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# Role of poly [DL-lactide-co-glycolide] in development of a sustained oral delivery system for antitubercular drug(s)

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**Abstract**

An oral formulation based on poly (DL-lactide-co-glycolide) (PLG) microparticles was developed for delivery of antituberculous drugs. PLG entrapped antitubercular drugs when administered orally, were found to release the drugs in a sustained manner. This formulation was found to be stable in the acidic environment of gastric fluid whereas, in the intestinal fluid the drug release was obtained up to 20 days as indicated by in vitro studies. Pharmacokinetic analysis of the data revealed changes in $C_{max}$; $AUC_{o-\alpha}$; $t_{1/2}$ (a) and $t_{1/2}$ (e) when drugs were given entrapped in PLG microparticles. Higher peak concentration, area under the concentration time curve and delayed elimination rate of entrapped drugs indicated the potential of PLG for effective treatment of tuberculosis. Further, work is being carried out to evaluate the chemotherapeutic efficacy of the antitubercular drugs encapsulated in PLG microspheres. © 2002 Elsevier Science B.V. All rights reserved.

*Keywords:* PLG; Oral drug delivery; Antitubercular drugs

## 1. Introduction

Tuberculosis still remains the cause of high mortality and morbidity; particularly in developing countries. It is curable to a large extent but has resurfaced due to emergence of multi-drug resistant strains and AIDS pandemic (Chretien, 1995). Short term chemotherapy involves daily administration of four drugs namely isoniazid, rifampicin, pyrazinamide and ethambutol for 6–8 months and this has many disadvantages like toxicity, decreased bioavailability at the target sites and most importantly non-compliance by the patient thus leading to drug resistance. Therefore, the shortcomings of this chemotherapy can be circumvented by developing a drug delivery system that would release the drugs in a sustained manner over a longer period of time.

Antitubercular drugs have been successfully entrapped and delivered in biodegradable polymers like PLG (Gangadharam et al., 1989; Kailasam et al., 1994). These polymers release drug in a controlled manner at therapeutic levels with minimal toxicity. In earlier studies, antituberculous drugs

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like isoniazid (INH) and rifampicin (RIF), entrapped in PLG polymers and injected as a single dose resulted in sustained release of drugs (6–7 weeks) in various organs of mice (Dutt and Khuller, 2001a,b). Although, the results obtained were encouraging but the oral delivery would avoid the pain and discomfort associated with subcutaneous routes of administration. Hence, in this communication, we report the pharmacokinetics of PLG encapsulated antituberculous drugs; orally administered either individually or in combination in mice.

## 2. Materials and methods

### 2.1. Chemicals and drugs

The materials, poly (DL-lactide-co-glycolide), 50:50 resomer RG 506, (MW 250) was purchased from Boehringer Mannheim (Germany); polyvinyl alcohol (87–89%) hydrolyzed (Av MW 13 000–23 000); isoniazid (INH), rifampicin (RIF) and pyrazinamide (PZA) were obtained from Sigma, St. Louis, MO. All other reagents used in the study were of highest purity.

### 2.2. Animals

Mice (Laca-strain) of either sex (4–5 weeks old) obtained from the Institute Animal House, PGIMER, Chandigarh were used. The animals were fed standard pellet diet and water ad libitum.

### 2.3. Preparation of PLG microspheres containing INH, RIF and PZA

Drug encapsulated PLG microparticles were prepared by the double emulsification solvent evaporation procedure as described earlier (Edwards et al., 1997). Briefly, 20 mg of rifampicin in 4 ml phosphate buffer saline (PBS) was emulsified on ice onto 400 mg PLG in 4 ml dichloromethane (DCM) by probe sonication in dark under reduced $N_2$ pressure. INH (40mg) in 1 ml PBS was emulsified on ice onto 27.0 mg PLG in 1 ml DCM by probe sonication. Similarly, 62.5 mg of PZA in 6 ml PBS was sonicated with 620 mg PLG in 6 ml DCM. The drug to polymer ratio in the emulsions was kept at 1:20 (w/v); 60:40 (w/v) and 1:10 (w/v) for RIF, INH and PZA, respectively. Different drug polymer ratios were used for three drugs as highest efficiencies were observed using these ratios (data not shown). The primary emulsions so formed were continuously stirred overnight after addition of 1 ml of 20% poly vinyl alcohol (PVA) dropwise into each formulation. Next day, the particles were collected by centrifugation at $10\,000 \times g$ for 30 min at 4 °C, washed several times with PBS and finally resuspended in same buffer. PLG microparticles containing INH or RIF or PZA were prepared separately and mixed at the ratio of 1:1:1 before administration for combination release profile studies.

