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Clinical Trial of Clarithromycin for Lepromatous Leprosy

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Clarithromycin was administered to nine previously untreated lepromatous leprosy patients. Patients received two 1,500-mg doses on the first day, followed by 7 days of no treatment, in order to evaluate the potential efficacy of intermittent therapy. Patients then received 1,000 mg daily for 2 weeks followed by 500 mg daily for 9 weeks. The efficacy of therapy was monitored clinically, by changes in morphological index, mouse footpad infectivity, and radiorespirometric activity of *Mycobacterium leprae* obtained from serial biopsies and by serum levels of phenolic glycolipid I. Clarithromycin was well tolerated, with only minor side effects noted in two patients. Most patients showed reductions in morphological index and radiorespirometry 1 week after the first two doses. Within 3 weeks of starting treatment (total of 17 g of clarithromycin), biopsy-derived *M. leprae* specimens from all patients had a morphological index of zero, were noninfectious for mice, and had less than 1% of the radiorespirometric activity of pretreatment specimens. Reductions in serum phenolic glycolipid I levels were observed for most patients at 3 weeks. Significant clinical improvement was evident after 4 weeks of treatment. All analyses indicate that clarithromycin is rapidly bactericidal for *M. leprae* in humans.

Clarithromycin is a new semi-synthetic macrolide with activity, pharmacokinetics, and gastric tolerance superior to those of erythromycin. Clarithromycin has demonstrated exceptional activity against *Mycobacterium leprae* both in vitro (3, 4) and in vivo (5, 7, 8, 11); its activity surpasses that of other macrolides (5, 8) and approximates that of rifampin (5, 7).

A recent clinical trial of 500 mg of clarithromycin daily against leprosy showed good activity as determined by loss of infectivity of biopsy-derived *M. leprae* for mice (10). Because of the exceptional tolerance of clarithromycin and increased half-life at increasing dosage (3) and considering the current use of intermittent rifampin therapy in leprosy, we evaluated the effect of both a single day's dose and a higher daily dose of clarithromycin for nine lepromatous leprosy patients and monitored efficacy by a number of rapid assays in addition to the mouse footpad assay.

MATERIALS AND METHODS

Patients and treatment. Nine male, previously untreated patients with multibacillary leprosy were recruited; all patients had at least one lesion with a bacteriologic index (BI) of >4+ and a morphologic index (MI) of >1% and of sufficient size so that five 6-mm skin punch biopsy specimens could be taken. By clinical evaluation, patients 1 to 3 were classified as borderline lepromatous and patients 4 to 9 were classified as polar lepromatous. Patients ranged from 14 to 56 (median, 31) years in age. Patients had neither a recent history nor signs or symptoms of lepra reactions upon admission. Examination procedures on admission and during the trial were as previously described (1).

Patients, who were hospitalized at the Research Institute for Tropical Medicine, Manila, Philippines, for the 8-week study, received 3,000 mg of clarithromycin in two divided doses orally on the first day of treatment (day 1). No treatment was given for the next 6 days in order to determine the effect of a single day's treatment. From day 8 to day 21, the patients received 1,000 mg of clarithromycin as a single daily dose. From day 22 to day 56, the single daily dose was 500 mg. This was followed by an additional 4 weeks of outpatient treatment with 500 mg of clarithromycin daily in order to evaluate the clinical response further.

Clinical observation and photography. Patients were observed weekly for clinical response and were monitored for signs and symptoms suggestive of adverse side effects to clarithromycin and for reversal or erythema nodosum leprosum reactions. The degree of clinical improvement or deterioration was assessed, with consideration given to erythema, swelling, diffuse infiltration, size or elevation of nodules and plaques, and nerve involvement. At the end of the study, the residual lesions and the appearance of new skin lesions or new nerve involvement were described carefully.

Serum and biopsy specimens were collected just prior to initiation of treatment and at 1, 3, 5, and 8 weeks following the first day's treatment. The BI was determined from skin slit smears from at least six sites. The MI was determined from slides prepared from homogenates of biopsy specimens.

PGL-I antigen in serum. The procedure of Cho et al. (2) for measuring phenolic glycolipid I (PGL-I) antigen levels was followed. Scoring was determined by the relative intensity of color development from the reaction of peroxidase with 4-chloro-1-naphthol.

