ANTIBIOTIC 6640.* III

BIOLOGICAL STUDIES WITH ANTIBIOTIC 6640, A NEW BROAD-SPECTRUM AMINOGLYCOSIDE ANTIBIOTIC

J. ALLAN WAITZ, EUGENE L. MOSS, Jr., EDWIN M. ODEN and MARVIN J. WEINSTEIN

Microbiology Department, Schering Corporation, Bloomfield, New Jersey, 07003, U.S.A.

(Received for publication October 3, 1970)

Antibiotic 6640 has an *in vitro* spectrum similar to gentamicin with a potency equal to or twice that of gentamicin, particularly against *Pseudomonas*. Antibiotic 6640 is more active at an alkaline pH but is little affected by the presence of serum. MBC values are near MIC values. Antibiotic 6640 has up to 5 times the activity of gentamicin in therapeutic and prophylactic antibacterial tests in mice. Serum levels of the two antibiotics are similar in mice, rats and dogs. Results of comparative ataxia tests in cats are reported.

Antibiotic 6640 is a new aminoglycoside antibiotic, produced by fermentation of a new species of the genus *Micromonospora*, *Micromonospora inyoensis*, first described by WEINSTEIN *et al.*[1] These authors presented initial data concerning the biological activity of antibiotic 6640 and showed it to have broad-spectrum antibacterial activity *in vitro* and *in vivo*. WAGMAN *et al.*[2] described procedures for isolation and purification of antibiotic 6640 and this report presents results of additional and more detailed biological studies with the antibiotic.

Materials and Methods

Antibiotic 6640 and gentamicin, which was employed as a reference material, were both used in the form of the sulfate, several lots of which assayed between 600~640 mcg/mg in terms of the base. All doses and tests results are expressed in terms of the base. *In vitro* and *in vivo* test procedures were similar to those described earlier (WAITZ *et al.*[3], WAITZ and WEINSTEIN[4]). The mice used were albino males of the CF-1 strain weighing approximately 20 g each; rats were albino CF-E males weighing 200 g; dogs were adult beagle-type mongrels weighing approximately 10 kg each; cats were mongrel males weighing 2.6~5.0 kg each. For therapeutic test in mice, groups of 7 animals each at 5 dose levels in addition to 10 controls were used. Mice were infected intraperitoneally with approximately 10^7 organisms per mouse. Untreated infected controls died in 18~24 hours with the exception of *Klebsiella*-infected mice which died 36~40 hours after infection. All treated mice were dosed subcutaneously with a volume of 0.1~0.2 ml. Two dose regimens were employed consisting of a single dose one hour after infection and another in which the total dose was divided into two and given 1/2 hour before and 4 hours after infection. Surviving mice were counted 48 hours after infection and PD_{50}

* Antibiotic 6640 was formerly known as rickamicin and has now been named sisomicin.

values were calculated by probit procedures. Serum and urine levels of antibiotic were determined according to the bioassay procedure described by WEINSTEIN *et al.*[1] Ataxia studies were done in cats in groups of 5 each dosed subcutaneously 7 days a week until the cats either died, or were sacrificed in a moribund condition, or at the end of the experiment (70 days). The animals were weighed daily and also examined daily for ataxia and righting reflex impairment. In comparative studies antibiotic 6640 and gentamicin were always run in parallel.

Results

In vitro Studies

Antibiotic 6640 has been shown to have a broad-spectrum of antibacterial activity with a potency *in vitro* similar to that of gentamicin. Further studies with an increased number of recent clinical isolates of *Pseudomonas aeruginosa* in tryptose phosphate broth are shown in Table 1. These data are expressed in terms of the number of the strains sensitive to various concentrations of either gentamicin or antibiotic 6640. Antibiotic 6640 shows some increased activity against *Pseudomonas*.

Table 1. *In vitro* activity of antibiotic 6640 and gentamicin against 27 clinical isolates of *Pseudomonas aeruginosa*

Antibiotic	Incubation time	No. strains sensitive (mcg/ml)					Total No. strains
		0.03	0.08	0.3	0.8	3.0	
Antibiotic 6640	24 hrs.	5	25	27			27
	48 hrs.	0	6	20	27		27
Gentamicin	24 hrs.	0	1	16	26	27	27
	48 hrs.	0	0	0	18	27	27

Tested in tryptose phosphate broth in a volume of 3 ml with an inoculum of 0.05 ml of a 1 : 1,000 dilution of an 18-hour broth culture.

