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Further Studies of the Killing of *M. leprae* by Aminoglycosides: Reduced Dosage and Frequency of Administration[1]

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There have been a number of reports demonstrating the activity of streptomycin against *Mycobacterium leprae* infections in mice and man ([2-7, 9, 11, 14, 16]). Previously, we reported ([7]) impressive killing of *M. leprae* by kanamycin, streptomycin, and amikacin, and a lack of significant bactericidal activity for gentamicin and tobramycin. We also reported ([7]) that the minimal effective mouse dietary concentration of dapsone did not potentiate the bactericidal activity of streptomycin for *M. leprae*. In these studies aminoglycosides were administered in high daily intraperitoneal doses. Unfortunately, aminoglycosides are not absorbed *per os*. Daily injections would not be operationally feasible in many leprosy endemic locales. Also, the high doses utilized in our previous mouse studies might potentially be associated with renal or acousto-vestibular toxicity, especially if required for prolonged periods. Thus the current studies, designed to investigate the bactericidal activity of streptomycin and kanamycin at decreased doses and frequencies of administration, were conducted. Also, because rifampin has proved the most potent bactericidal agent against *M. leprae* infections in mice and man and because for certain other experimental mycobacterial infections of mice the combination of an aminoglycoside and rifampin has proved synergistic ([15]), the bactericidal activity of streptomycin and kanamycin combined with rifampin was also assessed.

MATERIALS AND METHODS

In these studies we employed the proportional bactericidal test ([1]). This method involves inoculating groups of mice with several dilutions of *M. leprae*, treating for a limited period, and then assessing bacterial growth after a sufficient time has elapsed for detectable growth to have occurred from any surviving bacilli.

For the control and each treatment group, four groups of ten female BALB/c mice were inoculated in both hindfeet with 10^1, 10^2, 10^3, and 10^4 *M. leprae*. For the streptomycin study, the *M. leprae* inoculum was a fast-growing strain of *M. leprae* originally obtained from an untreated lepromatous patient and long maintained in mouse passage. For the kanamycin study, the inoculum was obtained directly from a skin biopsy of a previously untreated lepromatous leprosy patient. In all instances, therapy commenced the day following infection and concluded 60 days later. In order to assess different dosage levels, streptomycin and kanamycin were administered intraperitoneally (i.p.) 5 times weekly at 100 mg/kg, 50 mg/kg, 25 mg/kg, and 12.5 mg/kg. In order to assess intermittent treatment, groups of mice were treated with streptomycin or kanamycin 100 mg 5 times weekly, 2 times weekly, weekly, every 2 weeks, and monthly. Also, both streptomycin and kanamycin 100 mg/kg i.p. were administered to groups of mice monthly (three doses) together with monthly rifampin 20 mg/kg by gavage (three doses). Groups of mice were also treated with both streptomycin and kanamycin 100 mg/kg i.p. 5 times weekly together with the same three monthly doses of rifampin. Lastly, the effect of rifampin alone, 20 mg/kg, by gavage monthly (three doses) was similarly studied.

One year after completion of therapy, *M. leprae* from usually ten foot pads from each group of mice were enumerated by standard techniques ([10]). Growth of *M. leprae* was presumed to have occurred when *M. leprae*/foot pad were $\geq 5 \times 10^4$. The percent decrease in the size of the viable *M. leprae* population, the percentage bactericide, was

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TABLE 1. *Streptomycin killing of* M. leprae.

