Clarithromycin Is Bactericidal Against Strains of *Mycobacterium leprae* Resistant and Susceptible to Dapsone and Rifampin[1]

Laura L. Walker, Rosalind M. Van Landingham, and Thomas M. Shinnick[2]

Hansen's disease is a chronic, infectious disease that afflicts 10–15 million people worldwide, mainly in developing countries ([1]). This disease results from an infection with the acid-fast bacillus *Mycobacterium leprae* and, if left untreated, can result in disability and deformity. The use of sulfones to treat Hansen's disease began in the 1940s, and dapsone has been used widely as monotherapy for over 40 years ([2]). In response to the appearance of dapsone-resistant isolates of *M. leprae,* the World Health Organization (WHO) began recommending multidrug therapy (MDT) with dapsone, rifampin, and clofazimine in 1982 ([18]). However, rifampin-resistant and dapsone- and rifampin-doubly resistant strains of *M. leprae* have been reported ([8, 9]). Although reports of clofazimine-resistant isolates of *M. leprae* are rare and have not yet been confirmed ([17]), there is a clear need for new anti-*M. leprae* drugs to combat the potential drug-resistant strains. Also, because rifampin is the only powerful bactericidal anti-*M. leprae* agent currently used, new bactericidal drugs are needed to improve the chemotherapeutic regimen.

Interest in the use of macrolides against intracellular pathogens such as *M. leprae* arose from the observation that erythromycin was active against *Chlamydia trachomatis* and *Legionella pneumophila* ([12]). Macrolides interfere with protein synthesis by blocking ribosomal function ([12]) which would represent a new target for anti-*M. leprae* agents. Franzblau and Hastings ([5]) surveyed several macrolides for activity against *M. leprae* using an *in vitro* radiometric assay of metabolic activity. The most active of these was clarithromycin. Clarithromycin differs from erythromycin in the methylation at the 6-hydroxy position of the macrolide ring ([7]).

In animal studies, clarithromycin was found to have bactericidal activity against pan-susceptible strains of *M. leprae* ([5, 6]). It has also been reported to be active against *M. avium in vitro* and *in vivo* ([3, 4]). We report here the activity of clarithromycin, alone and in multidrug combinations with dapsone and rifampin, against pan-susceptible, dapsone-resistant, rifampin-resistant, and dapsone + rifampin-resistant strains of *M. leprae* in the mouse foot pad model of infection.

MATERIALS AND METHODS

Strains. The strains used in these studies and their sources are listed in Table 1. The pan-susceptible and the dapsone-resistant strains were maintained in regular mouse passage ([14]) without drug treatment. Strains resistant to rifampin and to dapsone + rifampin were maintained on a diet containing 0.01% rifampin in regular mouse passage.

Antimicrobial agents. Clarithromycin (Abbott-56268; TE-031) was discovered by Taisho Pharmaceutical Co., Japan, and was donated by Abbott Laboratories, Abbott Park, Illinois, U.S.A. Rifampin was purchased from Sigma Chemical Company, St. Louis, Missouri, U.S.A., and dapsone was purchased from ICN Biomedicals, Inc., Plainview, New York, U.S.A. The drugs were mixed with ground commercial mouse chow (Rodent Lab Chow 5001; Purina Mills, Inc., Richmond, Indiana, U.S.A.) in a twin-shell blender (Patterson-Kelly Co., East

[1] Received for publication on 13 October 1992; accepted for publication in revised form on 7 January 1993.

[2] L. L. Walker, B. S.; R. M. Van Landingham, M.A.; T. M. Shinnick, Ph.D., Division of Bacterial and Mycotic Diseases, Mail Stop G35, Centers for Disease Control, 1600 Clifton Road NE, Atlanta, Georgia 30333, U.S.A.

