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The bactericidal activity of various aminoglycoside antibiotics against *Mycobacterium leprae* in mice

R H GELBER*, P R HENIKA† & J B GIBSON‡
US Public Health Service Hospital, 15th Avenue and Lake Street, San Francisco, California 94118, USA

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Summary The killing potential of various aminoglycoside antibiotics for *Mycobacterium leprae* infection of the mouse foot-pad was studied, utilizing daily intraperitoneal therapy. Kanamycin (100 mg/kg), streptomycin (150 mg/kg), and amikacin (100 mg/kg) resulted in impressive killing of bacilli (99·7%, 97% and 96% bactericidal, respectively). Gentamicin (20 mg/kg) and tobramycin (20 mg/kg) were much less active (60% and 37% bactericidal). The bactericidal activity of these very high doses of kanamycin, streptomycin and amikacin compared favourably with those of other agents previously studied in a similar manner at relatively lower dosage levels.

Introduction

Aminoglycoside antibiotics have received only limited experimental and/or clinical attention for their potential role in the therapy of leprosy.[1–9] In 1964 the first study[8] on the activity of streptomycin in the treatment of experimental *Mycobacterium leprae* infection of the mouse foot-pad was reported. In this study, 2 mg of streptomycin injected subcutaneously 5 times weekly, continuously from the time of infection, prevented multiplication of *M. leprae* for the $15\frac{1}{2}$-month study duration. In a later study (1968)[7] *M. leprae*-infected mice were treated by the 'kinetic method' with 2 mg of streptomycin 3 times a week from day 30 to 86 following infection. The growth of *M. leprae* was found to be inhibited during the period of drug administration but resumed promptly when therapy was discontinued, suggesting that streptomycin was purely bacteriostatic. More

Present addresses:
* Seton Medical Center, 1900 Sullivan Avenue, Daly City, California 94015 (415/991-6652).
† 225 Redding Avenue, Castro Valley, California 94546.
‡ Department of Anatomy, Box 3011, Duke Univ. Med. Cen., Durham, North Carolina 27710.

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recently (1978), the activity of streptomycin against *M. leprae*-infected mice was studied.[6] It was found that 50–100 mg/kg was the minimal effective dose and that 100 mg/kg twice weekly to *M. leprae*-infected mice beginning 2 and 22 days after infection resulted in 93 and 81% killing respectively, as determined by the 'proportional bactericidal test'.[10] The only study of the activity of other agents of this class in experimental leprosy so far was published in 1967;[5] it found gentamicin 'partially' active in the treatment of *M. leprae*-infected mice by the subcutaneous route at a dose of 165 mg/kg 5–7 times weekly continuously from the time of infection. Because of the paucity of information, particularly of a quantitative nature, concerning the activity of various aminoglycosides against *M. leprae*, we initiated this study to compare the killing potential of high-dose daily therapy with a number of these agents against *M. leprae*-infected mice.

Materials and methods

The methods used in this study to assess bactericidal activity are necessitated because *M. leprae* has not yet been successfully cultivated in artificial media or tissue culture. In this study we utilized the 'proportional bactericidal test', first used by Hilson & Banerjee[11] with *Mycobacterium lepraemurium*, as Colston *et al.*[10] adapted it previously in order to assess the activity of ingested antimicrobials against *M. leprae*. Basically, this method involves inoculating groups of mice with serial dilutions of *M. leprae* in the foot-pads, treating the mice for a limited period, and then assessing bacillary growth after sufficient time has elapsed for detectable growth to have occurred from any surviving bacilli.

For the control and each treatment, 3 groups of 10 female BALB/c weanling mice were inoculated in both hind feet with 10^1, 10^2 or 10^3 *M. leprae*. Control mice were left untreated. Aminoglycosides were given as 60 daily (days 2–61) intraperitoneal injections in alternating sites, with each dose being given in 0·2 ml of sterile normal saline. Weekly average animal weights were used to maintain dosages at 150 mg streptomycin/kg (approximately 3·1 mg), 100 mg kanamycin/kg, 100 mg amikacin/kg, 20 mg tobramycin/kg or 20 mg gentamicin/kg. In addition, similar groups of mice were treated with 0·0001% dietary dapsone (the minimally effective dose[12]) or with a combination of 0·0001% dietary dapsone and daily streptomycin (150 mg/kg) injections. Generalized seizures immediately after drug administration, leading to death, occurred in up to 50% of the streptomycin and streptomycin + dapsone treated animals during the fifth week of therapy. This prompted discontinuation of streptomycin therapy in these groups on day 38.

