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Evaluation of the Activity of Streptomycin on *Mycobacterium leprae* in Mice

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The effect of streptomycin on *Mycobacterium leprae* was studied in the conventional mouse model. The drug has a relatively high bactericidal activity that places it between dapsone and ethionamide or prothionamide. Its effect is more pronounced when administered immediately after the experimental infection than when treatment is started at a later time. This is probably the result of the higher activity of streptomycin on leprosy bacilli located outside cells. It is concluded that streptomycin could be a valuable companion drug during the initial treatment of dapsone resistant leprosy in countries with limited resources. Streptomycin as monotherapy is not suited for the short course treatment of paucibacillary leprosy.

Introduction

There are at least two reasons why there is a need for additional antileprosy drugs. The first is that it is now evident that at least some forms of the disease will require combined treatment (Pattyn, 1972; Ellard, 1974; Pattyn, 1977; Colston *et al.,* 1978). The second is the necessity for reserve drugs for the treatment of dapsone resistant cases. To be useful these drugs should fulfil a number of criteria: easy administration, well tolerated, low price and possibility of supervised administration with convenient intermittent dosage.

The widely available streptomycin (SM) undoubtedly has an antibacterial activity against *Mycobacterium leprae* as shown by Shepard and Chang (1964) and Gaugas (1967) in mice, who found SM active in dosages of 80 and 100 mg/kg body weight respectively. Later Shepard (1967) found SM at

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80 mg/kg in the mouse to be bacteriostatic. No further work appears to have been done on the experimental chemotherapy of leprosy with SM.

Observations in man have shown that SM is active in leprosy (Faget and Erickson, 1947; Driesbach and Cochrane, 1958; Doull, 1954; Doull and Wolcott, 1956; Floch, 1966; Schultz *et al.,* 1966; Edwards *et al.,* 1972, Waters, 1974). However, Hastings *et al.* (1969) and Jacobson and Hastings (1976) presented evidence for the development of resistance after 23–21 months of monotherapy. Unfortunately SM resistance was not proven by mouse inoculation in these cases.

We therefore decided to investigate in more detail the value of SM against *M. leprae* in the mouse model.

There are at present four techniques to evaluate the antibacterial activity of a drug in the experimentally infected mouse:

(1) The continuous method (Shepard and Chang, 1964). Drug is administered during the whole observation period, until the multiplication of *M. leprae* in the control mice has reached the plateau level. This technique demonstrates whether a drug has any antileprosy activity and enables the minimal effective dose (MED) to be measured. If corresponding drug levels in mouse plasma are known or measured, the minimal inhibitory concentration (MIC) can be estimated.

(2) The kinetic method (Shepard, 1967). Drug is administered during the first 90 days after inoculation of the mice. The growth curve in treated mice is compared with that in the controls. This technique enables the drug's activity to be characterized as being bacteriostatic or of a bactericidal type.

(3) The proportional bactericidal test (Colston *et al.,* 1978). In this test drug is administered for a limited period to groups of mice inoculated with logarithmic dilutions of *M. leprae*. This test measures the proportion of bacilli killed by the drug.

(4) The total minimal inhibitory test (Pattyn and Saerens, 1975). Drug is administered during different periods of time to different groups of mice. The suppression of the bacterial multiplication of *M. leprae* is monitored. This technique gives information concerning the minimal period of time paucibacillary *M. leprae* infections have to be treated.

The antileprosy effect of SM was studied in the continuous method, in the proportional bactericidal (PBT) and the total minimal inhibitory (TMIT) tests.

Material and Methods

Mice of strain OF1 (Lyon, France) were inoculated with *M. leprae* strain 17547, previously described (Pattyn, 1972). All experiments were performed in duplicate, starting treatment respectively on days 1 and 22, after inoculation of the mice.

Except for the PBT all inocula were calculated to contain between 1 and 5×10^3 *M. leprae* per foot pad. Bacterial multiplication was monitored by the counting method as described by Shepard (1960).

