Chemotherapeutic Potential of Orally Administered Poly(Lactide-Co-Glycolide) Microparticles Containing Isoniazid, Rifampin, and Pyrazinamide against Experimental Tuberculosis

Qurrat ul-Ain, Sadhna Sharma, and G. K. Khuller*

Department of Biochemistry, Postgraduate Institute of Medical Education & Research, Chandigarh 160 012, India

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Poly(lactide-co-glycolide) (PLG) microspheres (diameter, less than 3 μm) containing isoniazid, rifampin, and pyrazinamide were used in a sustained oral drug delivery system to treat murine tuberculosis. Drug levels above the MIC were observed up to 72 h in plasma and for 9 days in various organs. Relative bioavailability of encapsulated drugs was greater than that of free drugs. Chemotherapy results showed better or equivalent clearance of bacilli in the PLG-drug-administered group (weekly) than with free drugs (daily).

Current short-term chemotherapy for tuberculosis requires daily administration of isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), and ethambutol (ETH) for a period of 6 months, which leads to patient noncompliance. Previous studies have shown that antitubercular drugs like INH and RIF, when entrapped in injectable poly(DL-lactide-co-glycolide) (PLG) microspheres (PLG-mps) and administered subcutaneously, released drug for 6 to 7 weeks and exhibited comparable or better clearance of bacilli in organs than free drugs (2–4). We now report the development and chemotherapeutic potential of PLG microparticles containing INH, RIF, and PZA, which can be administered orally with sustained release of drug(s) to treat experimental tuberculosis.

PLG-mps containing antituberculosis drugs (INH, RIF, and PZA) were prepared by following a double-emulsification solvent evaporation procedure (2, 5). PLG-mps were evaluated in particle size analysis, drug encapsulation, and in vivo drug release studies as described earlier (2). Mice (laca strain) 6 to 8 weeks old of either sex were orally administered single doses of free and PLG-encapsulated drugs (INH, 10 mg/kg of body weight; RIF, 12 mg/kg; and PZA, 25 mg/kg, which approximates doses being given to 50-kg humans). Controls consisted of mice administered empty PLG-mps drugs mixed merely with PLG-mps and phosphate-buffered saline. Mice were killed at various time intervals, and drug concentrations were determined in plasma and 20% (wt/vol) organ homogenates. The INH concentration was estimated spectrofluorimetrically (10), while the concentration of RIF in plasma and organ homogenates was estimated by microbiological assay (9), and PZA was measured spectrophotometrically with a sensitivity of 5 $\mu\text{g/ml}$ (6). Plasma time concentration data of free and PLG-encapsulated drugs were evaluated for peak plasma concentration (C_{max}) and time to reach peak level (T_{max}). The area under the curve [$\text{AUC}_{(0-\infty)}$] was calculated using the trapezoidal rule, and the relative bioavailability of encapsulated drugs was calculated from the ratio of the $\text{AUC}_{(0-\infty)}$ of encapsulated drugs and the $\text{AUC}_{(0-\infty)}$ of respective free drugs.

Mice were infected with 1.5×10^5 viable bacilli of *Mycobacterium tuberculosis* H₃₇Rv intravenously. Fifteen days postinfection (confirmed by Ziehl-Neelsen staining of tissue homogenates of three to four animals), mice were divided into different groups, each containing seven to eight animals. Group I received PLG-mps containing INH, RIF, and PZA weekly (six doses), group II was administered free INH, RIF, and PZA daily (35 doses), and group III received phosphate-buffered saline, which served as a control. Drugs were administered orally for 5 weeks. Animals were sacrificed 1 week after the last dose of chemotherapy (42nd day), and different organs were processed for CFU enumeration (2).

Drug-to-polymer ratios of 1.5:1, 1:2, and 1:10 for INH, RIF, and PZA, respectively, were found to be optimum with drug encapsulation efficiency of 8 to 10% (INH), 20 to 30% (RIF), and 9 to 13% (PZA). The size of PLG microparticles containing INH, RIF, and PZA varied from 1.11 to 2.20 μm . Drug loading in the PLG microspheres relative to 1 g of polymer matrix was found to be 135 mg for INH, 100 mg for RIF, and 10.5 mg for PZA. Oral administration of PLG-encapsulated antituberculosis drugs in combination resulted in a sustained release of INH, RIF, and PZA in plasma up to 72 to 120 h.