Table. 2. Effect of serum on the *in vitro* activity of antibiotic 6640 and gentamicin

	Organism	Gentamicin				Antibiotic 6640			
		0%*	25%	50%	100%	0%	25%	50%	100%
M.I.C. mcg/ml	*Escherichia coli* 10536	0.2	0.2	0.3	0.3	0.2	0.2	0.3	0.3
	Pseudomonas aeruginosa 8709	0.2	0.3	0.6	0.6	0.2	0.2	0.3	0.3
	Pseudomonas aeruginosa 8689	0.3	0.3	0.6	0.6	0.3	0.2	0.3	0.2
	Proteus vulgaris 121	0.6	0.3	0.3	0.3	0.6	0.3	0.3	0.2
	Proteus vulgaris 9921	0.3	0.3	0.2	0.3	0.6	0.3	0.3	0.3
	Staphylococcus aureus 209P	0.3	0.3	0.2	0.3	0.3	0.3	0.3	0.6
	Staphylococcus aureus 11631	0.3	0.6	0.3	0.3	0.2	0.2	0.3	0.6
	Streptococcus pyogenes C	>3.2	2.4	2.4	1.2	>3.2	2.4	1.2	0.6
	Streptococcus faecalis 10541	1.2	1.2	1.2	1.2	1.2	1.2	0.6	0.3
M.B.C. mcg/ml	*Eschherichia coli* 10536	>1.6	0.3	0.6	0.6	0.6	0.2	0.3	0.3
	Pseudomonas aeruginosa 8709	>1.6	0.3	0.6	0.6	0.2	0.2	0.3	0.3
	Pseudomonas aeruginosa 8689	>1.6	0.6	1.2	1.2	>1.6	0.3	0.6	0.3
	Proteus vulgaris 121	0.6	0.3	0.3	0.6	0.6	0.3	0.2	0.2
	Proteus vulgaris 9921	0.3	0.6	0.3	0.3	0.6	0.4	0.3	0.6
	Staphylococcus aureus 209P	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.6
	Staphylococcus aureus 11631	0.6	0.6	0.6	0.6	0.3	0.2	0.3	0.6
	Streptococcus pyogenes C	>3.2	2.4	2.4	2.4	>3.2	2.4	1.2	1.2
	Streptococcus faecalis 10541	1.2	1.2	1.2	2.4	1.2	1.2	0.8	0.3

* Percent horse serum. Yeast beef broth pH 6.7.

Table 3. Effect of pH on the *in vitro* activity of antibiotic 6640 and gentamicin

	Organism	pH and M.I.C. (mcg/ml)						
		6.7	7.0	7.2	7.4	7.6	7.8	8.0
Antibiotic 6640	*Staphylococcus aureus* 209P	0.3	0.1	0.1	0.03	0.02	0.02	0.02
	Staphylococcus aureus Gray	0.03	0.02	0.02	0.01	0.005	0.005	0.005
	Streptococcus pyogenes C	3.5	0.9	0.9	0.9	0.5	0.5	0.5
	Escherichia coli 10536	1.2	0.5	0.5	0.5	0.5	0.5	0.5
Gentamicin	*Staphylococcus aureus* 209P	0.3	0.1	0.1	0.03	0.03	0.3	0.03
	Staphylococcus aureus Gray	0.03	0.03	0.01	0.01	0.005	0.005	0.005
	Streptococcus pyogenes C	3.5	0.9	0.9	0.5	0.5	0.5	0.5
	Escherichia coli 10536	0.6	0.5	0.5	0.5	0.5	0.2	0.2

Medium : yeast beef broth.

A slight shift in sensitivity was noted with both antibiotics with increased incubation from 24 to 48 hours. All of the 27 strains were sensitive to 3 mcg/ml or less of gentamicin and 1 mcg/mg or less of antibiotic 6640.

Table 4. Effect of inoculum size on *in vitro* activity of antibiotic 6640*

Organism	Strain	Inoculum $\sim 5\times10^7$	Inoculum $\sim 5\times10^6$	Inoculum $\sim 5\times10^5$	Inoculum $\sim 5\times10^4$
Staphylococcus aureus	209P	0.3	0.3	0.3	0.08
	69	1.2	1.2	0.3	0.3
	362	1.2	1.2	0.6	0.6
	542	9.6	2.4	0.3	0.3
Escherichia coli	851	19.2	4.8	1.2	1.2
	384	9.6	2.4	2.4	2.4
	420	9.6	9.6	4.8	2.4
	4	9.6	4.8	2.4	1.2
Pseudomonas aeruginosa	Sc.	9.6	4.8	4.8	1.2
	456	1.2	1.2	0.6	0.15
	641	19.2	19.2	19.2	9.6
	651	0.6	0.6	0.3	0.3

* Inoculum added to tubes containing 3 ml of tryptose phosphate broth pH 7.2.