Treatment	MFP[a] with viable *M. leprae*/MFP without live *M. leprae*				*M. leprae* killed ±S.E.[b]
	10^4	10^3	10^2	10^1	
Controls	8/0	9/1	10/0	8/2	
Streptomycin 100 mg/kg 5× weekly	9/1	10/0	4/6	0/10	96% ± 2%
Streptomycin 50 mg/kg 5× weekly	10/0	4/0	5/3	1/3	85% ± 12%
Streptomycin 25 mg/kg 5× weekly	6/0	9/1	6/4	0/10	94% ± 4%
Streptomycin 12.5 mg/kg 5× weekly	8/0	8/0	8/2	0/10	87% ± 6%
Streptomycin 100 mg/kg 2× weekly		6/0	7/0	3/5	76% ± 16%
Streptomycin 100 mg/kg 1× weekly	8/0	4/0	10/0	0/2	80% ± 9%
Streptomycin 100 mg/kg once every 2 weeks	8/0	10/0	10/0		≤80% ± 8%
Streptomycin 100 mg/kg once monthly		6/0	9/1		≤84% ± 7%
Rifampin 20 mg/kg day 1, 30, 60	9/1	7/3	9/1		94% ± 4%
Streptomycin 100 mg/kg day 1, 30, 60 plus rifampin 20 mg/kg day 1, 30, 60	2/8	1/9	0/10		99.96% ± 0.02%
Streptomycin 100 mg/kg 5× weekly plus rifampin 20 mg/kg day 1, 30, 60	3/5	3/7	0/10	0/10	99.91% ± 0.06%

[a] MFP = mouse foot pad.
[b] The percentage bactericide was calculated by the Spearman and Kärber method ([13]).

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RESULTS

The results of the study with streptomycin are presented in Table 1. The strain of *M. leprae* was 96 ± 2% killed by streptomycin 100 mg/kg 5 times weekly, which is remarkably similar to the 97 ± 2% bactericide found by us previously for another strain of *M. leprae* following treatment with streptomycin 150 mg/kg daily. A reduced 5 times weekly dosage of 50 mg/kg, 25 mg/kg, and 12.5 mg/kg retained bactericidal activity of 85 ± 12%, 94 ± 4%, and 87 ± 6%, respectively, which although less active than 100 mg 5 times weekly, was not significantly so. Reducing the frequency of administration of streptomycin from five times weekly to twice weekly and once weekly was associated with significant but reduced bactericidal activity ($p < 0.02$). Unfortunately, all of the mice injected with 10^1 *M. leprae* and treated with streptomycin 100 mg every 2 weeks and monthly died; hence, the percentage bactericide can only be expressed as ≤80% and ≤84%, respectively. It is noteworthy that while monthly rifampin was found 94 ± 4% bactericidal, the combinations of monthly rifampin with monthly streptomycin and monthly rifampin plus 5 times weekly streptomycin were more bactericidal, 99.96 ± 0.02% and 99.91 ± 0.06% bactericidal, respectively, than either agent alone ($p < 0.01$).

The kanamycin study was seriously flawed by the poor viability of the *M. leprae* strain utilized. In this study, kanamycin 100 mg/kg 5 times weekly was found 89 ± 6% bactericidal. Decreased dosages (50 mg/kg, 25 mg/kg, and 12.5 mg/kg) 5 times weekly appeared inactive. A decreased frequency of administration was also associated with no significant bactericidal activity. In this study, rifampin 20 mg/kg monthly resulted in significant killing, 89 ± 7% bactericide. For the two regimens of kanamycin plus rifampin, even those mice inoculated with 10^4 *M. leprae* did not demonstrate viable bacilli after therapy, and thus the percent bactericide is expressed as ≥93% and ≥94%. Although not statistically significant, once again the two combination regimens of kanamycin and rifampin were found more active than either drug alone.

DISCUSSION

Previously, Pattyn ([9]) found no significant difference in streptomycin's ability to inhibit *M. leprae* multiplication in mice if it was given 1, 2, or 3 times weekly. In our studies, despite reduced dosage and frequency of administration, streptomycin's bactericidal activity for *M. leprae* in mice, though reduced, is largely retained. This does not appear to be the case for kanamycin.

TABLE 2. *Kanamycin killing of* M. leprae.

