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Activity of Repository Sulfones Against *Mycobacterium leprae* In Mice.* (31757)

CHARLES C. SHEPARD
Communicable Disease Center, Public Health Service, United States Department of HEW, Atlanta, Ga.

It was reported(1) recently that the growth of *Mycobacterium leprae* is suppressed in mice by very small dosages of 4,4′-diaminodiphenylsulfone (DDS). This drug, the most effective one in the treatment of leprosy and against which drug-resistance is very rare(2), has ordinarily been given in doses of 50 to 100 mg a day. Such intake produced 1-3 μg/ml blood and tissue(3) (with daily intake the drug level is relatively constant throughout the 24 hours). The result in mice indicated that much lower blood levels might be effective, and raised the possibility of innovation in the drug regimen.

One such possibility lies in repository forms. The drug 4,4′-diacetyldiaminodiphenylsulfone (DADDS) has recently been studied as a repository injection against malaria parasites(4), and a number of other repository sulfones have been synthesized for the same purpose(5). All these repository forms have one or both of the amino groups combined with other radicals. They are all poorly soluble in aqueous solution, and in the vehicle in which they are suspended. Antibacterial activity apparently depends upon cleavage by tissue enzymes to release DDS, or other possibly active compounds with only one of the amino groups free.

Materials and methods. The general procedure was to infect mice with a few thousand *M. leprae* and then to follow the growth curve in untreated mice until the bacterial population passed 1×10^6. This event signaled the onset of the plateau phase(6), so counts were then made from each treated and control group. Counts were repeated 3 months later. The methods for working with *M. leprae* in mice are described elsewhere(1,7). All counts were made on pools of the foot pad tissue of 4 mice, except for a few instances in the second harvest in Table I (at *ca.* 300 days) where only 2 mice remained.

The injected drugs (Tables I and II) were suspended in 40% benzyl benzoate and 60% castor oil. DADDS was supplied as a sterile suspension; the other compounds were heated to 150°C for 1¼ hours just before use and the sterile vehicle added. In the experiments listed in Table III the drugs were mixed in the powdered diet as described(1).

The mice were of the CFW strain of the Communicable Disease Center. They were fed commercial chow in the experiments of Tables I and II. The same diet, but unpelleted, was used for the experiments of Table III.

Results. Activity of 7 repository sulfones and DDS: In this experiment (Table I) all of the repository sulfones suppressed growth completely even when given every 2 months. DDS was completely effective when given twice a month, but allowed partial growth when given once a month, and nearly complete growth when given every 2 months. In other words DDS had a duration of activity

* The drugs were suggested by Dr. Paul E. Thompson on the basis of their repository activity against *Plasmodium berghei* in mice and were supplied by Dr. E. F. Elslager and Mr. D. F. Worth, all of the Research Division of Parke, Davis and Co., Ann Arbor, Mich.

of 3 to 4 weeks, and the repository drugs 2 months or more.

Minimal effective dose of DADDS and cycloguanil pamoate: In this experiment (Table II) DADDS was given at approximately 2-month intervals, at least during the

TABLE I. Activity Against *M. leprae* of 7 Different Repository Sulfones and DDS.

Mouse No.	Drug*	Interval between injections	AFB/mouse 218-224 days	AFB/mouse 294-300 days
1-20, 21-40, 41-60	PAM 1367	½ mo, 1 mo, 2 mo	$<2 \times 10^4$	$<2 \times 10^4$
61-80	Vehicle only	2 mo	1.3×10^6	1.7×10^6
81-100, 101-120, 121-140	PAM 1431	½ mo, 1 mo, 2 mo	$<2 \times 10^4$	$<2 \times 10^4$
141-160	Nil		3.2×10^6	1.5×10^6
161-180, 181-200, 201-220	PAM 1435	½ mo, 1 mo, 2 mo	$<2 \times 10^4$	$<2 \times 10^4$
221-240	Nil		2.4×10^6	2.0×10^6
241-260, 261-280, 281-300	PAM 1470	½ mo, 1 mo, 2 mo	$<2 \times 10^4$	$<4 \times 10^4$†
301-320	Nil		3.9×10^6	5.0×10^6
321-340, 341-360, 361-380	PAM 1481	½ mo, 1 mo, 2 mo	$<2 \times 10^4$	$<2 \times 10^4$
381-400	Nil		4.6×10^6	5.0×10^6
401-420, 421-440, 441-460	PAM 1503	½ mo, 1 mo, 2 mo	$<2 \times 10^4$	$<4 \times 10^4$
461-480	Nil		3.6×10^6	5.5×10^6
481-500, 501-520, 521-540	PAM 1513	½ mo, 1 mo, 2 mo	$<2 \times 10^4$	$<2 \times 10^4$
541-560	Nil		6.4×10^6	5.9×10^6
561-580	DDS	½ mo	$<2 \times 10^4$	
581-600	"	1 mo	5.8×10^4	3.3×10^4
601-620	"	2 mo	2.5×10^6	3.0×10^5
621-640	Nil		3.6×10^6	1.8×10^6

The mice were infected with 5.0×10^3 AFB (acid-fast bacteria), 18% of which stained solidly(6). The first injection of drugs was 400 mg/kg given at 58 days; subsequent injections were 200 mg/kg at intervals of either ½, 1, or 2 months. The growth curve of *M. leprae* in untreated controls was as follows: 7.3×10^4 at 108 days, and 7.1×10^5 at 158 days. Harvests were then made from all groups and repeated 3 months later; the average count for all untreated mice was 3.9×10^6 at 218 days, and 4.0×10^6 at 296 days.

