NOTES

Oral Therapy Using Nanoparticle-Encapsulated Antituberculosis Drugs in Guinea Pigs Infected with *Mycobacterium tuberculosis*

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Received 28 April 2005/Returned for modification 26 May 2005/Accepted 2 August 2005

We evaluated the efficacy of nanoparticle-encapsulated antituberculosis drugs administered every 10 days versus that of daily nonencapsulated drugs against *Mycobacterium tuberculosis* aerosol infection in guinea pigs. Both treatments significantly reduced the bacterial count and lung histopathology, suggesting that the nanoparticle drug delivery system has potential in intermittent treatment of tuberculosis.

The current standard regimen for treatment of tuberculosis requires 6 to 9 months of daily drug combination treatment in which patient noncompliance often results in treatment failure (1). One approach to improve patient compliance is to develop an enhanced drug delivery system aimed at sustained release of antituberculosis drugs currently available. Previous studies using poly(DL-lactide-co-glycolide) nanoparticles (PLG-NP) containing rifampin (RIF), isoniazid (INH), and pyrazinamide (PZA) have demonstrated that 5 doses of drug-loaded PLG-NP had the same efficacy as 46 conventional doses in mice and guinea pigs infected with *Mycobacterium tuberculosis* via intravenous and intramuscular routes, respectively (6, 7). Here, we evaluated the efficacy of the three-drug PLG-NP formulation against the guinea pig aerosol infection model of *M. tuberculosis*. This is also the first report comparing the histopathology of the lungs of guinea pigs treated with PLG-NP-encapsulated drugs to that of the lungs of guinea pigs treated with nonencapsulated drugs.

Drug-loaded PLG-NP were prepared by a multiple-emulsion and solvent evaporation procedure allowing coencapsulation of RIF, INH, and PZA, as described before (7). Characterization of the vacuum-dried formulation showed a particle size of 186 to 290 nm and drug encapsulation efficiency of 60 to 70% for all three drugs (6–8). Prior to efficacy testing, the formulation was evaluated for sustained tissue levels of at least 10 days, as described before (8) (data not shown). Female outbred Hartley guinea pigs (~500 g body weight) (Charles River Laboratories [North Wilmington, MA]) were exposed to an aerosol of *M. tuberculosis* H37Rv (Trudeau Institute, Sara-

nac Lake, NY) by using a Madison chamber aerosol generation device calibrated to deliver approximately 20 bacilli per guinea pig (4). Thirty days postinfection, two guinea pigs were euthanized to determine the bacterial load at the start of treatment. Remaining animals were allocated into four groups of five animals each, treated with the following: (i) sucrose control, i.e., daily administration of 50% (wt/vol) sucrose; (ii) empty PLG-NP (250 mg/kg of body weight) every 10 days for six weeks (5 doses), (iii) nonencapsulated drug combination (RIF, INH, and PZA) daily for 6 weeks (46 doses), and (iv) drug-loaded PLG-NP (250 mg/kg of body weight) every 10 days for 6 weeks (5 doses). In both drug-treated groups, RIF was given at 12 mg/kg of body weight, INH at 10 mg/kg, and PZA at 25 mg/kg. All doses were prepared in 50% sucrose to increase palatability. Animals were orally treated by administering each dose in the back of the mouth. Five days after the completion of chemotherapy, the animals were euthanized and lungs, spleens, and mediastinal lymph nodes (MLN) were removed. The number of viable organisms was determined by serial dilution of organ homogenates on nutrient Middlebrook 7H11 agar plates (GIBCO BRL, Gaithersburg, MD) and viable *M. tuberculosis* CFU were counted after 3 to 4 weeks incubation at 37°C, as described before (2, 4). Lung tissues were fixed in 10% phosphate-buffered formyl saline and embedded in paraffin wax. Sections were read blindly by a veterinary pathologist.

Treatment with empty PLG-NP did not change the bacterial load significantly in any of the organs compared to the untreated controls ($P > 0.05$) (Table 1). Following 6 weeks of therapy, a significant reduction in CFU was obtained in the lungs for both nonencapsulated drug and drug-loaded PLG-NP groups, with no significant difference between both groups ($P > 0.05$) (Table 1). In the spleens of both drug-treated groups, the effect was more pronounced, again, with no significant difference between both groups ($P > 0.05$) (Table

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TABLE 1. Number of viable *M. tuberculosis* organisms recovered from whole organs of drug-treated guinea pigs following 6 weeks of treatment^a