TABLE 1. Organ levels of INH, RIF, and PZA after oral administration of PLG microparticles containing antituberculosis drugs in combination

Treatment group	Time period	Concn ($\mu\text{g/ml}$) ^a			
		Lungs	Liver	Spleen	Intestine
PLG-INH	Day 3	4.96 $\pm$ 0.47	5.56 $\pm$ 0.19	6.70 $\pm$ 0.14	10.33 $\pm$ 0.47
	Day 7	2.76 $\pm$ 0.04	3.46 $\pm$ 0.04	4.93 $\pm$ 0.09	3.71 $\pm$ 0.14
	Day 9	0.96 $\pm$ 0.04	0.70 $\pm$ 0.04	1.80 $\pm$ 0.04	2.06 $\pm$ 0.47
PLG-RIF	Day 3	3.20 $\pm$ 0.15	3.50 $\pm$ 1.94	3.40 $\pm$ 1.03	4.00 $\pm$ 0.98
	Day 7	1.95 $\pm$ 0.23	2.28 $\pm$ 0.23	2.10 $\pm$ 0.25	2.90 $\pm$ 0.15
	Day 9	0.50 $\pm$ 0.10	0.90 $\pm$ 0.10	0.80 $\pm$ 0.10	1.00 $\pm$ 0.15
PLG-PZA	Day 3	39.00 $\pm$ 0.81	57.00 $\pm$ 0.81	59.00 $\pm$ 0.47	60.30 $\pm$ 0.12
	Day 7	35.30 $\pm$ 0.47	45.33 $\pm$ 0.47	55.33 $\pm$ 1.9	20.33 $\pm$ 0.47
	Day 9	20.12 $\pm$ 0.21	23.00 $\pm$ 0.47	25.00 $\pm$ 0.47	2.83 $\pm$ 0.23

* Corresponding author. Mailing address: Department of Biochemistry, PGIMER, Chandigarh 160 012, India. Phone: 0172-747 585, ext. 5174-75. Fax: 0172-744 401, 745 078. E-mail: gkkhuller@yahoo.co.in.

^a All values are mean $\pm$ standard deviation for 10 animals. Drugs above MIC levels were detected in lungs, liver, spleen, and intestine up to 9 days when administered alone in PLG microparticles.

FIG. 1. Log CFU in lungs, liver, and spleen after chemotherapy for *M. tuberculosis*-infected mice with INH, RIF, and PZA. Free drugs were administered daily, whereas PLG drugs were given weekly for 5 weeks. Values are mean $\pm$ SEM for seven to eight animals. ***, $P < 0.001$ compared to results for control (determined by Student's unpaired t test).

Free drugs as well as empty PLG microparticles merely mixed with free drugs were cleared from plasma within 12 to 24 h. The C_{max} of PLG-INH ($12.11 \pm 0.86 \mu\text{g/ml}$) was observed at 12 h (T_{max}) and exhibited an $AUC_{0-\infty}$ of $212.00 \pm 17.15 \mu\text{g/ml} \cdot \text{h}$, while free INH showed a C_{max} ($10.22 \pm 0.30 \mu\text{g/ml}$) at 6 h with an $AUC_{0-\infty}$ of $27.66 \pm 0.16 \mu\text{g/ml} \cdot \text{h}$. The C_{max} of PLG-RIF ($4.98 \pm 0.02 \mu\text{g/ml}$) and that of free RIF ($3.92 \pm 0.67 \mu\text{g/ml}$) were observed at 12 and 6 h, with AUC values of 91.5 ± 2.92 and $29.11 \pm 0.29 \mu\text{g/ml} \cdot \text{h}$, respectively. The C_{max} of PZA ($119.00 \pm 12.95 \mu\text{g/ml}$) was observed at 12 h (T_{max}) and exhibited an $AUC_{0-\infty}$ of $3,368.00 \pm 25.10 \mu\text{g/ml} \cdot \text{h}$, while for free PZA a peak level ($40.65 \pm 2.43 \mu\text{g/ml}$) was observed at 8 h with an $AUC_{0-\infty}$ of $397.00 \pm 14.20 \mu\text{g/ml} \cdot \text{h}$. Relative bioavailabilities of PLG-encapsulated INH, RIF, and PZA increased by 7.8-, 3.14-, and 8.48-fold, respectively.

