ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 1993, p. 1869–1872
0066-4804/93/091869-04$02.00/0

Vol. 37, No. 9

Liposome-Encapsulated Gentamicin Treatment of *Mycobacterium avium-Mycobacterium intracellulare* Complex Bacteremia in AIDS Patients

STEPHEN D. NIGHTINGALE,[1]* STEPHEN L. SALETAN,[2] CHRISTINE E. SWENSON,[2] A. JEAN LAWRENCE,[1] DURWARD A. WATSON,[1] FRANK G. PILKIEWICZ,[2] EDWARD G. SILVERMAN,[2] AND STANLEY X. CAL[1]

Division of General Internal Medicine, University of Texas Southwestern Medical School, Dallas, Texas 75235-9103,[1] and The Liposome Company, Princeton, New Jersey 08540[2]

Received 23 March 1993/Returned for modification 10 May 1993/Accepted 30 June 1993

TLC G-65, a liposome-encapsulated gentamicin, was given intravenously twice weekly for 4 weeks to AIDS patients with *Mycobacterium avium-M. intracellulare* complex (MAC) bacteremia at 1.7 mg of gentamicin per kg of body weight per infusion (4 patients), 3.4 mg/kg (10 patients), and 5.1 mg/kg (7 patients). MAC colony counts in blood fell by 75% or more in all three groups ($P < 0.005$). Drug resistance did not emerge during the study period. Transient renal insufficiency developed in one patient; no other adverse effects were detected. Liposome-encapsulated gentamicin is a potential therapy for MAC infections in AIDS patients.

Disseminated *Mycobacterium avium-M. intracellulare* complex (MAC) infection frequently causes morbidity and mortality in AIDS patients. While the incidence of MAC bacteremia is only about 5% at the time of AIDS diagnosis, it is over 50% by 30 months after AIDS diagnosis. It appears that all human immunodeficiency virus-positive patients who do not succumb to some other opportunistic event will eventually develop MAC infection (9).

Aminoglycosides are among the recommended treatments for disseminated MAC infection (3, 7). Recently, liposome-encapsulated aminoglycosides, including amikacin (1, 2, 4, 6), gentamicin (8, 11), and streptomycin (5), have been shown to be more effective than their unencapsulated counterparts in animal models of MAC infection. Those reports led us to perform a phase I/II safety and dose-finding study of TLC G-65, a liposome-encapsulated gentamicin, in AIDS patients with MAC bacteremia.

MATERIALS AND METHODS

Human immunodeficiency virus-infected men and nonpregnant women over 18 years of age with MAC cultured from their blood within 8 weeks of the time of entry into the study were eligible for the study. Patients were required to have a serum creatinine level of <1.5 mg/dl, normal audiometry and vestibular functions, no antimycobacterial therapy within 28 days of entry into the study, and liver function tests less than five times the upper limit of normal. Written informed consent was obtained prior to entry into the study.

TLC G-65 is a plurilamellar liposome made from egg phosphatidylcholine by The Liposome Company, Princeton, N.J. TLC G-65 contains 50 mg of phospholipid and 5 mg of gentamicin per ml. Over 80% of the gentamicin is encapsulated in the liposome, and 85% of the liposomes are between 1.2 and 10.0 μm in diameter. The preparation is stable at 5°C for 18 months.

Subjects were infused with TLC G-65 on study days 1, 4, 8, 11, 15, 18, 22, and 25. Infusions were performed at a uniform rate over a 2-h period. The first subjects received 1.7 mg gentamicin per kg of body weight per infusion. Dose escalations of 1.7 mg of gentamicin per kg were mandated by the protocol whenever (i) less than four of six patients receiving a given dose experienced less than a 99% reduction in MAC colony counts in blood by the end of the study and (ii) no serious or life-threatening toxicities were observed at that dose level.

