BIOLOGICAL ACTIVITY OF MEGALOMICIN A PHOSPHATE, A WATER-SOLUBLE SALT OF MEGALOMICIN A

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Megalomicin A phosphate is a new, stable, water-soluble salt of the new *Micromonospora*-produced macrolide antibiotic megalomicin A. The studies reported here suggest that the phosphate salt has the same spectrum of activity and *in vitro* potency as the insoluble base. Of particular interest is the substantial degree of activity seen against several gram-negative bacteria in comparison to that usually seen with other macrolides. Mouse protection tests suggest that the salt is more potent than the base when given subcutaneously, but this is undoubtedly due to the greater availability of drug when given in its soluble form. Solutions of megalomicin A phosphate were well tolerated intramuscularly by monkeys, dogs, cows and rats. Compared to commercially available forms of lincomycin, erythromycin and oleandomycin in dogs, megalomicin A phosphate produces serum levels with equal or greater peak values and significantly prolonged duration. The prolonged duration of serum levels was also seen with megalomicin A base as reported in earlier studies. Initial tolerance and toxicity tests in dogs and rats suggest that megalomicin A phosphate will be safe and well tolerated. This antibiotic preparation shows promise as a new injectable antibiotic in man and also for use in the treatment of diseases of veterinary importance.

Megalomicin A is one of the components of the megalomicin complex, a new *Micromonospora*-produced macrolide antibiotic complex first described by WEINSTEIN *et al.*[1,2]. Details of the biological activity of the complex as well as the individual components were described by WAITZ *et al.*[3]. In these studies[3], it was found that intramuscular administration of megalomicin A base to dogs resulted in a "depot" effect in which increased doses resulted in similar peak levels but with longer duration. This suggested the possibility that a soluble salt of megalomicin A for parenteral use might give higher peak serum levels. Extensive chemical efforts, to be described elsewhere, led to the development of megalomicin A phosphate, the potassium dihydrogen phosphate salt of megalomicin A base. This paper presents the results of biological studies with megalomicin A phosphate in comparison with several reference macrolide antibiotics.

Materials and Methods

Two lots of megalomicin A phosphate were used most extensively. These assayed, in terms of megalomicin A base, 700 mcg/mg and 650 mcg/mg respectively. For comparative purposes the following preparations were used: megalomicin A base, Schering lot

4106-34-I, 1,000 mcg/mg ; erythromycin ethyl succinate (erythromycin IM), Abbott lot 823-1007, 50 mg/ml ; lincomycin hydrochloride (lincocin IM), Upjohn lot XS 256 C8, 300 mg/ml and oleandomycin phosphate (oleandomycin IM), Roerig lot 86929, 100 mg/ml, and erythromycin base, USP, Farmaceutici SpA, 980 mcg/mg.

In vitro and *in vivo* test procedures were similar to those described by WAITZ *et al.*[3] Protection tests were done using groups of 7~10 male CF-1 albino mice weighing approximately 20 g each, with control groups of 10 mice each. Treatment was given shortly before and 4 hours after intraperitoneal infection with approximately 10^7 organisms per mouse. Control infected mice died 18~24 hours after infection, while survivors in treated groups were determined 48 hours after infection. PD_{50} values were calculated by probit procedures. All drugs were given as solutions in sterile distilled water, when possible, or as suspensions in 0.5 % aqueous carboxymethyl cellulose which were ultrasonicated to reduce particle size. The dogs used were beagle-type mongrels of both sexes weighing approximately 10 kg each. Rats were of the CF-E strain with males weighing approximately 170 g each and females weighing approximately 150 g each. The monkeys used were mature rhesus females, weighing between 6~8 kg. The cows used were normal Holstein, weighing approximately 1,300 lb (590 kg) each. Serum, milk, urine and feces levels of megalomicin were determined by the procedure described in WEINSTEIN *et al.*[1] Other antibiotics were determined as described in the U. S. Code of Federal Regulations.

Results and Discussion

In Vitro Activity

The *in vitro* activity of megalomicin A phosphate in comparison with erythromycin base and lincomycin hyrochloride is shown in Table 1. The spectrum of activity and potency of the megalomicin A phosphate is quite similar to that described earlier for megalomicin A base[1~3]. Megalomicin A phosphate is approximately equal to or slightly less active than erythromycin and lincomycin. As described by WAITZ *et al.*[3], megalomicin A is cross resistant with erythromycin and is bound by serum to approximately 25 %. Similar data were obtained for megalomicin A phosphate.