One year after the completion of drug therapy, mice were killed and *M. leprae* were enumerated by standard techniques[13] in each of 10 foot-pads from all but 2 groups of mice. *M. leprae* from only 8 foot-pads were counted in 1 streptomycin-treated group because of early deaths, and *M. leprae* from only 8 foot-pads were

counted in 1 amikacin-treated group because of random animal mortality throughout the study period. For purposes of calculation, growth of *M. leprae* was presumed to have occurred when foot-pad counts were 5×10^4. Percent decrease in the size of the *M. leprae* population ('killing') was calculated by a 'most probable number' calculation[14] and the Spearman-Kärber calculation described by Shepard.[15]

Results

The foot-pad results and the resultant 'percent of bacteria killed' from this study are presented in Table 1 by means of both the most probable number and the Spearman-Kärber calculation. There was remarkable concurrence in the results found by the 2 methods, the Spearman-Kärber calculation having the advantage of allowing the expression of confidence limits. Kanamycin apparently was the most active agent ($99{\cdot}7 \pm 0{\cdot}2\%$ bactericidal), but both streptomycin and amikacin had impressive activity as well ($97 \pm 2\%$ and $96 \pm 3\%$ bactericidal respectively). Gentamicin was minimally active ($60 \pm 20\%$), while tobramycin's activity ($37 \pm 22\%$) was not significantly different from controls ($P = 0{\cdot}27$). The slightly decreased activity with the addition of dapsone to streptomycin, when compared to streptomycin, is within the error limits of the experimental system ($P = 0{\cdot}40$).

Table 1. Aminoglycoside antibiotics foot-pad results

	Positive/negative			Killed (%)	
	10^3	10^2	10^1	Most probable No. technique	Spearman–Kärber
Control	—*	10/0	9/1		
Streptomycin	10/0	3/5	0/10	97·2	97 ± 2
Kanamycin	2/8	2/8	0/10	99·8	$99{\cdot}7 \pm 0{\cdot}2$
Tobramycin	—*	10/0	7/3	28·7	37 ± 22
Gentamicin	—*	10/0	5/5	58·5	60 ± 20
Amikacin	9/1	6/4	0/8	97·3	96 ± 3
Dapsone	—*	—*	10/0		
Dapsone + streptomycin	10/0	5/5	1/9	95·7	95 ± 2

* Not counted.

Table 2 allows comparison of the killing potential towards *M. leprae* of the tested aminoglycosides in very high dosage with that of other agents previously studied[10] in dosages more nearly approximating those chronically tolerated in man. It is noteworthy that streptomycin, amikacin and kanamycin, in the high doses used in this study, are more active than certain of those drugs commonly used to treat leprosy patients, particularly dapsone.

Table 2. Killing potential of various antibiotics by the proportional bactericidal test

Drug (dietary concentration)	'Killed' (%)
Thiambutosine (0·1%)*	0
Thiocarlide (0·1%)*	0
Thiacetazone (0·1%)*	42
Dapsone (0·01%)*	78, 72
Clofazimine (0·01%)*	98
Clofazimine (0·003%)*	99, 96
Prothionamide (0·1%)*	98·6
Ethionamide (0·1%)*	98·6
Ethionamide (0·2%)*	97·4
Rifampicin (0·003%)*	99·9
Rifampicin (0·01%)*	100
Streptomycin	97·2
Amikacin	97·3
Kanamycin	99·8

* Data from Colston *et al.*[10]

Discussion

This study has demonstrated the significant antimicrobial activity against *M. leprae* of very high doses of streptomycin, kanamycin and amikacin and the inactivity of gentamicin and tobramycin. Cultivable mycobacteria have been demonstrated to exhibit a similar pattern of aminoglycoside sensitivity, with kanamycin and especially amikacin being particularly effective. It has been found[17] that amikacin at clinically achievable levels inhibited all 54 strains tested of *M. fortuitum* (MIC < 2 μg/ml) and all 11 strains of *M. chelonei* (MIC < 16 μg/ml). Though kanamycin inhibited all strains of *M. fortuitum* and *M. chelonei* at 16 μg/ml, the median MIC to it was 8 μg/ml and 1 μg/ml for amikacin. However, as in our study, Wallace found gentamicin and tobramycin less active: only 28% of the *M. fortuitum* strains were inhibited by clinically achievable levels of gentamicin and tobramycin (4 μg/ml); all strains were resistant to streptomycin (MIC > 32 μg/ml). It was found[17] that strains of *M. fortuitum* were universally susceptible to < 1 μg/ml amikacin, but that *M. chelonei* required as much as 32 μg/ml for inhibition. In this study kanamycin was more active against *M. chelonei* and less active against *M. fortuitum*.