Results

A. MINIMAL EFFECTIVE DOSE

In Experiment 1 (Table 1) the minimal effective dose was 50 mg/kg. In Experiment 2 all mice given 100 mg/kg body weight (b.w.) 3 times weekly (3x/w) died from an unidentified toxic effect after about 2 months, a few animals in the groups treated 2x/w and 1x/w with this dosage survived. There were also some irregular results in this experiment, but the minimal effective dose ranged between 50 and 100 mg/kg. The different frequencies of administration did not profoundly influence the results.

TABLE 1

Determination of the minimal effective dose (MED) of streptomycin (SM) against M. leprae

SM dose	Experiment 1 Treatment started day 1 Twice weekly	Experiment 2 Treatment started day 22 Thrice weekly	Twice weekly	Once weekly
100 mg/kg b.w.†	N.T.‡	—	0/3*	0/4
50 mg/kg b.w.	0/8	4/17	1/7	1/7
25 mg/kg b.w.	2/9	1/8	2/9	4/8
12.5 mg/kg b.w.	6/8	2/9	5/10	2/8
6.25 mg/kg b.w.	4/8	8/9	1/9	3/9
MED 50	50 mg	50 mg	100 mg	100 mg

* Number of mice showing multiplication/number of mice inoculated.
†b.w. = body weight
‡N.T. = Not tested.

B. THE PROPORTIONAL BACTERICIDAL TEST (PBT)

In this test 100 mg/kg b.w. of streptomycin was administered 2 times a week for 60 days.

Treatment for 60 days starting 24 h after infection killed 93% of the inoculum, when the same treatment was started 3 weeks after infection the killing rate was 81%.

C. TOTAL MINIMAL INHIBITORY TEST (TMIT)

Table 3 shows the results of the total minimal inhibitory test. Treatment three times a week during 3 or 6 weeks was unable to prevent the multiplication of *M. leprae*. Treatment for 8 weeks gave a growth delay of *M. leprae*.

In all groups of mice treated twice a week for periods from 8 to 24 weeks *M. leprae* multiplied in some of the animals.

D. EFFECT OF TREATMENT DURING DIFFERENT PHASES OF THE GROWTH CURVE

To illustrate the effect of treatment during different phases of the growth curve, the following experiment was performed: mice were inoculated with *M.*

TABLE 2
Activity of streptomycin (SM) on M. leprae *in the proportional bactericidal test (PBT)*

Dry regimen (twice weekly	Inoculum size (*M. leprae* per footpad) 10^4	10^3	10^2	10	1	MPN* of viable *M. leprae*	Survival (%)	Killing (%)
Control	4/4†	4/4	4/4	5/7	4/9	1750		
SM 100 mg/kg b.w.‡ Starting day 2	5/5	3/3	4/5	0/5	0/5	130	0.07	93
SM 100 mg/kg b.w. Starting day 22	5/5	5/5	5/5	1/5	0/5	340	0.19	81

* Most probable number of bacteria in the highest inoculum.
† Number of mice showing multiplication/number of mice inoculated.
‡ b.w. = Body weight.

TABLE 3
Total minimal inhibitory test (TMIT) of streptomycin (SM) on M. leprae

Dose SM 100 mg/kg b.w.	Duration (weeks)	Number of mice showing growth of *M. leprae*/number inoculated at plateau*	+ 4 weeks
Thrice weekly	3	5/10	8/10
	6	2/10	10/10
	8	0/9	4/5
Twice weekly	8	15/18	—
	18	7/9	—
	24	2/6	—

* Mice examined when the number of bacilli reached $10^{5.5}$ in the controls.

leprae and divided into one control and three treatment groups, the latter receiving SM 100 mg/kg b.w. for 10 weeks, but treatment starting on days 2, 29 and 57 respectively. As can be seen from Fig. 1, all treatment regimens caused growth delay but the administration of SM from the start of the experiment had a greater effect than the treatments started on days 29 or 57.