Mouse footpad assay. Infectivity of *M. leprae* from skin biopsy homogenates was determined as previously described (1). Groups of 10 mice were inoculated in both hind footpads with 5,000 acid-fast bacilli (AFB). For pretreatment biopsies, additional groups of 10 mice were inoculated with 500, 50, and 5 AFB. Growth was assessed after 1 year by pooling tissue homogenates from both hind footpads and examining Ziehl-Neelsen-stained preparations for AFB. The observation of any AFB was considered a positive result.

Radiorespirometry. Radiorespirometric measurement of

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TABLE 1. Clinical improvement during treatment[a]

% Improvement	No. of patients with result at wk:			
	2	4	8	12
<50	8			
50–75	1	9	3	
>75			6	9

[a] Graded according to changes in erythema, swelling, diffuse infiltration, size or elevation of nodules and plaques, and nerve involvement.

the oxidation of [1-^{14}C]palmitic acid by biopsy-derived *M. leprae* was determined as previously described (1). Briefly, *M. leprae* in the biopsy homogenates was pelleted and resuspended in 7H12 medium, and then 10^6 AFB were added to 1 ml of 7H12 medium–1 μCi of [1-^{14}C]palmitic acid. $^{14}CO_2$ evolved during incubation of cultures for 1 week at 33°C was trapped on a NaOH-treated filter paper contained in the outer vial of a double-vial assembly. The inner culture vials were discarded, and liquid scintillation fluor (LKB) was added to the outer vials. Counts per minute (cpm) were determined in a liquid scintillation counter. Mean cpm from replicate heat-killed controls were subtracted from the mean cpm of replicate test cultures to yield a net cpm and standard deviation.

Statistics. Significance of the difference in pretreatment and posttreatment BI, MI (see Table 2), and radiorespirometry (see Table 4) was determined by the Wilcoxon signed-rank test. Mouse footpad viability (see Table 3) was determined by the method of Spearman-Karber (13), and the proportion of viables was calculated as 0.69/50% infective dose (9). Estimates of maximum percent viable bacilli of posttreatment samples from nontitrated inocula were made by using the assumptions described by Grosset et al. (9).

RESULTS

Clinical results. Clarithromycin was well tolerated, with only minor side effects being noted. After the initial dose of 1,500 mg on day 1, two patients complained of intermittent abdominal discomfort with nausea. Patient 8 developed a moderate reversal reaction during the fifth week; the reversal reaction was characterized by moderate swelling and erythema of the lesions of the face, accompanied by intermittent low-grade fever and malaise. The patient was treated with paracetamol and a short course of prednisone. Significant improvement of lesions was observed after 4 weeks of treatment in all patients (Table 1). By 8 to 12 weeks, all patients were graded at >75% clinical improvement.

Laboratory results. There was a modest but significant difference between the pretreatment median BI of 5 and the 8-week posttreatment BI of 4.5 ($P = 0.0078$). There was a significant reduction in median MI 1 week after the first two doses (Table 2). Only two patients with a relatively low pretreatment MI of 2 did not show a reduction. After an additional 2 weeks of daily treatment, all patients had an MI of zero.

With one exception (bacilli from patient 9), bacilli from all pretreatment patient biopsies were highly infectious for mice (Table 3). The two 1,500-mg doses on day 1 failed to produce a detectable change in *M. leprae* viability on the basis of biopsies taken 1 week later. However, after an additional 2 weeks of treatment of 1,000 mg daily, none of the harvested *M. leprae* were capable of infecting mice with an inoculum of 5,000 bacilli.

The radiorespirometric activity of bacilli harvested 1 week following two doses of 1,500 mg of clarithromycin (3,000 mg total) showed a reduction of 52 to 79% (Table 4). Bacilli recovered after an additional 2 weeks of 1,000 mg of clarithromycin daily demonstrated little or no activity.

PGL-I antigen levels in the serum samples of all nine patients showed a time-dependent decrease, with reductions in some patients evident as early as 1 week posttreatment (Table 5). By 3 weeks, a reduction was detected in eight of nine patients.

TABLE 2. MI of biopsy-derived *M. leprae*

Patient	MI at wk:	
	0	1
1	4	1
2	3	1
3	7	1
4	2	2
5	5	0
6	2	1
7	2	2
8	2	0
9	3	0
Median	3	1[b]

[a] MI at weeks 3, 5, and 8 was zero for all patients.
[b] Significantly less than pretreatment value ($P < 0.01$). (This applies to week 3, 5, and 8 values as well.)