* PAM 1367 = *p*-acetamidophenyl *p*-(benzylideneamino)phenyl sulfone; PAM 1431 = polymer of *p*-aminophenyl *p*-(allylideneamino)phenyl sulfone; PAM 1435 = bis[*p*-[3-(*p*-sulfanilylanilino)allylideneamino]phenyl] sulfone hydrate; PAM 1470 = *p*-acetamidophenyl *p*-(*p*-acetamidobenzylideneamino)phenyl sulfone; PAM 1481 = *p*-acetamidophenyl *p*-(3,5-dichlorosalicylideneamino)phenyl sulfone; PAM 1503 = 4′,4′′′-[*p*-phenylenebis(methylidyneimino-*p*-phenylenesulfonyl)]bisacetanilide hydrate; PAM 1513 = polymer of *p*-(methyleneamino)phenyl *p*-(benzylideneamino)-phenyl sulfone hydrate.

† The counts at 297 days were $<2 \times 10^4$ for #241-260, 3.1×10^4 for #261-280, and 3.8×10^4 for #281-300.

TABLE II. Activity Against *M. leprae* of the Repository Sulfone DADDS (4,4′-Diacetyldiaminodiphenylsulfone) and CI501 (Cycloguanil Pamoate).

Mouse No.	Drug	Dosage (mg/kg)	AFB/mouse 165-169 days	AFB/mouse 258-264 days
1- 20	Nil		4.1×10^6	2.5×10^6
21- 40	DADDS	400	$<2 \times 10^4$	2×10^4
41- 60	"	100	$<2 \times 10^4$	$<2 \times 10^4$
61- 80	"	25	$<2 \times 10^4$	4×10^4
81-100	Nil		7.7×10^5	7.6×10^6
101-120	DADDS	6	$<2 \times 10^4$	8×10^4
121-140	"	1.5	2.0×10^5	1.5×10^5
141-160	"	0.4	2.3×10^6	2.4×10^6
161-180	Nil		5.1×10^5	2.9×10^6
181-200	DADDS	0.1	6.6×10^5	1.1×10^6
201-220	CI501	400	9.3×10^5	2.6×10^6
221-240	"	100	9.2×10^5	3.8×10^6
241-260	Vehicle only		6.2×10^5	1.8×10^6

Mice were infected with 5.0×10^3 AFB (12% solidly staining). Drugs were given at 28, 90, and 200 days. The growth curve of *M. leprae* in untreated controls was as follows: $<2 \times 10^4$ at 92 days; 1.4×10^5 at 119 days; 1.2×10^6 at 152 days. Harvests were then made from all groups and repeated 3 months later; average count for all untreated mice was 1.8×10^6 at 167 days and 4.3×10^6 at 261 days.

TABLE III. Sensitivity Tests of 4 Strains of *M. leprae* to DDS in Diet.

Exp	Solid AFB in inoculum	DDS in diet	Harvest: Day	Harvest: AFB/mouse
A	18%	Nil	135	7.6×10^6
		.0001%	136	$<2 \times 10^4$
		.00001%	136	4.5×10^6
B	26%	Nil	119	7.5×10^6
		.0001%	119	2×10^4
		.00001%	119	7.5×10^6
C	12%	Nil	132	2.4×10^6
		.00001%	159	1.2×10^6
		.000001%	159	2.5×10^6
D	44%	Nil	119	3.5×10^6
		.00001%	145	2.6×10^6
		.000001%	145	7.6×10^6

After inoculation with 5.0×10^8 AFB the mice were placed in cages with the diets shown. After 120 to 140 days AFB were counted in controls. If AFB were greater than 5×10^6 counts were done on treated mice. If AFB were less than 5×10^6 counts were not done on treated mice until enough time had passed to allow bacterial growth to pass 1×10^6, assuming a rate of 12.5 days/generation (6).

critical logarithmic growth phase. The delay in the third injection probably did not affect the counts of the first harvest, but may have allowed some growth before the second harvest. The dose of drug was varied, and it was found that 1.5 mg/kg gave partial inhibition and 6 mg/kg nearly complete inhibition. Cycloguanil pamoate (4,6-diamino-1(*p*-chlorophenyl)-1,2-dihydro-2,2-dimethyl-s-triazine pamoate) was inactive. A possible relationship between triazines and sulfones had been indicated by the experiences with malaria parasites(8).

