	ANT (2')	4	>64	16	4
	APH (3')-III				
16	AAC (6') + APH (2')	8	64	32	8

^a MIC values on Mueller-Hinton broth.

be seen that Sch 22591 is a highly potent antibiotic. Its activity against *Escherichia coli*, *Enterobacter*, *Klebsiella*, and *Serratia* are equal to or twofold greater than that of gentamicin. Against *Providencia*, the activity of Sch 22591 was four- to eightfold greater than that of gentamicin. The activity of Sch 22591 against indole-positive *Proteus* was 2- to 16-fold greater than that of gentamicin, whereas its activity against *Proteus mirabilis* seemed to be equal to that of gentamicin. Against *Pseudomonas aeruginosa*, Sch 22591 was fourfold more potent than gentamicin.

Results of comparative in vitro tests performed in Mueller Hinton broth with Sch 22591 and gentamicin, tobramycin, and amikacin are shown in Table 2. It can be seen from these studies that Sch 22591 was active against a large number of strains that were resistant to gentamicin or tobramycin. Sch 22591 was active against resistant strains which possessed

the following aminoglycoside-inactivating enzymes. (i) Phosphorylating strains [APH (3')-I and II], which were resistant to neomycin and kanamycin; (ii) adenylylating strains [ANT (2'')], which were resistant to gentamicin and tobramycin and sensitive to amikacin; (iii) adenylylating strains [ANT (4')], which were resistant to amikacin and tobramycin but susceptible to gentamicin; (iv) many acetylating strains [AAC (2')] in *Providencia*, which were resistant to gentamicin and tobramycin but susceptible to amikacin; and (v) acetylating strains [AAC (3)-I], which were resistant to gentamicin and susceptible to tobramycin and amikacin.

Sch 22591 was not active against resistant strains that possessed the following aminoglycoside-inactivating enzymes: (i) acetylating strains [AAC (3)-II], which were resistant to gentamicin and tobramycin, but susceptible to amikacin; and (ii) acetylating strains [AAC (6'')], which were resistant to tobramycin and

FIG. 1. Comparative activity of Sch 22591, gentamicin, tobramycin, and amikacin against recent clinical isolates of gram-negative bacteria selected for aminoglycoside resistance. MIC values were determined in Mueller-Hinton agar.

amikacin, but susceptible to gentamicin. Sch 22591 was also not highly active against strains that are resistant to all of the aminoglycosides and that apparently owe their resistance to a decreased permeability to aminoglycosides. However, Sch 22591 is among the most active compounds against this type of resistant strain and might be clinically useful if sufficiently high drug concentrations could be attained in vivo.

We also tested Sch 22591 in direct comparison with amikacin, tobramycin, and gentamicin against a selection of approximately 200 recent clinical isolates of gram-negative bacteria by using agar dilution techniques. These were strains submitted to us as being aminoglycoside resistant. The results with these organisms are shown in Fig. 1. Against non-*Pseudomonas* gram-negative organisms, Sch 22591 was more potent than either gentamicin, tobramycin, or amikacin and was active against the majority of gentamicin- and tobramycin-resistant strains. Its spectrum appeared to be similar to that of amikacin, except that it was several times more potent. With *Pseudomonas* strains, Sch 22591 was active against many gentamicin- and tobramycin-resistant strains. It was also substantially more potent than any of the other three antibiotics. A few strains were resistant to Sch 22591 and partially susceptible to amikacin.

From the results of the above studies, it appears that Sch 22591 has not only good activity against many resistant strains, but is also an extremely potent aminoglycoside. We therefore carried out several experiments designed to verify this potency against a variety of aminoglycoside-susceptible bacteria. These in vitro studies included a comparison of the activities of Sch 22591, gentamicin, sisomicin,

tobramycin, and amikacin against a group of 36 aminoglycoside-susceptible *P. aeruginosa* strains under a variety of growth conditions.

Tests were performed in Mueller-Hinton agar, with results as shown in Table 3. The median MIC values, as well as the range, are shown. Use of Mueller-Hinton broth instead of agar resulted in a fourfold decrease in the MIC values of the four antibiotics. Also the reduction of pH from 7.2 to 6.6 resulted in an increase in the MIC values of the four antibiotics. Addition of horse serum to Mueller-Hinton broth had little effect. Finally, adulteration of the media by addition of calcium and magnesium raised the MIC values of all four antibiotics.