### 2.4. Determination of drug content of microparticles

The entrapment of drugs in PLG microparticles was determined by lysing them with 5% sodium dodecylsulphate (SDS) (w/v) in 0.1NNaOH. Briefly, the released RIF was estimated by measuring OD at 475 nm ($\lambda_{max}$). PZA was estimated spectrophotometrically by using alkaline, sodium nitroprusside, that forms a brown coloured solution with PZA, which absorbes at 495 nm (Gurumurthy et al., 1980). INH forms a fluorescent compound with isobutanol in presence of acetaldehyde and acetic acid, which is measured spectrofluorometrically (Scott and Wright, 1967). Plasma samples of mice were assayed for RIF by microbiological assay as described by Saito and Tonioke (1989).

### 2.5. Sizing of PLG particles

The size of PLG microparticles was determined on a particle size analyzer, CSI-I, Galai, Israel at the Talwar Research Laboratories, New Delhi.

### 2.6. In vitro drug release studies

In vitro drug release studies were carried out in simulated gastric and intestinal fluids without enzymes. They were prepared according to the method given in USP XXII (Hari et al., 1996).

Gastric fluid was composed of 0.2 M NaCl adjusted to pH 2. Intestinal fluid was composed of 0.8 M $NaH_2PO_4$; 0.2 M NaOH; 10 mM bile salts pH adjusted to 7.6. PLG microparticles with entrapped drugs were suspended in simulated fluids respectively and incubated at 37 °C for 20 days. Supernatant was collected at 6, 12, 24 h and then daily after centrifugation at 10 000 rpm. The supernatant was assayed for released drugs by the methods described above.

### 2.7. In vivo drug disposition studies

PLG microparticles with antituberculous drugs encapsulated individually were prepared as described above. The dose of INH, RIF and PZA given to mice was 10, 12 and 25 mg/kg body weight respectively. INH, RIF and PZA were given in combination as 10, 12 and 25 mg/kg body wt., respectively, estimated equivalent to human dose of a 50–60 kg body wt. Mice were divided into following groups containing 12 animals in each group and the dose of the drug was administered orally in 100 μl volume. Groups 1, 2 and 3 were given free INH, RIF and PZA, respectively. Group 4 was given combination of three drugs in the free form. Group 5, 6 and 7 were given INH, RIF and PZA encapsulated in PLG microparticles respectively. Group 8 animals were administered the combination of three drugs in PLG microparticles. Mice were bled at different time intervals of 2, 4, 6, 12, 24, 48, 96 and 108 h. Plasma was collected and assayed for different drugs as described earlier.

### 2.8. Comparison of the pharmacokinetics of PLG encapsulated drugs and free drugs (alone as well as in combination)

$C_{max}$ (peak plasma concentration) and $T_{max}$ (time to reach peak concentration) were calculated from the actual plasma data. The elimination rate constant ($K_{el}$) was calculated by least square regression analysis. Elimination half-life ($t_{1/2}$ e) was calculated using the formula $0.693/K_{el}$. The absorption rate constant ($K_a$) was calculated by the residual method and the absorption half-life ($t_{1/2}$ a) was determined by using the formula $0.693/K_a$. The area under the concentration time curve ($AUC_{0-t}$) was calculated by trapezoidal rule and the AUC $(t-\alpha)$ by dividing the last drug concentration by respective $K_{el}$. The AUC $(0-\alpha)$ is the sum of AUC $(0-t)$ and AUC $(t-\alpha)$.

### 2.9. Statistical analysis

Values were presented as mean ± S.E.M. The students unpaired $t$-test was applied for the statistical analysis of the data.

## 3. Results and discussion

In the present study, ability of biodegradable poly (DL-lactide-co-glycolide) (PLG) particles to release antituberculous drugs (as a single oral dose) was studied using three front line drugs viz., INH, RIF and PZA. The drug encapsulation efficiency of the microparticles was found to be 8–10% for PZA; 10–11% for INH and 12–18% for RIF. The entrapment of rifampicin was more because of its hydrophobic nature as these molecules are entrapped better in polymers than hydrophilic substances (Hajima and Okada, 1995).

Particle size analysis for the PLG microparticles in terms of volume mean diameter (in μm) was found to be 1.11 for INH, 1.4 for RIF and 2.20 for PZA microparticles, respectively.