Treatment	MFP[a] with viable *M. leprae*/MFB without live *M. leprae*				*M. leprae* killed ±S.E.[b]
	10^4	10^3	10^2	10^1	
Controls	5/5	10/10	0/6	4/12	
Kanamycin 100 mg/kg 5× weekly	3/13	0/18	0/10	0/18	89% ± 6%
Kanamycin 50 mg/kg 5× weekly	6/4	4/6	0/10		44% ± 42%
Kanamycin 25 mg/kg 5× weekly	5/5	4/6	2/8	0/10	29% ± 58%
Kanamycin 12.5 mg/kg 5× weekly	5/5	4/6	4/6	0/4	11% ± 75%
Kanamycin 100 mg/kg 2× weekly	8/2	4/6	0/10		11% ± 64%
Kanamycin 100 mg/kg 1× weekly	8/2	7/3	2/6	1/9	75% ± 21%
Kanamycin 100 mg/kg every other week	10/0	4/6	1/9	0/10	44% ± 39%
Kanamycin 100 mg/kg 1× monthly	9/1	7/3	1/9	1/9	72% ± 21%
Rifampin 20 mg/kg day 1, 30, 60	2/8	1/9	0/10		89% ± 7%
Rifampin 20 mg/kg plus kanamycin 100 mg/kg day 1, 30, 60	0/10	1/9	0/8	0/10	≥93% ± 4%
Rifampin 20 mg/kg day 1, 30, 60 plus kanamycin 100 mg/kg 5× weekly	0/10	0/10	0/10		≥94% ± 3%

[a] MFP = mouse foot pad.
[b] The percentage bactericide was calculated by the Spearman and Kärber method ([13]).

The differences between reduced dosages of streptomycin and kanamycin found in these two studies could be a result of varying aminoglycoside sensitivities of the two strains studied or, alternatively, a significant difference between streptomycin and kanamycin pharmacokinetics in mice. While 100 mg streptomycin i.p. in mice results, after distribution, in peak plasma levels approximating those in man following the customary 1.0 g intramuscular (i.m.), 40 μg/ml ([8, 17]), and is cleared from the plasma with a half-life of 2–3 hr in both species ([8, 17]), kanamycin's pharmacokinetics in man are quite different from those in the mouse. While in mouse and man once again peak plasma levels in the two species following 100 mg i.p. in mice and a customary 500 mg i.m. dose in man are quite similar ([8, 18]), 30 μg/ml, kanamycin has a plasma half-life in man of 4 hr ([18]) and a plasma half-life in mice of less than 0.5 hr ([8]). Thus, it might be that in these studies very rapid clearance of kanamycin in mice accounts for its loss of activity by both decreased dosage and frequency of administration; while a sufficient time-concentration product at the ribosomal site of drug action is retained for streptomycin by similar reductions in dosage and frequency of administration. Furthermore, the more favorable pharmacokinetics of kanamycin in man might negate the differences found in these animal studies by reduced dosages between kanamycin and streptomycin, resulting in kanamycin's being an equally potent agent for *M. leprae* in man by such dosage manipulations.

Of considerable importance is that even the largest doses of both streptomycin and kanamycin used in these studies yielded plasma levels in mice that are no greater than those experienced by patients receiving the usual and customary i.m. doses of these agents. Also, encouraging for the clinical application of certain aminoglycosides to the therapy of leprosy is the clinical trial in lepromatous leprosy in Malaysia, wherein daily i.m. streptomycin resulted in clinical improvement comparable to dapsone, a fall in the morphological index similar to that following dapsone, and a loss of mouse foot pad infectivity of skin-biopsy specimens that was somewhat faster than with dapsone ([16]).

Most noteworthy in this study is the consistently increased bactericidal activity found for rifampin plus aminoglycosides as compared to either agent alone. Such combinations appear at least additive and likely synergistic. The same cannot be said to be consistently true in mice for combinations of rifampin with dapsone or ethionamide ([17]). It is encouraging in these studies that the increased activity of rifampin with kanamycin or streptomycin can be demonstrated in mice even by monthly administration. Since monthly supervised rifampin has been recommended by the World Health Organization ([19]) and is frequently

utilized in the therapy of leprosy, the addition of monthly streptomycin might prove even more efficacious. Furthermore, streptomycin acts at a different locus than established antimicrobial agents that have been found to be similarly active against *M. leprae* and, hence, may be an acceptable alternative agent in multidrug regimens. Because undesirable side effects and intolerance to dapsone, clofazimine, and ethionamide are certainly not uncommon, the options that aminoglycosides offer in the therapy of leprosy require consideration in selected cases. Monthly supervised rifampin and streptomycin would also have the advantage of supervised multidrug therapy without necessarily employing an agent such as clofazimine, which remains cosmetically unacceptable in many parts of the world.