DISCUSSION

The rapid rate of clinical improvement with clarithromycin was similar to that observed in a recent clinical trial of sparfloxacin against leprosy (1). Moderate improvement was observed as early as 2 weeks and in most cases at 4 weeks after initiation of therapy, and clinical improvement continued until about 6 to 8 weeks, after which the rate of improvement slowed considerably. With the current World Health Organization multidrug therapy of dapsone, rifampin, and clofazimine, an equivalent response usually commences after 6 to 8 weeks and

TABLE 3. Mouse footpad infectivity of biopsy-derived *M. leprae*[a]

Patient	No. of positive mice/10 mice at wk (inoculum of AFB)							
	0				1 (5,000)	3 (5,000)	5 (5,000)	8 (5,000)
	5	50	500	5,000				
1	6	9[b]	9[b]	10	10	0	0	0
2	3	9	9[b]	10	10	0	0	0
3	7	10	10	10	10	0	0	0
4	5	7[b]	10	10	7[b]	0	0	0
5	5[c]	6[b]	10	8[b]	9	0	0	0[d]
6	5	10	10	10	8	0	0	0[b]
7	6	10	10	10	10	0	0	0
8	0	6	10	10	9	0[b]	0	0
9	1	0	1	4	1	0	0	0

[a] The median percent viability, calculated by the method of Shepard (13), was 13.77 for week zero, not determined for week 1, and <0.01 for weeks 3, 5, and 8. Proportion of viables = 0.69/50% infective dose. The median percent kill was not determined for week 1 and >99.9 for weeks 3, 5, and 8. All post-treatment values are estimates based on conventions of Grosset et al. (9), but estimates for week 1 were considered invalid because of the high percentage of positive footpads at the 5,000-AFB dose.
[b] Only nine mice were evaluated because of mortality.
[c] Only eight mice were evaluated because of mortality.
[d] Only seven mice were evaluated because of mortality.

TABLE 4. Radiorespirometric activity of biopsy-derived *M. leprae*

Patient	Mean (SD) cpm/10^6 AFB at wk:				
	0	1	3	5	8
1	4,895 (403)	1,214 (323)	−21 (80)	−25 (31)	17 (26)
2	2,334 (589)	494 (191)	−13 (15)	23 (33)	1 (7)
3	2,904 (511)	1,397 (224)	32 (48)	3 (14)	4 (2)
4	8,680 (1,417)	790 (74)	5 (15)	0 (8)	−2 (7)
5	6,446 (799)	504 (65)	−3 (6)	7 (15)	−3 (10)
6	4,914 (748)	2,122 (245)	17 (11)	4 (17)	−3 (7)
7	6,995 (871)	2,935 (405)	37 (15)	18 (19)	12 (9)
8	2,584 (61)	1,160 (321)	18 (12)	0 (11)	10 (5)
9	249 (37)	24 (11)	−2 (8)	5 (7)	1 (7)
Median	4,895	1,160[a]	5[b]	4	1
% Decrease		76	>99	>99	>99

[a] Significantly different from pretreatment value ($P = 0.002$).
[b] Significantly different from 1-week value ($P = 0.002$).

continues until 12 to 16 weeks posttreatment before tapering off.

Overall the laboratory evaluation suggested a marked reduction in viability after 2 weeks of 1,000 mg of clarithromycin daily, equivalent to that after 4 weeks with 200 mg of sparfloxacin daily (1) and similar to that observed in recent trials of 100 mg of minocycline daily (6, 10) and 400 mg of ofloxacin daily (9). As observed in the sparfloxacin trial (1), there was good overall agreement between the standard mouse footpad results and the more rapid MI, PGL-I, and radiorespirometry assays. The radiorespirometry result, because of the lack of subjectivity, appears especially promising as a means of quantitating the response to therapy.

For reasons discussed previously (1), we used a cutoff of 5.2 $\times$ 10^3 AFB for positivity in the mouse footpad assay (Table 3). However, if the more commonly employed value of 10^5 AFB were used, the results would be essentially the same: i.e., all biopsies (with the exception of that of patient 9) were still infectious for mice 1 week after the single day's dose, and all were negative after an additional 2 weeks of 1,000 mg daily. Comparison of the results of this trial with those of the 500-mg daily clarithromycin trial of Ji et al. (10) is complicated by differences in dosing schedules and biopsy schedules and a lack of standardization of clinical and MI evaluations.