Discussion

It is almost impossible to extrapolate the MED of SM as determined in the mouse to drug levels obtained in man, because the rate of elimination of SM in both species is so different. The mouse excretes SM much more rapidly than man. In mice the half-life for the elimination of SM is about 0.4 hr (J. M. Dickinson, personal communication) while in man it is of the order of 3 to 4 hr. In experimental tuberculosis in mice SM has to be administered in doses of 100–200 mg/kg body weight in order to be effective, whereas tuberculosis patients are routinely treated with a daily dose equivalent to about 20 mg/kg.

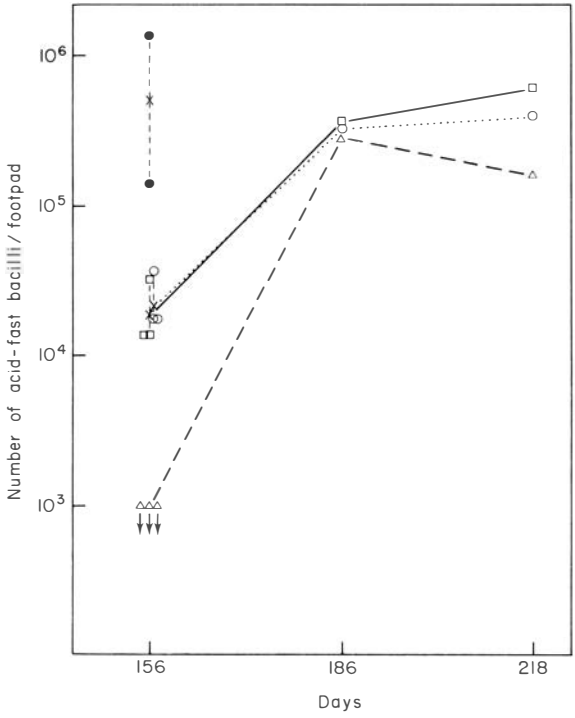

Fig. 1. Growth curves of *M. leprae* in mouse pads of untreated animals and animals treated with streptomycin (SM). ●, untreated; △, SM once weekly for days 2–72; ○, SM once weekly for days 29–99; □, SM once weekly for days 57–127.

The cause of death for the mice receiving 100 mg/kg in Experiment 2 of Table 1 has not been determined and later on this dosage was administered without any harm.

It is generally accepted that SM in experimental tuberculosis in mice is less effective than in man because, in an established murine infection, in contrast with man almost all tubercle bacilli are located intracellularly, and the drug is known to be less active intracellularly (Suter, 1952; MacKaness and Smith, 1953; Grumbach, 1965).

M. leprae has an exceptionally long lag phase (Shepard, 1960). During the latter part of this phase the bacilli are situated intracellularly, and although the site of the bacilli during the early phase after inoculation is uncertain (Levy *et al.*, 1974; Desikan, 1975) it could well be partly extracellular. In the PBT administration of doses of 100 mg/kg b.w. of SM twice a week starting on day 1 had a high killing effect (93%). When treatment started after 3 weeks the killing effect was only 81%.

The higher antileprosy activity of SM when administered from day 2, as compared with treatment started on day 22, could be the result of its greater

activity on at least part of the inoculum situated extracellularly during the early phase of the infection. The effect of a delayed start of treatment was also apparent in the experiment depicted in Fig. 1, when treatment started on day 29, although starting treatment even later was no worse.

In terms of bactericidal activity SM falls between the activities of dapsone and ethionamide or prothionamide (Colston *et al.*, 1978) when treatment is started early during the experimental infection and near to the activity of dapsone when started later. However, it is the latter situation that more closely resembles the condition in man where treatment is started when the infection is well established and almost all *M. leprae* bacilli are in an intracellular situation.

Due to its side effects and the inconvenience of long periods of intramuscular injections of streptomycin, it would be difficult to use SM in monotherapy for long periods of time in the treatment of human leprosy. It could be a companion drug during the initial phase of treatment of multibacillary leprosy to prevent the emergence of resistance, and a component of regimens used in the treatment of dapsone resistant leprosy in countries with limited resources.

Finally the results of the TMIT indicate that SM in monotherapy would not be suited for short course treatment of paucibacillary leprosy in man, since SM did not sterilize the paucibacillary infection in the mouse within 24 weeks, when administrated twice a week.

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