INH, RIF, and PZA were released in a sustained manner in the lungs, liver, spleen, and intestine (Table 1). Peak levels of drugs were observed on day 3 in all the organs and were detected above the MIC until 9 days, indicating sustained release behavior of PLG drugs. Free drugs and free drugs merely mixed with empty PLG microparticles were cleared from all the organs within 24 h post-oral administration. Further, repeated administration of PLG-mps drugs on the 7th, 14th, 21st, and 28th day did not reveal any accumulation effect of drugs in organs.

Since PLG-encapsulated drugs revealed sustained release of the drugs in the organs until 7 to 9 days above the MIC, the weekly therapeutic regime for the delivery of PLG-entrapped drugs was followed. Figure 1 reveals the log CFU obtained in organs after chemotherapy for *M. tuberculosis*-infected mice with free and PLG-entrapped drugs for 5 weeks. Treatment for both the groups resulted in a significant ($P < 0.001$) clearance of bacilli compared to results for controls. Daily treatment with free drugs and once-weekly treatment with PLG-encapsulated drugs resulted in the clearance of bacilli by 1.70 and 1.80 log units in lungs, respectively, compared to controls. Liver and spleen exhibited equivalent reduction in CFU in free-drug-treated and PLG-encapsulated-drug-treated groups. The chemotherapeutic efficacy of PLG-encapsulated drugs given orally was equivalent to that of subcutaneously injected drugs (Table 2), as reported earlier (4). Study of lymphocyte proliferation as an index of cellular immune response indicated a marginal increase in empty PLG microparticle-immunized mice compared to results for nonimmunized animals. Further, equivalent numbers of bacilli were recovered from the infected organs of control (nonimmunized) and empty PLG microparticle-immunized groups, thus confirming that PLG itself is not inducing any immune response. Previous studies using PLG-based injectable or implantable drug delivery systems showed efficacy in mice (8) and rabbits (7). These carrier systems,

TABLE 2. Comparison of log CFU after oral and subcutaneous administration of PLG-encapsulated drugs

Group	log CFU					
	Lungs		Liver		Spleen	
	Oral ^a	Subcut ^b	Oral	Subcut	Oral	Subcut
Controls	6.19 $\pm$ 0.07	6.22 $\pm$ 0.05	5.78 $\pm$ 0.32	6.47 $\pm$ 0.04	5.58 $\pm$ 0.35	5.66 $\pm$ 0.02
PLG-encapsulated drugs	4.39 $\pm$ 0.30	4.75 $\pm$ 0.06	4.24 $\pm$ 0.39	4.60 $\pm$ 0.09	4.49 $\pm$ 0.37	4.82 $\pm$ 0.02

^a Oral, results of this study when drugs were administered orally (INH, 10 mg/kg; RIF, 12 mg/kg; PZA, 25 mg/kg).

^b Subcut, results obtained when drugs were administered as subcutaneous injections (INH, 37.5 mg/kg; RIF, 42.5 mg/kg) as reported previously (4).

however, have practical limitations. Here we report the use of PLG-based microspheres with mean diameters of 1.11 to 2.20 μm for oral administration. The microparticles of size $<5 \mu\text{m}$ are likely to maintain prolonged contact with intestinal mucosa or likely to cross intestinal barriers via M-cells in Peyer's patches, resulting in increased bioavailability (1). The PLG microspheres can function as an effective sustained-release carrier of antituberculosis drugs up to 9 days in all the target organs. Therefore, 35 doses of free drugs have been reduced to 6 doses of encapsulated drugs administered weekly, which achieve similar or better results.

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