To determine whether the increased in vitro activity against *Pseudomonas* could be demonstrated in vivo, a group of 12 *Pseudomonas* strains were used to produce experimental infections in mice. In each of these studies, the four antibiotics were compared in parallel, using a single subcutaneous dose administered 1 h after infection. The same inoculum and large groups of mice were used to permit fairly accurate determinations of PD₅₀ values. The PD₅₀ values varied substantially from strain to strain (Table 4); however, Sch 22591 was the most potent against these strains, as was suggested from the in vitro tests. The median PD₅₀ for Sch 22591 was about one-half that of tobramycin and one-sixth that of gentamicin.

The acute toxicity of Sch 22591 was evaluated in mice by a variety of routes of administration (Table 5). The observed LD₅₀ values were similar to those of sisomicin, although our data suggest that Sch 22591 may be 5 or 10% more toxic. The nature and time of death of the mice observed in these experiments was a typical effect of aminoglycosides and, presumably, the cause of death was neuromuscular blockade.

TABLE 3. Comparison of the in vitro activities of Sch 22591, gentamicin, sisomicin, tobramycin, and amikacin against 36 aminoglycoside-susceptible *Pseudomonas* in a variety of media

Media	Median MIC (range) (μ g/ml, 24 h)				
	Sch 22591	Gentamicin	Sisomicin	Tobramycin	Amikacin
Mueller-Hinton agar	0.5(0.125-4)	2(0.125-4)	1(0.125-4)	0.5(0.06-8)	4(0.25-16)
Mueller-Hinton broth					
pH 6.6	0.5(0.125-4)	1(0.25-8)	0.5(0.25-4)	0.5(0.25-4)	4(1-32)
pH 7.2	0.125(<0.03-1)	0.5(0.125-2)	0.25(0.06-2)	0.125(<0.03-1)	1(<0.03-4)
Mueller-Hinton broth plus					
1% Horse serum	0.125(<0.03-5)	0.5(0.125-2)	0.5(0.125-2)	0.25(0.03-1)	1(0.03-4)
5% Horse serum	0.25(<0.03-1)	1(0.25-4)	0.5(0.125-1)	0.25(0.03-1)	2(0.125-8)
10% Horse serum	0.25(0.03-1)	1(0.25-4)	0.5(0.125-2)	0.5(0.125-2)	1(0.03-8)
Calcium and magnesium	2(0.03-16)	8(0.5-64)	4(0.5-32)	2(0.03-8)	16(0.125-64)

TABLE 4. Comparison of the *in vivo* activities of Sch 22591, gentamicin, sisomicin, tobramycin, and amikacin against aminoglycoside-susceptible *Pseudomonas*

Strain	Mouse protection tests, PD ₅₀ (mg/kg)				
	Sch 22591	Gentamicin	Sisomicin	Tobramycin	Amikacin
St. Michael 762	0.4	1.5	0.3	0.2	<0.05
St. Michael 1395	0.3	2	1.5	1	0.4
Delaware 6	2	2.5	3	3	35
NRRL 3223	2	4	1.5	1	2.5
Stone 39	<0.05	1.5	<0.05	0.25	0.05
Stone 20	<0.05	1.0	0.5	0.05	1
Pittsburgh 8	0.5	15	2.5	4	15
Cohen Cole	2	3	4	4	>25
Cohen Shore	5	0.5	1	4	25
Cohen Robi, M.	0.3	5	2	0.3	4
Kaiser 1	5	20	5	10	6
Kaiser 2	1.5	10	5	15	25
Median	0.5	3	2	1	4
Range	(<0.05–5)	(0.5–20)	(<0.05–5)	(0.15–15)	(<0.05–>25)

TABLE 5. Comparison of the acute toxicities of Sch 22591, gentamicin, tobramycin, and amikacin in mice