TABLE 1. Mycobacterium leprae *strains used in the study.*

Strain	Drug resistance[a]	Source
B2602	None	USPHS Hospital[b]
N2538	None	USPHS Hospital[b]
B2631	Dapsone	USPHS Hospital[b]
B3000	Dapsone	Russell[c]
B2000	Dapsone	Levy[d]
B3026	Rifampin	Jacobson[e]
B81030	Rifampin	Grosset[f]
B82061	Rifampin + dapsone	Grosset[f]

[a] Growth of susceptible strains is inhibited by including 0.0001% dapsone or 0.01% rifampin in the diet. Dapsone-resistant strains are resistant to 0.01% of the drug in the diet. Rifampin-resistant strains are resistant to 0.1% rifampin in the diet. The dapsone + rifampin-resistant strain is resistant to 0.01% dapsone and 0.03% rifampin in the diet.

[b] United States Public Health Service Hospital, Carville, Louisiana, U.S.A.

[c] Dr. Douglas Russell, Brisbane, Australia (World Health Organization).

[d] Dr. Louis Levy, United States Public Health Service Hospital, San Francisco, California, U.S.A.

[e] Dr. Robert Jacobson, United States Public Health Service Hospital, Carville, Louisiana, U.S.A.

[f] Dr. Jacques Grosset, Pitié-Salpêtrière School of Medicine, Paris, France.

Stroudsburg, Pennsylvania, U.S.A.), and concentrations were expressed as weight/weight percentages of the diet. A concentration of 0.001% in the diet corresponds to a dosage of about 1.0 mg/kg mouse weight/day. Control groups were fed diets containing no drug.

Evaluation of drug activities *in vivo*. The kinetic method for determining drug activity against *M. leprae* in the mouse foot pad model was used as previously described ([14]). Briefly, approximately 5000 *M. leprae* cells were injected into the right hindfoot pad of 3-week-old CFW female mice. Mice were fed a diet containing drugs in various concentrations alone and in multidrug combinations from day 70 after inoculation through day 126, which is approximately the logarithmic phase of growth ([14]). Growth of the bacilli in the foot pads of mice fed a drug-free diet was monitored monthly beginning at day 70 by pooling foot pad tissue from four mice and enumerating the bacilli. When bacilli in this group reached approximately 10^6 bacilli per foot pad or a plateau level (similar numbers in two consecutive harvests), foot pads from five mice in each group were removed individually and bacilli were enumerated in each. This first time point varied from 5 to 7 months after challenge. A second assay was done 5–6 months after the first assay, and a third assay was done 3–6 months later if mice were available. The numbers of bacilli per foot pad in the various drug groups were compared statistically to their respective control group using the Wilcoxon rank sum test ([15]). Differences of $p < 0.05$ were considered statistically significant. Because the *M. leprae* had grown to 10^4 to 10^5 acid-fast bacilli (AFB) per foot pad by day 70 (the start of drug administration), growth of the *M. leprae* after drug treatment was considered significant if the foot pads contained $> 2 \times 10^5$ AFB per foot pad.

Results

Activity against pan-susceptible strains. In a preliminary experiment, mice were infected with the pan-susceptible strain N2538 and fed diets containing clarithromycin at concentrations of 0.1%, 0.01%, or 0.001%. Clarithromycin at 0.01% and 0.001% in the diet did not significantly inhibit the growth of the *M. leprae* at any of the time points (7, 11, or 13 months postchallenge; data not shown). In contrast, no bacilli were detected in the foot pads of mice fed the 0.1% concentration even at 340 days post-inoculation (214 days after cessation of treatment). Therefore, in the subsequent studies, clarithromycin was administered in concentrations of 0.03% and 0.1% in the diet.