In Vivo Activity

The protective activity of megalomicin A phosphate is shown in comparision with

Table 1. *In vitro* activity of megalomicin A phosphate, erythromycin base and lincomycin hydrochloride in yeast beef broth pH 7.4

Organism	No. Strains	MIC (mcg/ml)		
		Megalomicin A phosphate	Erythromycin base	Lincomycin HCl
Staphylococcus aureus	11	0.3	0.01~0.75	0.03~0.75
Streptococcus pyogenes	11	0.3~7.5	0.3~0.75	0.3~7.5
Streptococcus faecalis	3	0.3~3.0	0.08	0.3~3.0
Diplococcus pneumoniae	6	3.0	3.0	0.3~0.75
Enterococcus sp.	5	0.75~3.0	0.3	0.3~0.75
Aerobacter sp.	2	7.5~>25	>25	—
Escherichia coli	5	0.3~7.5	7.5~>25	—
Haemophilus influenzae	3	3.0	2.5	0.3
Klebsiella pneumoniae	3	0.75~>25	7.5~>25	—
Neisseria gonorrheae	3	0.75~3.0	1.0	0.075~0.3
Proteus sp.	3	0.3~0.75	17.5~>25	—
Pseudomonas aeruginosa	5	0.75~7.5	12.5~>25	—
Salmonella paratyphi B	2	0.3~>25	7.5~>25	—
Mycobacterium smegmatis	1	0.8	7.5	0.08
*Mycoplasma gallisepticum**	1	0.05	0.1	—

* PPLO broth and agar.

Table 2. Protective activity and toxicity of megalomicin A phosphate, erythromycin base and lincomycin hydrochloride in mice

Organism		Treatment route	Megalomicin A phosphate	Erythromycin base**	Lincomycin HCl
Protective activity PD_{50} (mg/kg)*	*Staphylococcus aureus* Gray	S. C.	1.5	30	3.0
	Staphylococcus aureus Smith	S. C.	4.0	40	—
	Staphylococcus aureus W	S. C.	1.5	20	2.0
	Streptococcus pyogenes C	S. C.	5.0	90	50
	Streptococcus pyogenes C 203	S. C.	18.0	134	50
	Streptococcus pyogenes 22	S. C.	2.5	50	2.5
	Escherichia coli ATCC 10536	S. C.	200.0	160	—
	Pseudomonas aeruginosa Sc	S. C.	75.0	130	—
Acute toxicity LD_{50} (mg/kg)		I. V.	75	—	225
		I. P.	350	500	1,200

* The drug was administered as a solution in sterile distilled water (megalomicin, lincomycin) or as an ultrasonicated suspension in 0.5% aqueous carboxy methyl cellulose (erythromycin). The total dose was divided into two and given at the time of and then 4 hours after intraperitoneal infection.

** From WAITZ *et al.*[3]

erythromycin base and lincomycin hydrochloride in Table 2. The lincomycin and megalomicin A phosphate data were obtained from parallel experiments. The PD_{50} values obtained against infection with gram-positive bacteria were considerably lower with the megalomicin A phosphate than those described earlier with megalomicin A base. This perhaps is due to the greater availability of antibiotic from the soluble salt as opposed to the insoluble base. Megalomicin A phosphate was equal to or superior to lincomycin hydrochloride and substantially superior to erythromycin base against the strains described in the table. Attempts were made to use the commercially available erythromycin ethyl succinate for protection tests but these were not successful due to the toxicity of the commercial preparation in mice at therapeutic doses. The acute intraperitoneal LD_{50} of megalomicin A phosphate was the same as that obtained for the base: 350 mg/kg. The acute intravenous LD_{50} was 75 mg/kg. Megalomicin A phosphate is thus slightly more toxic than erythromycin base and 3~4 times as toxic as lincomycin hydrochloride in acute tests in mice.