The effect of serum on the *in vitro* activity of antibiotic 6640 and gentamicin was studied in yeast beef broth with varying concentrations of added horse serum. Both MIC (minimal inhibitory concentration), and MBC (minimal bactericidal concentration) values were determined for a group of 9 selected gram-positive and gram-negative organisms. These data are shown in Table 2. Serum had only a minor influence on the *in vitro* activity of the two antibiotics. There are some indications that antibiotic 6640 has greater bactericidal activity than gentamicin in the presence of serum, particularly against strains of *Streptococcus*, *Pseudomonas* and *E. coli*. The effect of the pH of the medium on the *in vitro* activity of antibiotic 6640 was compared with the effect on gentamicin using a small group of organisms in yeast beef broth at several pH levels. These results are shown in Table 3 and indicate that antibiotic 6640 like gentamicin is more active at an alkaline pH than at a pH below neutrality.

The effect of inoculum size was tested by varying the number of organisms added to 3 ml of tryptose phosphate broth for a selected group of strains. The results (Table 4) suggest a modest effect with several strains.

In vivo Activity

The therapeutic activity of antibiotic 6640 and gentamicin was compared against a number of experimental bacterial infections in mice. The results of tests against a variety of gram-positive and gram-negative bacterial infections are shown in

Table 5. Therapeutic activity of antibiotic 6640 and gentamicin against gram-positive infections in mice. All mice were dosed subcutaneously

Infecting organism	PD_{50} (mg/kg)					
	Single dose 1 hour after infection		Total dose divided and given 1/2 hour before and 4 hours after infection		Gentamicin PD_{50}/antibiotic 6640 PD_{50}	
	Antibiotic 6640	Gentamicin	Antibiotic 6640	Gentamicin	1 dose	2 doses
Staphylococcus aureus Gray	1.8	2.4	7.0	7.5	1.3	1.1
Staphylococcus aureus Smith	1.0	3.3	10.0	8.0	3.3	1.3
Staphylococcus aureus W	15.0	20.0	10.0	12.0	1.3	0.8
Streptococcus pyogenes C	6.0	14.0	10.0	20.0	2.3	2.0
Streptococcus pyogenes C 203	21.0	46.0	4.0	8.0	2.2	2.0
Enterococcus sp. 801	7.5	18.0	5.8	17.0	2.4	2.9
Enterococcus sp. 804	7.0	41.0	5.8	17.0	5.9	2.9

Table 6. Therapeutic activity of antibiotic 6640 and gentamicin against gram-negative infections in mice. All mice were dosed subcutaneously

Infecting organism	PD_{50} (mg/kg)					
	Single dose 1 hour after infection		Total dose divided and given 1/2 hour before and 4 hours after infection		Gentamicin PD_{50}/antibiotic 6640 PD_{50}	
	Antibiotic 6640	Gentamicin	Antibiotic 6640	Gentamicin	1 dose	2 doses
Escherichia coli Sc.	1.7	3.2	1.4	1.5	1.9	1.1
Escherichia coli No. 1	1.5	2.5	0.2	0.5	1.7	2.5
Klebsiella pneumoniae Sc.	0.84	0.84	0.8	0.9	1.0	1.1
Proteus vulgaris 120.4	1.7	1.5	1.5	1.8	0.9	1.2
Proteus vulgaris No. 2	5.7	3.1	3.0	8.4	0.5	2.8
Pseudomonas aeruginosa Sc.	1.1	1.7	0.5	1.5	1.5	3.0
Pseudomonas aeruginosa 9027	0.3	0.5	—	—	1.7	—
Pseudomonas aeruginosa No. 3	13.1	50.0	50.0	>50.0	3.9	>1.0
Pseudomonas aeruginosa No. 5	4.9	13.0	2.8	9.1	2.7	3.2
Pseudomonas aeruginosa No. 6	2.5	2.5	2.0	4.4	1.0	2.2
Pseudomonas aeruginosa No. 11	26.0	50.0	6.0	7.5	2.0	1.3
Salmonella schottmuelleri Sc.	2.0	11.2	1.5	1.8	5.6	1.2
Salmonella schottmuelleri No. 13	1.7	4.8	2.8	3.3	2.8	1.2
Serratia sp. A	3.5	15.0	—	—	4.3	—
Serratia sp. B	1,0	2.5	—	—	2.5	—

Tables 5 and 6 using two different treatment regimens. These tables shows the PD_{50} values in terms of mg/kg of the two antibiotics in parallel tests and the relative potency of the two antibiotics. The larger the relative activity value, the greater the activity of antibiotic 6640. In general, antibiotic 6640 was 1~4 times more active than gentamicin in these protective tests. It should be noted that the high PD_{50} values of gentamicin against some of the *Pseudomonas* strains reflect the deliberate selection of strains with reduced sensitivity to gentamicin in order to determine the presence or absence of cross-resistance. As with sensitive *Pseudomonas* strains, antibiotic 6640 was more active *in vivo* against strains with reduced sensitivity to gentamicin than was gentamicin itself. The two treatment regimens were performed on separate tests and are not directly comparable due to variation in inocula and mice between tests.