Growth of the pan-susceptible strain B2602 was inhibited ($p < 0.05$) at both time points by clarithromycin at a dietary concentration of 0.1% (Table 2). Furthermore, no growth of *M. leprae* was observed in the foot pads of any of the mice in this treatment group assayed 368 days after inoculation. Clarithromycin when tested at a concentration of 0.3% in the diet (Table 2)

TABLE 2. *Activity of clarithromycin against the pan-susceptible strain B2602 and the dapsone + rifampin-resistant strain B82061.*

Drugs[a]			B2602				B82061			
			Day[b] 179		Day 368		Day 176		Day 368	
DDS	CLA	RA	AFB[c] × 10^5	(+/t)	AFB × 10^5	(+/t)	AFB × 10^5	(+/t)	AFB × 10^5	(+/t)
0.01	—	—	—	(0/5)	41–96	(5/5)	13–30	(5/5)	7–107	(5/5)
—	0.03	—	—	(0/5)	51–88	(3/5)	—	(0/5)	2–8	(3/5)
—	0.1	—	—	(0/5)	—	(0/5)	—	(0/5)	—	(0/5)
—	—	0.01	—	(0/5)	—	(0/5)	9–15	(5/5)	2–49	(5/5)
—	0.1	0.01	—	(0/5)	—	(0/5)	—	(0/5)	—	(0/5)
0.01	0.1	—	—	(0/5)	—	(0/5)	—	(0/5)	—	(0/5)
0.01	—	0.01	—	(0/5)	8–11	(2/5)	9–30	(5/5)	2–111	(5/5)
0.01	0.1	0.01	—	(0/5)	—	(0/5)	—	(0/5)	—	(0/5)
—	—	—	12–39	(5/5)	12–44	(5/5)	12–37	(5/5)	6–28	(5/5)

[a] The dose (% w/w) of dapsone (DDS), clarithromycin (CLA), and rifampin (RA) included in the diet; — = no drug included in the diet.

[b] Number of days postchallenge at which foot pads were harvested.

[c] The range of bacilli per foot pad displaying > 2 × 10^5 AFB and, in parentheses, the number of foot pads with > 1 × 10^5 AFB/total number of mice assayed at that time point.

significantly inhibited ($p < 0.05$) the growth of B2602 in the foot pads of all mice assayed at the first time point (day 179), but prevented growth in only 2 of the 5 mice assayed 368 days after inoculation.

Dapsone and/or rifampin (Table 2) included in the diet did not antagonize the ability of clarithromycin at 0.1% in the diet to completely prevent growth of this pan-susceptible strain. Interestingly, growth was observed at the day 368 time point in foot pads of 2 of the 5 mice fed diets containing 0.01% dapsone + 0.01% rifampin, but not in the foot pads of any of the five mice fed diets containing only 0.01% rifampin. This is consistent with the suggested antagonism between dapsone and rifampin ([13]).

Activity against dapsone-resistant strains. As expected, the dapsone-resistant strains (B2000, B2631, B3000) grew in the foot pads of mice fed a diet containing 0.01% dapsone (Table 3). Dapsone at this concentration in the diet did not interfere with the ability of clarithromycin at 0.1% in the diet to prevent growth of strain B3000 in the foot pads of all mice assayed. When tested at the 0.03% concentration, clarithromycin prevented growth of this strain in 4 of the 5 mice assayed 405 days postchallenge.

In vivo growth of two of the dapsone-resistant strains, B2631 and B2000, was rapid and already at detectable numbers when treatment started on day 70. Since bacilli are cleared very slowly from the foot pad ([16]), anti-*M. leprae* activity is assessed in this case by the absence of an increase in the number of bacilli per foot pad. Clarithromycin when fed at a concentration of 0.1% alone or in combination with 0.01% dapsone prevented any significant increase ($p < 0.05$) in the numbers of strain B2631 (day 294) or strain B2000 (day 362). Clarithromycin when fed at a concentration of 0.03% significantly inhibited ($p < 0.05$) the growth of strains B2631 and B2000 in the foot pads of all mice assayed at the first time points (day 152 and 154) but in none of the foot pads of five mice infected with strain B2000 and assayed 362 days postchallenge, and in the foot pad of only 1 of 5 mice infected with strain B2631 and assayed 294 days postchallenge. In addition, growth of strain B2631 was not inhibited in the foot pads of any of four mice assayed 480 days postchallenge (data not shown).