Sensitivity of more strains of M. leprae *to DDS (Table III).* In 2 experiments *M. leprae* was completely inhibited by 0.0001%, but not by 0.00001% DDS. In 2 other experiments there was little if any inhibition with 0.00001% or 0.000001% DDS.

Discussion. When the experiments with repository sulfones were begun it was not realized that *M. leprae* might be so sensitive to DDS, and it was thought only a relatively rapidly absorbing repository sulfone would be active. Instead, the picture that has emerged is one of great bacterial sensitivity and all the repository sulfones were active for at least 2 months in a dose of 200-400 mg/kg. DADDS was nearly completely suppressive in a dose of 6 mg/kg/2 months.

The earlier work(1) found 0.00001% DDS in the diet to be completely suppressive to a strain *M. leprae.* The experiments of Table III found 2 strains to require 0.0001% for complete suppression. Extrapolation from intakes that produce measurable concentrations of DDS in blood(1) to 0.0001% in the diet would indicate 0.03 μg DDS/ml as the minimal inhibitory concentration for these strains of *M. leprae.* This concentration of DDS is about 1/100 as much as that produced in man by the standard 100 mg/day, and about 1/300 as much as the toxic level of DDS in man, which is about 10 μg/ml of blood(3).

Most of our work in chemotherapy of *M. leprae* in animals has measured suppression of multiplication, rather than bacterial killing. However, recently an experiment was carried out(9) in which infections were allowed to establish themselves at the plateau level before DDS was started, at a level of 0.1% in the diet. Viability of the *M. leprae* was then measured at intervals by harvests of bacilli, observation of solid-staining ratios, and sub-inoculation of the bacilli into new groups of animals. Bacillary viability was found to be unaffected by treatment for 64 days, but it was seriously decreased after 87 days of treatment. The interpretation of some of the present experiments also hinges on whether or not bacteria were killed. The results with DDS in Table I are most simply interpreted on the basis that no killing occurred, and that the multiplication of *M. leprae* merely halted after the injection of DDS and then resumed at a full rate when the level of DDS fell below a critical concentration. An analysis of these results, assuming that the generation time of *M. leprae* was 12.5 days(6), indicates that for the mice receiving monthly injections the population of 5.8×10^4 AFB at 218 days could have been reached if DDS had been completely suppressive for 21 days after each injection and had then allowed 7 days of unrestricted growth. Similarly for the mice receiving bimonthly injections a completely suppressive period of 21 days after each injection would allow time for the population of 2.5×10^6

to be reached at 218 days. Other interpretations are possible with present knowledge, and additional studies of the influence of duration and level of drug on bacterial killing are being carried out.

The duration of the activity of DADDS in mice and monkeys was studied by Thompson, Olszewski, and Waitz(4), who gave a single injection of drug and challenged with malaria parasites at intervals thereafter. In mice doses of 100 to 400 mg/kg in benzyl benzoate and castor oil suppressed *Plasmodium berghei* for 6 to 14 weeks; 50 mg had little activity at one week. In monkeys doses of 50 mg/kg in the same vehicle prevented infection of *P. cynomologi* for 9 to 38 weeks. In man DADDS has suppressed falciparum malaria infections for about 60 days(10).

In leprosy effective repository drugs might have special usefulness, both in treatment and chemoprophylaxis, because of the very limited medical facilities in many of the important endemic areas.

Summary. 1. Seven discrete repository sulfones were tested against *M. leprae* in mice, and all were found completely suppressive when injected at 2-month intervals. DDS was inactive when given at 2-month intervals, partially suppressive at one month, and completely suppressive at half-month intervals. 2. The repository sulfone 4,4′-diacetyldiaminodiphenylsulfone was titrated in mice against *M. leprae*. The least dose giving nearly complete suppression was 6 mg/kg/2 months. Cycloguanil pamoate, a repository triazine antimalarial, was inactive in a dose of 400 mg/kg/2 months. 3. Further tests of the sensitivity of *M. leprae* to DDS were carried out by feeding the drug in the diet. Two strains were completely inhibited by 0.0001%; the same two and two other strains were insensitive to 0.00001%.

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Sites of Production of Primate Serum Proteins Associated with the Complement System.* (31758)

VERA J. STECHER,† JANE H. MORSE,‡ AND G. JEANETTE THORBECKE§

Department of Pathology, New York University School of Medicine, and Department of Medicine, College of Physicians and Surgeons, Columbia University, New York

Various complement components have been characterized as individual serum proteins which are distinguished by their physiochemical and immunological properties. Four of the components can be identified in immunoelectrophoretic patterns. Among these are C′3 as β_{1C}(1,2), C′4 as β_{1E}(3), C′5 as β_{1F}(4), and C′1q as an 11S globulin of γ-mobility(5,6,7). The technique employing autoradiography of immunoelectrophoretic

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