Serum levels of antibiotic 6640 and gentamicin were determined in groups of rats

Table 7. Serum levels of antibiotic 6640 and gentamicin in mice and rats

	Preparation	Dose mg/kg	Route	No. Replicates	Average serum levels in mcg/ml at times after dosing (hours)						
					1/4	1/2	1	2	4	6	24
Mice	Antibiotic 6640	50	s.c.	2	74	42	13	2.8		<0.04	0
	Gentamicin	50	s.c.	2	66	35	14	2.3		<0.04	0
	Antibiotic 6640	25	s.c.	3	30	14	6.6	0.1		<0.04	0
	Gentamicin	25	s.c.	3	25	14	6.8	0.5		<0.04	0
	Antibiotic 6640	5	s.c.	2	6.5	4.8	1.5	0.1		0	0
	Gentamicin	5	s.c.	2	6.7	4.6	1.0	0.06		0	0
Rats	Antibiotic 6640	4	s.c.	1	17	12.5	9.3	3.1	<0.5	0	
	Gentamicin	4	s.c.	1	14.5	11.5	6.3	1.6	<0.5	0	
	Antibiotic 6640	4	i.m.	1	12.5	11.0	5.0	1.9	<0.5	0	
	Gentamicin	4	i.m.	1	13.0	10.5	3.9	1.4	0	0	

and mice after single subcutaneous or intramuscular doses and the results are shown in Table 7. These data demonstrate that gentamicin and antibiotic 6640 produced quite similar serum levels in both mice and rats at the several dose levels studied. Peak levels were found as early as one quarter hour after dosing and declined very rapidly in both species. Serum and urine levels of antibiotic 6640 were also determined in 4 dogs after a single intramuscular dose of either 8 or 16 mg/kg in a volume of 0.5~1.0 ml. The injection was well tolerated in all dogs. Assay results (Table 8) showed that peak serum levels were produced rapidly and that these quickly declined. Urine levels indicate a recovery of 55~77% of the dose in 24 hours and a total of 57~83% of the dose in 48 hours. This is a reflection of the rapid excretion of antibiotic 6640 by the kidneys into the urine. Serum and urine levels of antibiotic 6640 in dogs are very similar to those reported for gentamicin at comparable dose levels.

Table 8. Serum and urine levels of antibiotic 6640 in dogs after a single intramuscular dose

Dog	Dose mg/kg	Serum levels (mcg/ml) at time after dosing (hours)						
		0	1/2	1	2	4	6	24
420	60			95		19.4		0.16
280	60			125		48		0.24
278	60			92		36		0.26
197	30			67.5		15.2		0.08
171	30			62.5		10.4		0.23
267	30			62.5		12.6		0.23
HE-6	16	0	31.5	36.0	21.0	2.8	0.7	0
90	16	0	43.5	34.5	20.0	2.8	0.5	0
144	15			30.0		3.75		0.04
286	15			26.3		2.91		0.04
277	15			25.0		3.75		A
290	8	0	21.9	19.8	13.5	1.9	0.4	0
17	8	0	18.9	18.0	10.0	0.7	0.2	0

Dog	Dose mg/kg	Urine levels					
		24 hours			24~48 hours		
		mcg/ml	Total mg excreted	% of Dose	mcg/ml	Total mg excreted	% of Dose
HE-6	16	274	103	64	10	1.9	1.2
90	16	586	100	62	14	1.9	1.2
290	8	130	44	55	6	1.4	1.7
17	8	426	62	77	31	4.3	5.4

The effect of chronic administration of antibiotic 6640 and gentamicin in cats was studied in order to compare the relative abilities of the two antibiotics to produce ataxia. Groups of 5 male cats each were given daily subcutaneous doses of 20, 40 or 60 mg/kg/day, 7 days a week, until the termination of the experiment.