An audiogram, blood culture for MAC, CD4 count, chest X ray, complete blood count, blood chemistry panel, and urinalysis were performed before the first infusion. Plasma gentamicin levels were measured before and after each infusion. Both total and free (nonliposomal) plasma gentamicin levels were measured separately after each infusion for patients who received the 5.1-mg/kg dose. The serum creatinine level was measured prior to each infusion. Continuous 24-h urine samples for protein, creatinine, and gentamicin determinations were collected from the day before the first infusion until 7 days after the last infusion. Quantitative blood cultures for MAC, a complete blood count, and blood chemistries were obtained weekly during the infusions and for 2 weeks thereafter. Audiograms were repeated 7 days after the last infusion.

Gentamicin levels in blood and urine were assayed by a fluorescence polarization immunoassay by using the Abbott TDx system (Abbott Laboratories, Abbott Park, Ill.). The total plasma gentamicin level was measured after disruption of liposomes by freezing and detergent treatment. The free gentamicin in plasma was separated from liposome-encapsulated gentamicin by ultrafiltration of plasma by using Centricon-30 microconcentrators (Amicon, Beverly, Mass.). This separation was completed less than 1 h after the sample was obtained from the patient (10).

Quantitative blood cultures for MAC were performed immediately before each infusion. Blood was collected in Isolator-10 tubes (Wampole Laboratories, Cranbury, N.J.) and was processed within 4 h of collection by plating serial 10-fold dilutions of blood in 0.85% saline on Middlebrook 7H10 agar. The plates were sealed to retard evaporation, incubated at 35 to 37°C in 8 to 10% CO_2, and examined weekly for up to 8 weeks.

Organisms from the first and the last positive cultures

* Corresponding author.

TABLE 1. MAC colony counts in blood, patient weights before and after the study, and gentamicin MICs pre- and posttherapy

TLC G-65 dose and study subject no.[a]	CD4 count	MAC colony count/ml of blood on day:						Initial wt (kg)	Wt change (kg)	Gentamicin MIC (μg/ml)	
		0	8	15	25	32	39			Pretherapy	Posttherapy
1.7 mg/kg											
1	7	6,900	1,300	1,300	450	130	160	69.5	−3.1	2	2
2	2	3,500	700	780	260	200	195	56.1	−2.0	<1	<1
3	5	3,200	1,600	2,600	450	ND[b]	5,200	76.8	−4.8	2	2
4	6	260	200	130	100	130	195	73.2	−2.7	<1	<1
3.4 mg/kg											
5	4	5,850	9,100	ND	ND	10,400	4,160	67.0	−1.1	2	ND
6	6	97	32	ND	65	—[c]	—	51.8	—	ND	—
7	1	325	390	260	260	ND	ND	55.5	+1.3	4	4
8	28	1	1	1	4	ND	2	68.2	−2.8	2	2
9	2	260	900	2,600	780	ND	260	60.0	−4.5	ND	ND
10	9	32	130	8	2	ND	6	51.0	−1.0	2	ND
11	15	130	65	65	30	1	32	58.0	−1.6	2	4
12	6	260	260	130	86	—	—	64.7	—	ND	—
13	9	182,000	—	—	—	—	—	47.3	—	ND	—
14	9	650	ND	36	10	56	30	71.4	+2.6	8	4
5.1 mg/kg											
15	4	11,700	9,100	—	—	—	—	40.9	—	ND	—
16	2	520	260	520	260	520	910	77.5	+0.4	<1	<1
17	1	520	390	130	65	65	30	47.7	−1.7	2	2
18	7	650	ND	130	2,600	325	60	64.5	−4.5	2	<1
19	17	130	10	22	8	2	ND	56.6	−6.6	8	ND
20	12	6,500	2,600	325	130	130	ND	38.6	−0.6	<1	<1
21	6	390	130	195	130	30	195	78.0	−6.2	2	ND

[a] Patients 13 and 15 died of progressive MAC infection during the second week of the study. Patients 6 and 12 declined to participate in the study after receiving their last dose of study drug on day 25.
[b] ND, not done because of laboratory accident.
[c] —, patient off study prior to scheduled test.

obtained from each patient were frozen in Middlebrook 7H9 broth at −70°C. The MIC of gentamicin for each of these isolates was determined at the National Jewish Center for Immunology and Respiratory Medicine, Denver, Colo.