Activity against rifampin-resistant strains. Rifampin-resistant strains B3026 and B81030 grew in the foot pads of mice fed a diet containing 0.01% of the drug (Table 4). Clarithromycin at a concentration of 0.1%, alone or in combination with 0.01% rifampin, prevented growth of these rifampin-resistant strains in the foot pads of all mice assayed. When tested at a concentration of 0.03%, clarithromycin significantly inhibited growth ($p < 0.05$) of B3026 in the foot pads of all five mice at the first time point (day 180) but did not inhibit growth in the foot pads of any of the mice assayed 377 days postchallenge. This concentration

TABLE 3. *Activity of clarithromycin against dapsone-resistant strains B2000, B3000, and B2631.*

Drugs[a]		B2000				B3000				B2631			
		Day[b] 154		Day 362		Day 209		Day 405		Day 152		Day 294	
DDS	CLA	AFB[c] × 10^5	(+/t)	AFB × 10^5	(+/t)	AFB × 10^5	(+/t)	AFB × 10^5	(+/t)	AFB × 10^5	(+/t)	AFB × 10^5	(+/t)
0.01	–	23–41	(5/5)	4–271	(5/5)	7–42	(5/5)	19–94	(5/5)	20–54	(5/5)	23–83	(5/5)
–	0.03	–	(0/5)	19–130	(5/5)	–	(0/5)	37	(1/5)	–	(0/5)	33–89	(4/5)
–	0.10	–	(0/5)	–	(0/5)	–	(0/5)	–	(0/5)	–	(0/5)	–	(0/5)
0.01	0.10	–	(0/5)	–	(0/5)	–	(0/5)	–	(0/5)	–	(0/5)	–	(0/5)
–	–	14–96	(5/5)	31–229	(5/5)	2–9	(5/5)	4–30	(5/5)	6–65	(5/5)	8–38	(5/5)

[a] The dose (% w/w) of dapsone (DDS) and clarithromycin (CLA) included in the diet; – = no drug included in the diet.
[b] Number of days postchallenge at which foot pads were harvested.
[c] The range of bacilli per foot pad displaying > 2 × 10^5 AFB and, in parentheses, the number of foot pads showing that growth/total number of mice assayed at that time point.

of clarithromycin in the diet also completely prevented the growth of B81030 since no bacilli were found in any of the five foot pads of the mice assayed 383 days postchallenge (Table 4) or in the foot pads of the two mice assayed 459 days postchallenge (data not shown).

Strain B82061, which is resistant to both dapsone and rifampin, grew well in the foot pads of mice fed diets containing 0.01% dapsone and 0.01% rifampin (Table 2). Growth of this doubly resistant strain was prevented in the foot pads of all mice assayed by clarithromycin at 0.1% in the diet alone or in combination with dapsone and/or rifampin. Clarithromycin, when fed at a concentration of 0.03%, significantly inhibited growth ($p < 0.05$) in the foot pads of all mice at the first time point (day 176) but in only 2 of 5 mice assayed 368 days postchallenge. Similarly, growth was prevented in only 1 of 4 foot pads assayed 501 days postchallenge (data not shown).

DISCUSSION

In the kinetic method of drug evaluation, drugs are administered only from day 70 through day 126 after inoculation. A drug that is bacteriostatic will delay the growth of *M. leprae* for the period that the drug is present in the foot pad tissue in effective concentrations. A drug that kills all of the bacilli will prevent any growth after removal of the drug. Killing a fraction of the bacilli will cause a growth delay greater than the period during which the drug is present. The length of the growth delay is roughly proportional to the number of bacilli killed.