Table 9. Sub-chronic toxicity tests of antibiotic 6640 and gentamicin in cats

Drug	Dose (mg/kg/day)	Cat No.	Initial weight (kg)	Day ataxic	Day righting reflex impairment	Day death
Antibiotic 6640	20	3585	4.8	16	15	17
		19623	4.1	22	22	33
		21606	3.8	26	25	55
		3613	3.5	23	23	36
		21467	3.1	27	27	43
		Mean		22.8	22.4	36.8
	40	3849	4.5	15	15	17
		5272	4.2	14	15	19
		3844	3.9	16	17	19
		5069	3.4	15	14	27
		3604	3.8	15	14	18
		Mean		15.0	15.0	20.0
	60	3576	4.5	10	11	12
		3774	4.4	12	13	15
		3931	3.9	10	11	13
		21667	3.8	10	10	11
		5395	3.1	13	10	14
		Mean		11.0	11.0	13.0
Gentamicin	20	5390	5.1	27	27	64
		21549	4.1	34	34	>70
		5385	4.0	35	34	>70
		5298	3.3	35	35	>70
		5137	2.6	26	36	40
		Mean		31.4	31.2	>62.8
	40	3923	5.0	18	17	28
		5250	3.5	19	19	>70
		3602	4.0	15	14	15
		5188	3.4	15	15	16
		5310	2.9	19	19	23
		Mean		17.2	16.8	>30.4
	60	3697	5.0	10	11	12
		3482	4.1	10	11	11
		5021	3.9	14	14	27
		5273	3.5	14	14	23
		3955	3.0	13	14	24
		Mean		12.2	12.8	17.4

The results of this study are shown in Table 9. These are given in terms of the number of days of dosing to produce ataxia, to produce righting reflex impairment, and to produce death. The experiment was terminated on day 70. A definite dose-response relationship was seen with both antibiotics in terms of the number of days to become ataxic and the number of days to show impairment or righting reflex. The day of death for the cats was more variable but it appeared to follow the appearance of ataxia more closely with antibiotic 6640 than with gentamicin. In all cats receiving both antibiotics at levels of 40 or 60 mg/kg/day a slight posterior muscular weakness was noted after the first and second doses but was not observed after subsequent doses. Statistical analysis of the results by use of parallel line assay procedures indicated that antibiotic 6640 is 1.3 times as ataxic as gentamicin in terms

of the length of time required to produce ataxia at the selected doses used.

Discussion

Antibiotic 6640 is a new aminoglycoside antibiotic with a spectrum of activity and potency quite similar to that of gentamicin *in vitro* and *in vivo*. The greater bactericidal activity of antibiotic 6640 relative to gentamicin against some strains of bacteria, particularly in the presence of serum may account for the better *in vivo* activity in spite of similar *in vitro* activity. The differences seen in effectiveness of treatment by a single dose as opposed to split dosing are undoubtedly due to variations between tests with particular regard to inoculum virulence. It is not possible therefore to compare the two treatment regimens. Since antibiotic 6640 was run in parallel with gentamicin in each test however, the comparisons between PD_{50} values of the two antibiotics on a particular test are valid. Thus, the ratio of the pairs of PD_{50} values are more appropriate for comparisons than the actual PD_{50} values themselves since they take into account between test variations. While both treatment regimens gave ratios favorable to antibiotic 6640, no consistent differences were found in the ratios between the two treatment regimens. The acute toxicity of antibiotic 6640 (WEINSTEIN *et al.*[1]) was approximately twice that of gentamicin in mice and more nearly resembled the acute toxicity values of neomycin. Chronic toxicity studies in cats suggest that antibiotic 6640 and gentamicin have similar toxicity, with antibiotic 6640 being approximately 1.3 times as toxic as gentamicin in terms of the time required for production of ataxia and impairment of the righting reflex. The absorption characteristics of antibiotic 6640 after parenteral dosing in mice, rats and dogs appeared to be quite similar to those of gentamicin.

References

1) WEINSTEIN, M. J.; J. A. MARQUEZ, R. T. TESTA, G. H. WAGMAN, E. M. ODEN & J. A. WAITZ: Antibiotic 6640, a new *Micromonospora*-produced aminoglycoside antibiotic. J. Antibiotics 23 : 551~554, 1970
2) WAGMAN, G. H.; R. T. TESTA & J. A. MARQUEZ: Antibiotic 6640. II. Fermentation, isolation and properties. J. Antibiotics 23 : 555~558, 1970
3) WAITZ, J. A.; E. L. MOSS, Jr., E. M. ODEN & M. J. WEINSTEIN: Biological activity of megalomicin, a new *Micromonospora*-produced macrolide antibiotic complex. J. Antibiotics 22 : 265~272, 1969
4) WAITZ, J. A. & M. J. WEINSTEIN: Recent microbiological studies with gentamicin. J. Infect. Dis. 119 : 355~360, 1969