The effects of dose and duration of therapy on MAC colony counts in blood were evaluated by regression analysis by using PC-SAS STAT software (SAS Institute, Cary, N.C.).

RESULTS

The results of the study are summarized in Table 1. The mean age of the 21 study participants was 34 years. Fifteen subjects were white, 4 were black, and two were hispanic; 20 were male. The median CD4 count at the time of entry into the study was 6/mm^3. All but four patients had median CD4 counts of <10/mm^3; the highest was 28/mm^3. Preenrollment audiograms were all within normal limits.

Dose escalations were implemented after four patients had received the 1.7-mg/kg dose and after 10 had received the 3.4-mg/kg dose without toxicity. Seven patients received the 5.1-mg/kg dose. Further dose escalation was not attempted because one patient developed transient renal insufficiency (see below).

Peak total plasma gentamicin levels, obtained within 10 min after each infusion was completed, averaged 2 μg/ml for the 1.7-mg/kg dose, 8 μg/ml for the 3.4-mg/kg dose, and 29 μg/ml for the 5.1-mg/kg dose. Free plasma gentamicin levels averaged 33% ± 8% (standard deviation) of the simultaneous total level in the plasma of patients who received the 5.1-mg/kg dose. Trough gentamicin levels were <1 μg/ml on 126 of the 140 occasions that they were measured. The elimination of total and free gentamicin from the plasma of one representative patient who received the 5.1-mg/kg dose is illustrated in Fig. 1.

The mean gentamicin balance (total milligrams infused − total milligrams excreted in urine) for each treatment group is illustrated in Fig. 2. The gentamicin balance increased linearly over time at each dose studied. After the eighth infusion, on study day 25, the mean gentamicin balance was 559 ± 113 mg (standard error) positive in the 1.7-mg/kg dose group, 775 ± 89 mg in the 3.4-mg/kg dose group, and 1,006 ± 153 mg in the 5.1-mg/kg dose group. When corrected for patient weight, these averages are 7.9 ± 1.2 mg/kg for the 1.7-mg/kg dose group, 12.7 ± 1.3 mg/kg for the 3.4-mg/kg dose group, and 16.4 ± 1.6 mg/kg for the 5.1-mg/kg dose group.

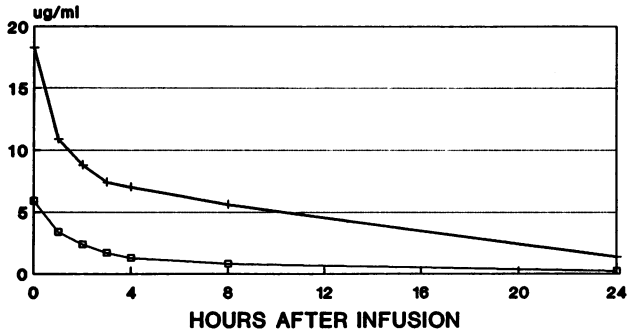

FIG. 1. Total (+) and free (□) gentamicin levels in plasma over 24 h after TLC G-65 infusion (5.1 mg of gentamicin per kg).

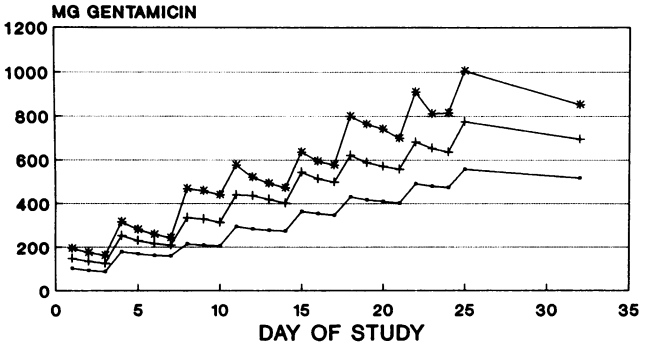

FIG. 2. Cumulative gentamicin balance (amount of gentamicin infused − amount excreted in urine) at the beginning of each study day for each dosing group. ■, 1.7-mg/kg dose; +, 3.4-mg/kg dose; *, 5.1-mg/kg dose.