Absorption and Excretion in Dogs - Single Dose

The absorption and excretion of megalomicin A phosphate after intramuscular administration to dogs was compared with megalomicin A base, erythromycin ethyl succinate, oleandomycin phosphate, and lincomycin hydrochloride. The latter 3 antibiotics were used in their commercially available form. All preparations were given as a single intramuscular dose of 100 mg which approximated 10 mg/kg. The results of these studies are shown in Table 3. With reference to the magnitude of peak serum levels, megalomicin A phosphate produced levels which were substantially higher than those obtained with megalomicin A base and erythromycin ethyl succinate but similar to those obtained with oleandomycin phosphate and lincomycin hydrochloride. With the exception of megalomicin A base which has been reported earlier (WAITZ *et al.*[3]), to produce serum levels with prolonged duration, megalomicin A phosphate showed greater duration of serum levels than any of the other antibiotics studied. Megalomicin A phosphate administered to dogs intramuscularly produced significant serum levels as long as 24 hours after dosing in contrast to that obtained in dogs given

Table 3 a. Serum levels in dogs after a single intramuscular dose of 100 mg (approximately 10 mg/kg) of several macrolides

Preparation	Dog. No.	Serum levels (mcg/ml) at hours after dosing						
		0	1	2	4	6	24	48
Megalomicin A phosphate aqueous solution 100 mg/ml	90	0	1.2	0.7	0.3	—	A**	—
	90	0	4.6	1.0	0.6	0.3	0.4	A
	266*	0	5.8	—	1.5	—	0.4	—
	268*	0	3.7	—	1.8	—	0.3	—
	94*	0	4.2	—	1.5	—	0.3	—
	Mean		3.9	0.8	1.13	0.3	0.35	A
Megalomicin A base aqueous suspension 100 mg/ml	6	0	0.3	0.5	0.3	0.4	0.3	A
	6	0	1.5	1.1	0.7	0.4	0.5	0
	89	0	0.6	2.0	1.3	0.7	0.4	A
	63	0	2.0	2.3	1.7	1.1	0.1	A
	Mean		1.1	1.47	1.0	0.65	0.3	A
Erythromycin ethyl succinate non-aqueous solution 50 mg/ml	236*	0	1.4	—	0.8	—	0	—
	206*	0	1.3	—	0.8	—	A	—
	52	0	0.8	—	0.8	—	0	—
	Mean		1.16	—	0.8	—	0	—
Oleandomycin phosphate aqueous solution 100 mg/ml	90	0	3.0	2.1	0.8	A	0	—
	290	0	3.1	2.0	0.9	A	0	—
	Mean		3.05	2.05	0.85	A	0	—
Lincomycin HCl aqueous solution 300 mg/ml	17	0	2.4	3.1	0.8	A	0	—
	6	0	4.4	2.7	0.7	0.6	0	—
	Mean		3.4	2.9	0.75	<.6	0	—

* See also Table 4.
** A—active but below the level of the assay procedure.

oleandomycin phosphate or lincomycin hydrochloride. All preparations were well tolerated by the dogs except for the erythromycin ethyl succinate which appeared to produce considerable pain upon injection. Urine levels for all antibiotics (Table 3 b) generally reflected what was seen with serum levels. Significant hepato-biliary excretion of all of the test antibiotics was suggested by the presence of significant antibiotic activity in the feces after intramuscular dosing. With reference to urine levels of antibiotic, megalomicin both as the base and the phosphate appeared to be most like lincomycin.

Absorption and Excretion in Dogs - Multiple Dosing

Serum, urine and feces levels of megalomicin A phosphate were obtained from a small number of dogs of both sexes from a probing four week intramuscular toxicity study. Included in this study were 3 dogs receiving megalomicin A phosphate intramuscularly at a level of 40 mg/kg/day, 3 dogs at 10 mg/kg/day, and 3 dogs which received erythromycin ethyl succinate at a level of 10 mg/kg/day. Two control dogs received saline intramuscularly. The serum, urine, and feces levels obtained after the first, 7th, 14th and 28th doses are shown in Table 4. As expected, serum levels

Table 3 b. Urine and feces levels in dogs after a single intramuscular does of 100 mg (approximately 10 mg/kg)