SUMMARY

The bactericidal activity of the aminoglycoside antibiotics streptomycin and kanamycin for *Mycobacterium leprae* in mice was assessed, both alone and in combination with rifampin, utilizing various dosage schedules. As in previous studies, 100 mg/kg five times weekly of streptomycin and kanamycin resulted, respectively, in 96% ± 2% and 89% ± 6% bactericide. Reducing the dosage of streptomycin to 50 mg/kg, 25 mg/kg, and even 12.5 mg/kg resulted in less but significant bactericidal activity. Such a reduction of kanamycin dosage resulted in no significant bactericidal activity. Reducing the frequency of administration of streptomycin (100 mg/kg) to twice weekly and once weekly resulted in a decreased but still significant killing of *M. leprae*; for kanamycin such a reduction in frequency of administration resulted in loss of bactericidal activity. Streptomycin when combined with rifampin was found more bactericidal than either drug alone, even when each was administered only once monthly.

RESUMEN

Se estudió la actividad bactericida de la estreptomicina y de la kanamicina sobre el *Mycobacterium leprae* en el ratón usando las drogas solas o en combinación con rifampina a varias dosis y esquemas de administración. Como en estudios previos, la estreptomicina y la kanamicina en dosis de 100 mg/kg cinco días a la semana, tuvieron una potencia bactericida del 96 ± 2% y 89 ± 6%, respectivamente. La reducción de las dosis de estreptomicina a 50 mg/kg, 25 mg/kg, y aún 12.5 mg/kg, dió como resultado una menor pero significativa actividad bactericida. Una reducción similar en la dosis de kanamicina hizo que ésta perdiera su actividad bactericida. Reduciendo la frecuencia de administración de estreptomicina (100 mg/kg) a dos y a una veces por semana, se encontró una disminuída pero aún significante capacidad bactericida sobre el *M. leprae*; para la kanamicina tal reducción en la frecuencia de administración dió como resultado una pérdida de la actividad bactericida. La estreptomicina combinada con rifampina fue más bactericida que cualquiera de las drogas solas, aún cuando su administración se hiciese sólo una vez por mes.

RÉSUMÉ

On a évalué chez la souris l'activité bactéricide pour *Mycobacterium leprae* de deux aminoglycosides dotés de propriétés antibiotiques, la streptomycine et la kanamycine, à des posologies variées, en administration exclusive ou en combinaison avec la rifampine. Ainsi qu'on l'avait noté dans les études antérieures, la streptomycine et la kanamycine à des doses de 100 mg/kg, cinq fois par semaine, ont témoigné d'activités bactéricides respectives de 96% ± 2% et de 89% ± 6%. Une réduction des doses de streptomycine à 50 mg/kg, à 25 mg/kg, et même à 12,5 mg/kg, a abouti à une activité bactéricide moindre mais toutefois encore notable. Une réduction similaire du dosage de la kanamycine supprimait l'activité bactéricide. Lorsqu'on diminuait la fréquence de l'administration de la streptomycine, à la dose de 100 mg/kg, à deux administrations hebdomadaires, et même à une seule administration au cours de la semaine, on observait une réduction dans le pouvoir bactéricide contre *M. leprae*, mais celui-ci était cependant encore significatif. Par contre, avec la kanamycine, une telle diminution de la fréquence d'administration a entraîné une perte du pouvoir bactéricide. La streptomycine, en combinaison avec la rifampine s'est révélée plus bactéricide que l'un ou l'autre de ces médicaments donnés seuls, et ceci même lorsque ces médicaments étaient administrés une seule fois par mois.

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