In the 7 days after the eighth infusion, 69% of the amount of gentamicin delivered during the eighth infusion was recovered in the urine of the patients who received the 1.7-mg/kg dose, 84% was recovered in the urine of the patients who received the 3.4-mg/kg dose, and 79% was recovered in the urine of the patients who received the 5.1-mg/kg dose. For all patients, 75% of the amount of gentamicin delivered during the eighth dose was recovered in the urine over the following 7 days.

The median MAC colony counts in blood at each week of the study are illustrated in Fig. 3. In each treatment group, the median colony count fell over 6 weeks by 75% or more from its baseline value (3,400 to 195/ml for the 1.7-mg/kg dose, 260 to 31/ml for the 3.4-mg/kg dose, and 520 to 130/ml for the 5.1-mg/kg dose), although no patient's blood became sterile. Figure 3 illustrates that the median colony count continued to fall through day 32, 7 days after the last dose of TLC G-65; however, the median colony count increased slightly in all three groups between days 32 and 39.

Figure 3 also demonstrates that the relationship between the logarithm of the median colony count and study day was linear in each dosage group. By using the base 10 logarithm of the MAC colony count in blood as the dependent variable in a multiple regression model and study day and dose of liposome-encapsulated gentamicin as independent variables, the decrease in the median colony count was significantly related to study day ($t = -3.01$; $P < 0.005$) but not to dose ($t = -1.27$; $P > 0.20$).

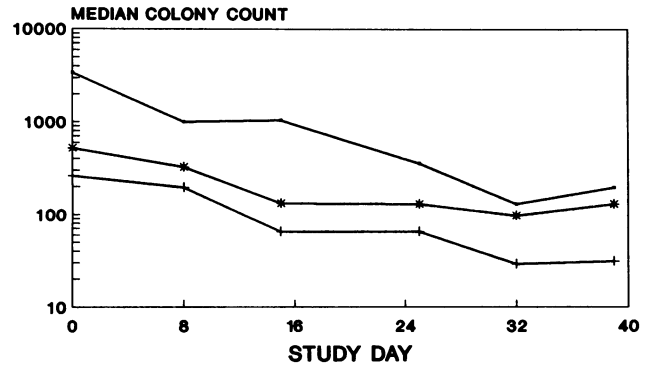

FIG. 3. Median MAC CFU per milliliter of blood at each week of the study for each dosing group. ■, 1.7-mg/kg dose; +, 3.4-mg/kg dose; *, 5.1-mg/kg dose.

The median MIC of gentamicin for pretreatment MAC isolates was 2 μg/ml. The median posttreatment MIC was also 2 μg/ml. No MIC was greater than 8 μg/ml, and there was no evidence that gentamicin resistance developed during the study period. Patients reported that fevers and night sweats were less frequent and less severe after than before treatment, but these changes did not achieve statistical significance. Patients lost an average of 2.3 kg of weight between days 1 and day 39. Posttreatment audiograms were not significantly changed from the baseline.

The only adverse event in the present study was renal insufficiency in one patient. This patient tolerated the 5.1-mg/kg dose well until the seventh infusion, when his serum creatinine level was found to be 3.4 mg/dl. His peak total plasma gentamicin levels had ranged from 15 to 42 μg/ml, and his peak free plasma gentamicin levels had ranged from 6.5 to 12 μg/ml. After liposome-encapsulated gentamicin was discontinued, the patient's serum creatinine rose to 6.1 mg/dl 3 days later and then spontaneously returned to its baseline value of 1.1 mg/dl over a 3-week period.

DISCUSSION

The phase I/II study described here demonstrated a beneficial effect of liposome-encapsulated gentamicin on MAC bacteremia at all doses tested. The effect of liposome-encapsulated gentamicin on MAC bacteremia was related to the duration of therapy rather than to the dose given. The net gentamicin balance increased throughout the study period. By the end of the study, for all three doses, the average amount of retained gentamicin was 8 μg/mg of body weight or greater. However, since gentamicin levels in specific tissues were not measured, it is uncertain whether or when therapeutic gentamicin levels were achieved in MAC-infected tissues or cells.