Using the kinetic method of drug evaluation in the mouse foot pad model, Gelber, *et al.* ([6]) showed that 0.1% clarithromycin in the diet prevented multiplication of a pansusceptible *M. leprae* strain in the foot pads of all mice assayed as long as 11 months after inoculation. In addition, using the proportional bactericidal technique ([6]), they reported that a dietary concentration of 0.1% clarithromycin was 96% ± 2% bactericidal since 0 of 10 foot pads inoculated with 10^3 *M. leprae* had detectable bacilli after 1 year. Ji, *et. al.*, also using the proportional bactericidal technique in mice ([10]), reported strong bactericidal activity of clarithromycin against *M. leprae* when administered by gavage as 20 daily doses at levels of 12.5 to

TABLE 4. *Activity of clarithromycin against the rifampin-resistant strains B81030 and B3026.*

Drugs[a]		B81030				B3026			
		Day[b] 215		Day 383		Day 180		Day 377	
CLA	RA	AFB[c] × 10^5	(+/t)	AFB × 10^5	(+/t)	AFB × 10^5	(+/t)	AFB × 10^5	(+/t)
—	0.01	5.7–19	(5/5)	8–52	(5/5)	45–68	(5/5)	29–342	(5/5)
0.03	—	—	(0/5)	—	(0/5)	—	(0/5)	13–45	(5/5)
0.10	—	—	(0/5)	—	(0/5)	—	(0/5)	—	(0/5)
0.10	0.01	—	(0/5)	—	(0/5)	—	(0/5)	—	(0/5)
—	—	2–20	(4/5)	5–15	(4/5)	12–46	(5/5)	23–102	(5/5)

[a] The dose (% w/w) of clarithromycin (CLA) and rifampin (RA) included in the diet; — = no drug included in the diet.

[b] Number of days postchallenge at which foot pads were harvested.

[c] The range of bacilli per foot pad displaying > 2 × 10^5 AFB and, in parentheses, the number of foot pads showing that growth/total number of mice assayed at that time point.

50 mg/kg of body weight (87.4%–94.9% bactericide). In our studies, 0.1% clarithromycin in the diet completely prevented growth of each of the eight strains tested. Assuming that growth begins when drug administration is stopped (day 126) and that the generation time of *M. leprae* is 12.5 days ([14]), the failure to observe growth by day 350 in our experiments indicates that no viable bacilli were present in the foot pad on day 126. If so, this suggests that the 0.1% clarithromycin treatment was > 99.9% bactericidal because there were 10^4 to 10^5 bacilli per foot pad at the beginning of treatment. Similarly, our data suggest that 0.03% clarithromycin was > 99.9% bactericidal for four strains (B2602, B3000, B81030, and B82016), approximately 99% bactericidal for strain B2631, and approximately 90% bactericidal for strains B2000 and B3026. However, it must be noted that this apparent bactericidal activity may reflect contributions both from drug activity and from a possible immune response since the experiments involved immunocompetent mice ([11]).

Neither 0.01% nor 0.001% clarithromycin in the diet inhibited the growth of the one strain (N2538) tested with these concentrations. In contrast, Franzblau and Hastings ([5]) reported that a dietary concentration of 0.01% clarithromycin was fully bactericidal against *M. leprae* when assayed using a minor modification of the kinetic method; that is, no bacilli (i.e., < 1.6 × 10^4/foot pad) were found in foot pads from six mice assayed 5 months after discontinuation of drug treatment. The differences in our observations may reflect variations in the susceptibility of individual strains to clarithromycin, such as seen with B3026 and B3000 in the present study. Overall, the data indicate that a dietary concentration of 0.1% clarithromycin (approximately 100 mg/kg/day/mouse) is necessary to ensure full bactericidal activity against all *M. leprae* strains.