Route of administration	LD ₅₀ (mg/kg)				
	Sch 22591	Gentamicin	Sisomicin	Tobramycin	Amikacin
Intravenous	45	65	50	110	400
Intraperitoneal	175	350	200	625	750
Subcutaneous	210	475	275	700	>1000

TABLE 6. Comparison of the chronic toxicities of Sch 22591 and gentamicin after daily subcutaneous doses of 40 mg/kg

Cat no.	Days to become ataxic	Drug concn in serum (μg/ml) 24 h after dose 9		Drug concn in serum (μg/ml) 24 h after last dose	
		Sch 22591	Gentamicin	Sch 22591	Gentamicin
Sch 22591					
8685	15	1.3		6.8	
09630	10	63.0		63.0	
09590	15	0.5		7.1	
8695	21	1.5		24.1	
6153	15	1.0		5.4	
Avg	15.2 ± 3.9				
Gentamicin					
8369	15		3.2		29.2
09634	15		0.6		4.4
6327	18		0.7		57
81848	14		11.0		28
09629	18		2.7		22
Avg	16.0 ± 1.9				

Table 6 gives the results of a single test of ataxia in cats, comparing Sch 22591 and gentamicin. At a dose of 40 mg/kg, subcutaneously, it took an average of 15 days for five cats to become ataxic when given doses of Sch 22591,

whereas with gentamicin under the same conditions, it took an average of 16 days. Thus, the chronic vestibular toxicity of Sch 22591 would seem to be similar to that of gentamicin. Measurement of drug concentration in the se-

rum 24 h after dose 9 and after the last dose suggested that Sch 22591 and gentamicin might be similarly nephrotoxic.

To study the effects of Sch 22591 and gentamicin on renal function, groups of six rats were given doses of either 80 or 160 mg/kg i.m. for 7 days. The results of these tests are shown mainly in terms of GFR (Fig. 2). At a dosage of 80 mg/kg, Sch 22591 appeared to cause a greater reduction in GFR than did gentamicin.

FIG. 2. Effect of aminoglycosides on GFR in rats. Each point represents the value from each individual animal; the horizontal bar represents the group mean. The duration of treatment (i.m.) for all groups was 7 days.

FIG. 3. Serum and cage fluid levels of Sch 22591 after a single 10-mg/kg (i.m.) dose to a male beagle dog.

In both cases, moderate elevation of fractional excretion of K^+ (FE_K) occurred (data not shown). Both drugs given at 160 mg/kg for 7 days produced severe kidney damage, as indicated by a GFR that was reduced to about 20% of normal values. Concurrent changes were glucosuria, aciduria, and markedly increased FE_K . The data indicate that the nephrotoxic potency of Sch 22591 is comparable to that of gentamicin in rats.

The results of an experiment in which Sch 22591 was given to a dog that had a stainless steel mesh cage surgically implanted under the skin are given in Fig. 3. Peak serum and cage fluid levels were similar to those observed with other aminoglycosides. Similarly, the absorption rate and serum half-life were similar to those seen with other aminoglycosides.

DISCUSSION

The in vitro potency and spectrum of Sch 22591 has been compared to other aminoglycosides in a variety of test systems. Sch 22591 appeared to be equal to or more potent than gentamicin against most gram-negative strains. It appeared to be equal to or slightly more potent than tobramycin against *Pseudomonas*. Sch 22591 was also active against many of the gentamicin- and tobramycin-resistant strains tested and appeared to have a spectrum of activity against aminoglycoside-resistant strains different from other previously described aminoglycosides. Although amikacin was active against some strains resistant to Sch 22591, the latter appeared to have good activity against some amikacin-resistant strains, including several *Providencia*, *Staphylococcus*, and *P. aeruginosa*. The high in vitro activity of Sch 22591 was confirmed in in vivo tests against *Pseudomonas* infections in mice. Although the acute toxicity of Sch 22591 in mice was greater than that of gentamicin, the effect on renal function after giving multiple doses of Sch 22591 to rats was about equal to that of gentamicin, as was its effect on vestibular function in cats. Thus, Sch 22591 appears to be an antibiotic with high potency and an unusual broad spectrum of activity, and it appears to have a toxic liability similar to that of gentamicin.

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