Preparation	Dog No.	Urine levels				Feces levels			
		0～24 hrs.		25～48 hrs.		0～24 hrs.		25～48 hrs.	
		mcg/ml	Total mg	mcg/ml	Total mg	mcg/ml	Total mg	mcg/ml	Total mg
Megalomicin A phosphate aqueous solution 100 mg/ml	90	99	25.6	39	7.0	40	3.4	110	2.3
	90	195	29.3	50	6.3				
	266*		24.7				0.2		
	268*		22.4				5.5		
	94		27.5				5.8		
	Mean	147	25.9	45	6.7	40	3.73	110	2.3
Megalomicin A base-aqueous suspension 100 mg/ml	6	175	35.0	37	8.3	48	1.3	84	4.2
	6	155	29.4	28	10.5				
	89		19.5		7.6		1.9		3.1
	63		24.5		6.0		1.2		3.4
	Mean	165	27.1	32	8.1	48	1.46	84	3.56
Erythromycin ethyl succinate non-aqueous solution 50 mg/ml	236*		9.9				5.2		
	206*		11.2				1.7		
	52		8.2				3.7		
	Mean		9.76				3.53		
Oleandomycin phosphate-aqueous solution 25 mg/ml	90	66	11.9			32	3.8		
	290	49	17.2			53	3.8		
	Mean	57.5	14.5			43	3.8		
Lincomycin HCl aqueous solution 300 mg/ml	17	220	37.4			77	6.8		
	6	180	41.4			75	2.8		
	Mean	200	39.4			76	4.8		

* See also Table 4.

with megalomicin A phosphate at the 40 mg/kg/day dose level were substantially higher than those obtained with the 10 mg/kg/day dose level, in contrast to megalomicin A base. The 10 mg/kg/day megalomicin A phosphate treatment produced substantially higher serum levels and with greater duration than those obtained with a similar dose of erythromycin ethyl succinate. With both megalomicin dose levels, but not with erythromycin, serum levels after the 7th day of dosing tended to be slightly higher than after the first dose but did not generally increase after this.

Fourteen Day Toxicity in Rats

An initial 14-day toxicity study was conducted in rats. Groups of 10 female and 10 male rats each, were given daily intramuscular doses of 10 mg/kg/day or 40 mg/kg/day of megalomicin A phosphate in a volume of 0.15～0.20 ml/dose. Groups of 5 male and 5 female rats each were utilized as saline-treated controls. Animals were weighed daily and observed for gross untoward effects. Aside from a slight dose related inhibition of weight gain, toxic effects were limited to some inflammation at the injection area in all treated and control groups. No obvious gross toxic effects were noted.

Table 4. Chronic intramuscular administration of megalomicin A phosphate and erythromycin ethyl succinate to dogs

	Dog	Day of test	Serum levels (mcg/ml) at hours after dosing			Total 24 hours recovery (mg)	
			1 hr.	4 hrs.	24 hrs.	Urine	Feces
Megalomicin A phosphate 40 mg/kg/day (200 mg/ml)	279	1	11.0	7.7	1.5	182.0	7.0
	322	1	14.5	8.0	1.2	115.7	1.7
	92	1	6.6	7.3	1.7	116.2	1.3
	Mean		10.7	7.7	1.5	137.9	3.3
	279	7	9.1	2.3	2.3	132.0	—
	322	7	6.8	3.6	3.4	210.0	—
	92	7	16.0	11.0	3.4	153.0	37.8
	Mean		10.6	5.6	3.0	165.0	37.8
	279	14	15.0	5.8	2.1	165.0	—
	322	14	21.0	7.8	2.4	83.0	—
	92	14	20.0	9.4	2.7	168.0	—
	Mean		18.7	7.7	2.4	138.6	—
	279	21	13.0	8.6	2.7	155.0	—
	322	21	14.0	9.0	1.9	119.0	—
	92	21	20.0	10.0	2.7	195.0	—
	Mean		15.7	9.2	2.5	156.3	—
	279	28	—	—	1.9	9.9	—
	322	28	—	—	2.6	34.5	—
	92	28	—	—	3.4	108.0	—
	Mean		—	—	2.6	50.8	—
Megalomicin A phosphate 10 mg/kg/day (50 mg/ml)	266	1	5.8	1.5	0.4	24.7	0.2
	268	1	3.7	1.8	0.3	22.4	5.5
	94	1	4.2	1.5	0.3	27.5	5.8
	Mean		4.5	1.6	0.3	24.8	3.8
	266	7	7.8	2.8	1.4	41.6	—
	268	7	7.8	2.7	1.9	25.2	—
	94	7	8.1	1.9	0.7	40.0	7.7
	Mean		7.9	2.5	1.3	35.6	7.7
	266	14	6.4	2.2	0.6	15.0	—
	268	14	5.0	2.2	0.6	16.1	—
	94	14	8.0	2.1	0.2	11.2	—
	Mean		6.5	2.2	0.5	14.1	—
	266	21	4.4	1.6	0.8	20.9	—
	268	21	7.4	2.3	0.7	25.2	—
	94	21	6.6	1.5	0.9	21.0	—
	Mean		6.1	1.8	0.8	22.4	—
	266	28	—	—	0.8	27.5	—
	268	28	—	—	0.9	30.0	—
	94	28	—	—	0.7	16.0	—
	Mean		—	—	0.8	24.5	—
Erythromycin ethyl succinate 10 mg/kg/day (50 mg/ml)	236	1	1.4	0.8	0	9.9	5.2
	206	1	1.3	0.8	<.06	11.2	1.6
	52	1	0.8	0.8	0	8.2	3.7
	Mean		1.2	0.8	0	9.8	3.5
	236	7	1.1	0.7	0	10.8	—
	206	7	0.9	0.3	0.07	13.2	2.4
	52	7	1.7	0.5	0	12.2	—
	Mean		1.2	0.5	0	12.1	2.4
	236	14	2.1	0.8	0	6.3	—
	206	14	1.0	0.9	0	4.4	—
	52	14	2.3	0.9	0	9.3	—
	Mean		1.8	0.9	0	6.7	—
	236	21	1.5	0.1	0	—	10.5
	206	21	1.2	1.0	0	—	11.3
	52	21	1.1	0.6	0	—	9.6
	Mean		1.3	0.6	0	—	10.5
	236	28	—	—	0	—	4.9
	206	28	—	—	0	—	9.0
	52	28	—	—	0	—	4.0
	Mean		—	—	0	—	5.9