Previous studies have suggested that dapsone can antagonize the bactericidal activity of rifampin ([13]). Our observation of the growth of strain B2602 by day 368 in the foot pads of 2 of 5 mice fed diets containing 0.01% rifampin + 0.01% dapsone, but no growth in the foot pads of any of the 5 mice fed diets containing 0.01% rifampin alone is consistent with such antagonism but our observed difference is not statistically significant. The addition of 0.1% clarithromycin to the diet containing 0.01% rifampin + 0.1% dapsone prevented growth of B2602 in the foot pads of all five mice assayed on day 368. Also, no antagonism was observed between clarithromycin and dapsone or rifampin in any of the combinations tested. This suggests that the addition of a second bactericidal drug, clarithromycin, to the currently recommended multidrug regimen may improve the killing of *M. leprae* bacilli even in the presence of a bacteriostatic drug such as dapsone.

An important result was that clarithromycin was effective when used alone against each of the rifampin-resistant and dapsone-resistant strains tested. In addition, clarithromycin was equally effective against these six drug-resistant strains when given in combination with dapsone and/or rifampin

as might be expected since each drug is thought to affect a different cellular process. This strongly suggests that clarithromycin would be a useful addition to the multidrug regimen to prevent the growth of dapsone-resistant or rifampin-resistant bacilli.

SUMMARY

The anti-*Mycobacterium leprae* activity of clarithromycin when administered alone and in combination with rifampin and dapsone in the diet was determined using the kinetic method of drug evaluation in mice. Clarithromycin when administered at a concentration of 0.1% (w/w) in the diet completely prevented growth of 2 pan-susceptible, 3 dapsone-resistant, 2 rifampin-resistant, and 2 rifampin and dapsone double resistant strains of *M. leprae*. A 0.03% (w/w) concentration also completely prevented growth of *M. leprae* in all mice infected with 2 of 7 strains tested, but in only some of the mice infected with the remaining 5 strains. No antagonistic drug interactions were observed between clarithromycin and dapsone or rifampin. The addition of clarithromycin to the currently recommended multidrug regimen should improve the rate of killing of *M. leprae* and help to prevent the growth of dapsone-resistant and rifampin-resistant strains.

RESUMEN

Utilizando el método cinético de evaluación de drogas en el ratón, se determinó la actividad anti-*Mycobacterium leprae* de la claritromicina administrada sola o en combinación con rifampina y dapsona. La claritromicina administrada en la dieta a la concentración del 0.1% (peso/peso) evitó completamente el crecimiento de 2 cepas de *M. leprae* resistentes a todo, 3 cepas resistentes a la dapsona, 2 resistentes a la rifampina, y 2 resistentes a la rifampina y a la dapsona. Una concentración del 0.03% también evitó completamente el crecimiento del *M. leprae* en todos los ratones infectados con 2 de las 7 cepas probadas y en algunos de los ratones infectados con las 5 cepas restantes. No se observaron interacciones antagonistas de las drogas entre la claritromicina, la dapsona y la rifampina. Es posible que la adición de claritromicina al tratamiento con poliquimioterapia recomendado por la OMS, ayude a mejorar su capacidad bactericida y a prevenir el crecimiento de cepas resistentes a la dapsona y de cepas resistentes a la rifampina.

RÉSUMÉ

L'activité anti-*Mycobacterium leprae* de la clarithromycine administrée seule dans l'alimentation et en combinaison avec la rifampicine et la dapsone a été évaluée chez la souris par la méthode cinétique. La clarithromycine administrée à une concentration de 0.1% (poids/poids) dans l'alimentation empêchait complètement la multiplication de 2 souches de *M. leprae* sensibles aux deux médicaments, de 3 souches résistant à la dapsone, de 2 souches résistant à la rifampicine et de 2 souches ayant la double résistance à la dapsone et à la rifampicine. Une concentration de 0.03% (poids/poids) empêchait également complètement la multiplication de *M. leprae* chez toutes les souris infectées par 2 des 7 souches testées, mais seulement chez certaines des souris infectées par les 5 autres souches. Aucune interaction antagoniste n'a été observée entre la clarithromycine et la dapsone ou la rifampicine. L'addition de clarithromycine au régime de polychimiothérapie actuellement recommandé devrait améliorer le pouvoir bactéricide vis-à-vis de *M. leprae* et aider à prévenir la multiplication de souches résistant à la dapsone et à la rifampicine.