Treatment	Regimen	Log ₁₀ CFU ± SEM		
		Lung	MLN	Spleen
Pretreatment controls	None	6.15 ± 0.31	6.10 ± 0.01	5.94 ± 0.03
Sucrose controls	Daily	5.48 ± 0.21	5.22 ± 0.05	5.33 ± 0.15
Empty PLG-NP	Every 10 days	5.01 ± 0.42	4.98 ± 0.17	5.22 ± 0.40
Nonencapsulated drugs	Daily	3.75 ± 0.18	2.11 ± 0.48	2.27 ± 0.65
Drug-loaded PLG-NP	Every 10 days	4.14 ± 0.23	3.28 ± 0.23	2.15 ± 0.37

^a Viable bacterial counts were converted to logarithms and analyzed by one-way analysis of variance followed by an all-pairwise multiple comparison procedure utilizing the Fisher least significant difference method for the analysis of lung data and the Student-Newman-Keuls method for MLN and spleen data (SigmaStat v.2.03, SPSS Inc.).

1). In the MLN, the reduction in bacterial load was greater than that observed in the lungs and similar to that in the spleen. Daily treatment with nonencapsulated drugs was significantly better in the MLN than treatment with drug-loaded PLG-NP every 10 days, resulting in a reduction of 3.12 log₁₀ CFU compared to 1.95 log₁₀ CFU ($P < 0.05$) (Table 1). One explanation for this result might be that PLG-NP does not penetrate lymphoid tissues as easily as nonencapsulated drugs. This is an important observation, as the MLN could be a source for dissemination if not completely sterilized. This result also suggests that a regimen of weekly dosing rather than dosing every 10 days might increase effectiveness. This alternative regimen will be tested in a future study as well as the determination of drug levels in lymphoid tissue.

This is the first report determining bacterial numbers in lymph nodes of guinea pigs to test the efficacies of compounds. Recent findings in our laboratory have shown that guinea pigs develop severe lymphadenopathy of the MLN 30 days following aerosol infection with *M. tuberculosis* (3). Taking into consideration the extent of the bacterial infection relative to the organ volume, the infection in the lymph nodes was more established and severe than the infection in the lungs and spleen at the time of sacrifice. We show here that the drug effect can be seen more dramatically in the MLN than in the lungs. Current guinea pig models for screening experimental antituberculosis drugs generally evaluate the efficacy of compounds by determining the bacterial loads in lungs and spleens following treatment (9, 10). This report shows the importance of including the MLN in a drug study, since it reveals additional information on bacterial clearance in lymphoid organs and can perhaps increase the sensitivity of this model.

Unlike in the mouse, the progression of pathology in the guinea pig model of experimental airborne infection with *M. tuberculosis* has many similarities with the disease process in humans, such as caseous necrosis and mineralization of the granuloma (5, 11). To evaluate the effect of the drug-loaded PLG-NP on lung pathology, lung tissue sections from infected

drug-treated guinea pigs were examined microscopically. Lung sections from untreated and empty-PLG-NP-control animals showed numerous severe lesions with extensive lung parenchymal involvement with evidence of necrosis and progression of disease (Fig. 1A, B, E, and F). Guinea pigs from both the drug-loaded PLG-NP and nonencapsulated drug groups had considerably fewer lung lesions characterized by discrete foci of fibrosis with minimal lung parenchymal involvement (Fig. 1C, D, G, and H). Both drug-treated groups were scored as having similar lung pathology, showing extensive improvement compared to the controls (Fig. 1).

The present study clearly documents the efficacy of polymeric nanoparticle-based antituberculosis chemotherapy in an aerosol infection model in the guinea pig and its promise for use in intermittent-treatment regimens.

We thank Robert Christensen, Steven Dreitz, and Nicholas Tompkin for their excellent technical assistance.

We acknowledge National Institutes of Health grant AI-054697 and the research and development contract NO1 AI-95385 (Barbara Laughon and Karen Near). We also acknowledge Department of Science and Technology, New Delhi, India, for providing financial support to Department of Biochemistry, PGIMER, Chandigarh, India.

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FIG. 1. Representative light photomicrographs of lungs from aerosol-infected guinea pigs following 6 weeks of chemotherapy. Sucrose control- (A) and empty-NP-treated (E) animals show numerous severe lesions with extensive parenchymal involvement (magnification, ×2). These lesions were characterized by evidence of necrosis with intralésional mineralization (arrow), thus reflecting progression of disease (B and F, respectively) (magnification, ×40). In contrast, nonencapsulated- (C) and encapsulated- (G) drug-treated animals had considerably fewer lesions with minimal lung parenchymal involvement (magnification, ×2). These lesions were characterized by discrete foci of fibrosis and mixed inflammation (D and H, respectively) (magnification, ×40). Sections were stained with hematoxylin and eosin.

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