It has been found[19] that all 33 tested strains of *M. fortuitum* and *M. chelonei* were inhibited by 2 μg/ml amikacin. Another study[19] found that amikacin and kanamycin were active against the 10 tested strains of *M. marinum* and that gentamicin was inactive. It has been found[20] that all isolates of *M. marinum* tested

were sensitive to amikacin and kanamycin and resistant to gentamicin and tobramycin. It has also been found[21] that amikacin was active against all 100 strains of a wide variety of atypical mycobacteria: 69 were sensitive to 1·6 μg/ml, 30 required 3·2 μg/ml and 1 was only sensitive to 6·7 μg/ml.

Few published or pharmaceutical-company references exist for chronic administration of high doses of aminoglycosides to mice, and fewer for prolonged intraperitoneal injection of the agents (data from computer-assisted searches of literature and in-plant information, James T. Baldini, Schering Corporation, Harold W. Brinkley, Bristol Laboratories, Robert J. Petrick, Pfizer Laboratories, written communications; and Medline search). Because of this dearth of literature, dosage schedules generally had to be extrapolated from subcutaneous administration schedules in mice or rats and intraperitoneal injection data in rats. Despite the potential for neuromuscular blockade, nephrotoxicity and resultant death, mice treated with kanamycin, amikacin, tobramycin or gentamicin retained sufficient renal function to survive for the year after the protracted high-dose course of antibiotics. Mice treated with streptomycin fared well until the fifth week, when their therapy was prematurely halted because of seizure-related deaths immediately after injection. All mice treated with streptomycin and surviving this therapeutic period had enough renal reserve to live until the completion of the study.

The practicality and usefulness of aminoglycosides in the treatment of human leprosy have not yet been established. A number of studies (judged by clinical criteria alone) have shown that streptomycin is active in human leprosy.[1–4] A small pilot trial in previously untreated lepromatous leprosy patients in Malaysia, utilizing streptomycin intramuscularly in a daily dose of 0·75–1·5 g, resulted in clinical improvement comparable to dapsone, a fall in the morphological index (percent of solid-staining bacilli in skin smears, generally correlating with viability) similar to that observed with dapsone and a loss of mouse foot-pad infectivity of skin biopsy specimens that was somewhat faster than with dapsone.[9] Five of the ten dapsone-resistant patients treated with dapsone and streptomycin by Hastings *et al.*[22] relapsed clinically, and new lesions showed high morphological indices after only 23–31 months of treatment with both drugs. Unfortunately, streptomycin resistance was not proved by mouse inoculation in these cases. No leprosy trials with other agents of this class have been published.

Aminoglycosides, as a class of antimicrobial agents, may have a place in the therapy of leprosy if pharmacological and toxic problems associated with their use can be circumvented. The potential for utilization of the aminoglycosides in treatment of infectious diseases, including leprosy, is limited by the necessity of their injection and by their pronounced oto- and nephrotoxicity in acute, subacute or chronic administrations. On the other hand, rifampin and streptomycin have been found to be truly synergistic against *M. kansasii* and *M. intracellulare* infections of mice.[23] If such synergism was found for *M. leprae*, the discovery would certainly rekindle enthusiasm for a reconsideration of a clinical

role for these agents. Pattyn *et al.*[6] have found once weekly streptomycin to be equally effective in inhibiting growth of *M. leprae* in mice to twice and thrice weekly. As WHO[24] has recommended intermittent supervised combination therapy, the use of aminoglycoside antibiotics might prove especially practical, if aminoglycoside therapy could be correspondingly spaced out to monthly intervals. Experiments are currently in progress in mice to assess the combined activity of certain of these active aminoglycosides with rifampin and the efficacy of decreasing the dose and frequency of aminoglycoside administration.

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