Acknowledgment. We would like to thank Abbott Laboratories for providing clarithromycin and to thank Dr. R. C. Good for his helpful discussions.

REFERENCES

1. Bloom, B. R. and Godal, T. Selective primary health care: strategies for control of disease in the developing world. V. Leprosy. Rev. Infect. Dis. **5** (1983) 765–780.
2. Centers for Disease Control. Increase in prevalence of leprosy caused by dapsone-resistant *Mycobacterium leprae*. Morbid. Mortal. Weekly **30** (1982) 637–638.
3. Dautzenberg, B., Truffot, C., Legris, S., Meyohas, M., Berlie, H. C., Mercat, A., Cheuret, S. and Grosset, J. Activity of clarithromycin against *Mycobacterium avium* infection in patients with the acquired immune deficiency syndrome; a controlled clinical trial. Am. Rev. Respir. Dis. **144** (1991) 564–569.
4. Fernandes, P. B., Hardy, D. J., McDaniel, D., Hanson, C. W. and Swanson, R. N. *In vitro* and *in vivo* activities of clarithromycin against *Mycobacterium avium*. Antimicrob. Agents Chemother. **33** (1989) 1531–1534.
5. Franzblau, S. G. and Hastings, R. C. *In vitro* and *in vivo* activities of macrolides against *Mycobacterium leprae*. Antimicrob. Agents Chemother. **32** (1988) 1758–1762.
6. Gelber, R. H., Siu, P., Tsang, M. and Murray, L. P. Activities of various macrolide antibiotics against *Mycobacterium leprae* infection in mice. Antimicrob. Agents Chemother. **35** (1991) 760–763.
7. Hardy, D. J., Hensey, D. M., Beyer, J. M., Vojtko, C., McDonald, E. J. and Fernandes, P. B. Comparative *in vitro* activities of new 14-, 15-, and 16-membered macrolides. Antimicrob. Agents Chemother. **32** (1988) 1710–1719.

8. JACOBSON, R. R. and HASTINGS, R. C. Rifampin resistant leprosy. Lancet **2** (1976) 1304–1305.
9. JI, B. Drug resistance in leprosy—a review. Lepr. Rev. **56** (1985) 265–278.
10. JI, B., PERANI, E. G. and GROSSET, J. H. Effectiveness of clarithromycin and minocycline alone and in combination against experimental *Mycobacterium leprae* infection in mice. Antimicrob. Agents Chemother. **35** (1991) 579–581.
11. LEVY, L. Superinfection in mice previously infected with *Mycobacterium leprae.* Infect. Immun. **11** (1975) 1094–1099.
12. MALMBORG, A.-S. The renaissance of erythromycin. J. Antimicrob. Chemother. **18** (1986) 293–295.
13. MILLAN, J. P. and MOULIA-PELAT, J. P. Antagonism between dapsone and rifampicin in experimental *Mycobacterium leprae* infection in mice. Res. Microbiol. **140** (1989) 143–150.
14. SHEPARD, C. C. A kinetic method for the study of activity of drugs against *Mycobacterium leprae* in mice. Int. J. Lepr. **35** (1967) 429–435.
15. SHEPARD, C. C. Statistical analysis of results obtained by two methods for testing drug activity against *Mycobacterium leprae.* Int. J. Lepr. **50** (1982) 96–101.
16. SHEPARD, C. C. and CHANG, Y. T. Effect of DDS on established infections with *Mycobacterium leprae* in mice. Int. J. Lepr. **35** (1967) 52–57.
17. WHO EXPERT COMMITTEE ON LEPROSY. Sixth Report. Geneva: World Health Organization, 1988. Tech. Rep. Ser. 768.
18. WHO STUDY GROUP. Chemotherapy of leprosy for control programs. Geneva: World Health Organization, 1982. Tech. Rep. Ser. 675.