TABLE 5. PGL-I antigen titer in serum during treatment

Patient	Titer[a] at wk:					
	0	1	3	5	8	12 or later
1	+++	++	++	+	+/−	−[b]
2	+++	++	++	++	+	+/−[c]
3	++	++	+	+/−	+/−	−
4	+++	+++	+++	++	+	−
5	+++	+++	++	+	+	+
6	+++	+++	++	+/−	+/−	−
7	+++	+++	++	+	+/−	−
8	+++	++	+/−	+/−	+/−	ND[d]
9	+++	++	++	+	+	ND

[a] Relative color intensity.
[b] 20 weeks.
[c] 16 weeks.
[d] ND, not determined.

Although administration of 3 g of clarithromycin in two divided doses on day 1 did not dramatically reduce bacillary viability as determined by mouse footpad analysis, detection of a more modest reduction in viability would have required bacillary titration of the 1-week biopsies (as was done for pretreatment biopsies). Indeed, the reductions observed in MI, radiorespirometry, and serum PGL-I at 1 week all suggested a moderate reduction in *M. leprae* viability.

Overall, the results obtained here would suggest that clarithromycin is a highly potent, well-tolerated, bactericidal antileprosy agent. While use is currently contraindicated in pregnancy (12), this drug would appear to have promise for use in multi-drug regimens for leprosy.

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REFERENCES

1. **Chan, G. P., B. Y. Garcia-Ignacio, V. E. Chavez, J. B. Livelo, C. L. Jimenez, M. L. R. Parrilla, and S. G. Franzblau.** 1994. Clinical trial of sparfloxacin in lepromatous leprosy. Antimicrob. Agents Chemother. **38:**61–65.
2. **Cho, S. N., R. V. Cellona, T. T. Fajardo, R. M. Abalos, E. C. dela Cruz, G. P. Walsh, J. D. Kim, and P. J. Brennan.** 1991. Detection of phenolic glycolipid-I antigen and antibody in sera from new and relapsed lepromatous patients treated with various drug regimens. Int. J. Lepr. **59:**25–31.
3. **Chu, S.-Y., L. T. Sennello, S. T. Bunnell, L. L. Varga, D. S. Wilson, and R. C. Sonders.** 1992. Pharmacokinetics of clarithromycin, a new macrolide, after single ascending oral doses. Antimicrob. Agents Chemother. **36:**2447–2453.
4. **Franzblau, S. G.** 1989. Drug susceptibility testing of *Mycobacterium leprae* in the BACTEC 460 system. Antimicrob. Agents Chemother. **33:**2115–2117.
5. **Franzblau, S. G., and R. C. Hastings.** 1988. In vitro and in vivo activities of macrolides against *Mycobacterium leprae*. Antimicrob. Agents Chemother. **32:**1758–1762.
6. **Gelber, R. H., K. Fukuda, S. Byrd, L. P. Murray, P. Siu, M. Tsang, and T. H. Rea.** 1992. A clinical trial of minocycline in lepromatous leprosy. Br. Med. J. **304:**91–92.
7. **Gelber, R. H., L. P. Murray, P. Siu, and M. Tsang.** 1992. Clarithromycin at very low levels and on intermittent administration inhibits the growth of *M. leprae* in mice. Int. J. Lepr. **60:**485–487. (Letter.)
8. **Gelber, R. H., P. Siu, M. Tsang, and L. P. Murray.** 1991. Activities of various macrolide antibiotics against *Mycobacterium leprae* in mice. Antimicrob. Agents Chemother. **35:**760–763.
9. **Grosset, J. H., B. Ji, C. C. Guelpa-Lauras, E. G. Perani, and L. N. N'Deli.** 1990. Clinical trial of pefloxacin and ofloxacin in the treatment of lepromatous leprosy. Int. J. Lepr. **58:**281–295.
10. **Ji, B., P. Jamet, E. G. Perani, P. Bobin, and J. H. Grosset.** 1993. Powerful bactericidal activities of clarithromycin and minocycline against *Mycobacterium leprae* in the treatment of lepromatous leprosy. J. Infect. Dis. **168:**188–190.
11. **Ji, B., E. G. Perani, and J. H. Grosset.** 1991. Effectiveness of clarithromycin and minocycline alone and in combination against experimental *Mycobacterium leprae* infection in mice. Antimicrob. Agents Chemother. **35:**579–581.
12. **Physicians' desk reference, 48th ed.** 1994. Biaxin, p. 405–407. Medical Economics Data Production Company, Montvale, N.J.
13. **Shepard, C. C.** 1982. Statistical analysis of results obtained by two methods for testing drug activity against *Mycobacterium leprae*. Int. J. Lepr. **50:**96–101.