Absorption in Monkeys

The absorption of megalomicin A phosphate was studied in two mature female monkeys each given a single intramuscular dose of 100 mg. This dose approximated 13 mg/kg in one monkey and 18 mg/kg in the second monkey. The data, shown in Table 5, indicate adequate serum and urine levels with peak serum values of 2.5~7.2

mcg/ml 1 hour after drug administration. The injection was well tolerated by both animals.

Serum and Milk Levels in Cows

The *in vitro* activity spectrum of megalomicin A coupled with the tolerance and absorption data on its soluble form megalomicin A phosphate, suggested the possible utility of this soluble form for parenteral treatment of mastitis in cows. As a result, a small study utilizing 2 normal non-mastitic Holstein cows weighing approximately 1,300 lbs (590 kg) each was conducted. Each cow was given a single intramuscular injection of megalomicin A phosphate in the form of an aqueous solution of 125 mg/ml. One cow was dosed at a level of 0.5 mg/lb (0.23 mg/kg) and the other was dosed at a level of 2.0 mg/lb (0.91 mg/kg). Milk and serum samples were drawn periodically and assayed for antibiotic content. The assay results of this study are shown in Table 6. These data indicate excellent excretion of megalomicin into the milk which was dose related. A dose related milk-out time of 36~48 hours was also indicated. Both cows tolerated the doses well.

Table 5. Absorption of megalomicin A phosphate in monkeys after a single intramuscular dose of 100 mg

Monkey	Weight (kg)	mg/kg	Serum levels (mcg/ml) at hours after dosing				
			0 hr.	1 hr.	2 hrs.	4 hrs.	6 hrs.
4	5.7	17.6	0	2.5	1.5	0.8	0.4
5	7.5	13.4	0	7.2	6.0	1.0	0.4
		mcg/ml	Urine levels 24 hours (Total mg)				
4		31	17.0				
5		31	14.9				

Table 6. Serum and milk levels (mcg/ml) of megalomicin A in cows after a single intramuscular dose of megalomicin A phosphate solution (125 mg base/ml)

Hours after dosing	Cow no. 2 (1,300 lbs) 0.5 mg/lb		Cow no. 3 (13,00 lbs) 2.0 mg/lb	
	Milk	Serum	Milk	Serum
0	0	0	0	0
1	0	1.5	0	5.0
2	1.9	0.5	4.0	3.0
4	—	0.2	—	1.5
8	7.5	0	56.0	0.2
12	3.0	0	33.0	0.5
24	1.0	0	4.5	0.2
36	0	0	4.5	0.2
48	0	0	0	A
60	0	0	—	A
72	0	0	0	0

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