The optimum dosing regimen for TLC G-65 remains to be determined. Future development of this drug will focus on this issue. Studies now in progress will evaluate both the efficacy and the safety of using various loading doses followed by weekly or biweekly maintenance doses, longer durations of therapy, and a combination of TLC G-65 with other antimycobacterial agents. The results of these studies will determine the role of liposome-encapsulated gentamicin in the treatment of disseminated MAC infection in AIDS patients.

ACKNOWLEDGMENT

The study was supported by a grant from The Liposome Company to the University of Texas Southwestern Medical Center.

REFERENCES

1. **Bermudez, L. E. M., M. Wu, and L. S. Young.** 1987. Intracellular killing of *Mycobacterium avium* complex by rifapentine and liposome-encapsulated amikacin. J. Infect. Dis. **156:**510–513.
2. **Bermudez, L. E., A. O. Yau-Young, J. P. Lin, J. Cogger, and L. S. Young.** 1990. Treatment of disseminated *Mycobacterium avium* complex infection of beige mice with liposome-encapsulated aminoglycosides. J. Infect. Dis. **161:**1262–1268.
3. **Chiu, J., J. Nussbaum, S. Bozzette, J. G. Tiles, L. S. Young, J. Leedom, P. N. R. Heseltine, J. A. McCutchan, and the California Collaborative Trials Group.** 1990. Treatment of disseminated *Mycobacterium avium* complex infection in AIDS with amikacin, ethambutol, rifampin, and ciprofloxacin. Ann. Intern. Med. **113:**358–361.

4. **Cynamon, M. H., C. E. Swenson, G. S. Palmer, and R. S. Ginsberg.** 1989. Liposome-encapsulated amikacin therapy of *Mycobacterium avium* complex infection in beige mice. Antimicrob. Agents Chemother. **33:**1179–1183.
5. **Duzgunes, N., D. R. Ashtekar, D. L. Flasher, N. Ghori, R. J. Debs, D. S. Friend, and P. R. J. Gangadharam.** 1991. Treatment of *Mycobacterium avium-intracellulare* complex infection in beige mice with free and liposome-encapsulated streptomycin: role of liposome type and duration of treatment. J. Infect. Dis. **164:**143–151.
6. **Duzgunes, N., V. K. Perumal, L. Kesavalu, J. A. Goldstein, R. J. Debs, and P. R. J. Gangadharam.** 1988. Enhanced effect of liposome-encapsulated amikacin on *Mycobacterium avium-M. intracellulare* complex infection in beige mice. Antimicrob. Agents Chemother. **32:**1404–1411.
7. **Horsburgh, C. R.** 1991. *Mycobacterium avium* complex infection in the acquired immunodeficiency syndrome. N. Engl. J. Med. **324:**1332–1338.
8. **Klemens, S. P., M. H. Cynamon, C. E. Swenson, and R. S. Ginsberg.** 1990. Liposome-encapsulated gentamicin therapy of *Mycobacterium avium* complex infection of beige mice. Antimicrob. Agents Chemother. **34:**967–970.
9. **Nightingale, S. D., L. T. Byrd, P. M. Southern, J. D. Jockusch, S. X. Cal, and B. A. Wynne.** 1992. Incidence of *Mycobacterium avium-intracellulare* complex bacteremia in human immunodeficiency virus-positive patients. J. Infect. Dis. **165:**1082–1085.
10. **Roberts, P., W. Palaitis, F. Pilkiewicz, and C. E. Swenson.** 1991. Separation of free from liposomal gentamicin in plasma and analysis of the behavior of TLC G-65 in vitro and in vivo. Program Abstr. 31st Intersci. Conf. Antimicrob. Agents Chemother., abstr. 152.
11. **Swenson, C. E., K. A. Stewart, J. L. Hammett, W. E. Fitzsimmons, and R. S. Ginsberg.** 1990. Pharmacokinetics and in vivo activity of liposome encapsulated gentamicin. Antimicrob. Agents Chemother. **34:**235.