In order to see the stability of PLG encapsulated drug formulation in gastro intestinal tract, in vitro drug release studies were conducted in simulated gastric and intestinal fluids without enzymes. Fig. 1 depicts that drug release is faster and relatively more in intestinal fluid (about 85% over a period of 12 days) while the drug release in gastric fluid was only 22–30% in 18 days. It could be because of erosion and diffusion of these polymers at alkaline pH due to the fibre formation whereas these polymers release the drugs only by diffusion at acidic pH (Rao and Kumar, 1997). In addition, it could also be due to the PVA, which was employed in the preparation of PLG particles accounting for the sustained release of drugs (Landry et al., 1997). These results thus implicate the stability of PLG microparticles containing antituberculous drugs in gastric environment.

Three antituberculous drugs viz. INH, RIF and PZA in hardened PLG microspheres at therapeutic dose of 10, 12 and 25 mg/kg body weight respectively were administered orally to mice to evaluate their in vivo release. Peak plasma levels for free INH and entrapped INH was observed at 6 and 12 h, respectively. Free INH remained in the circulation for 24 h, while PLG entrapped INH remained in the body for 72 h (Fig. 2a). Fig. 2(b) depicts plasma concentration of free INH and PLGA encapsulated INH at different time intervals when given in combination with PZA and RIF. Peak plasma concentration for free drug was observed at 6 h while encapsulated INH in combination showed peak level at 12 h. Entrapped drugs remained in circulation up to 72 h as compared to free drugs, which were eliminated within 24 h. It is worth mentioning that level of PLG encapsulated INH were found to be always higher than its MIC value (0.1 μg/ml).

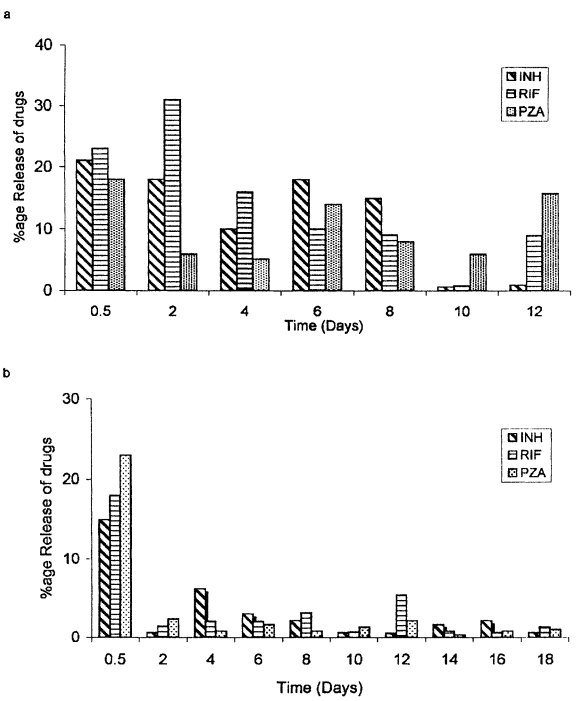


Fig. 1. In vitro % age release of drugs in (a) simulated intestinal fluid; (b) simulated gastric fluid at different time points. The values are average of two independent set of experiments.

Fig. 3(a) reveals plasma levels of free and PLG encapsulated RIF. Peak drug levels for both the formulations was observed at 12 h, however, $C_{max}$ for free drug was significantly lower than for entrapped RIF. Fig. 3(b) shows plasma concentration of free and PLG entrapped RIF in combination with INH and PZA at different time points. RIF levels were seen up to 72 h above MIC (0.2 μg/ml) for PLG entrapped RIF.

Plasma concentration of free PZA and entrapped PZA at different time points when given alone are represented in Fig. 4(a). Peak concentration of free PZA was seen at 6 h while entrapped drug peaks at 24 h. Similar observations were obtained when PZA was administered in combination with INH and RIF (Fig. 4b). However, entrapped PZA remains in the circulation for up to 108 h (values were above MIC, i.e. 8 μg/ml at all time points).

It is, thus clear from that drug levels remain in the body for 3–5 days when given in PLG microparticles, exhibiting a sustained release pattern of the drug in plasma as compared to free drugs, which are eliminated within 24–48 h. In addition, drug levels were detected in organs up to 9–12 days when PLG encapsulated drugs were administered orally (unpublished observations). The particle size of PLGs prepared in this study is of 1.10–2.20 μm in size. Since the size of PLG microparticles is less than 10 μm, there is possibility of uptake of these microparticles through M cells in intestinal mucosa. These observations are supported by earlier findings of Damge et al. (1996) who have demonstrated that PLG microparticles less than 10 μm can cross the intestinal mucosa. In addition, PLG microparticles may stick to intestinal mucosa for an extended period of time due to their bioadhesive nature and drug may be released in a sustained manner as reported by Mathiowitz et al. (1997).

Table 1 depicts the pharmacokinetic parameters of INH when given alone or in combination in free or encapsulated form. The bioavailability of INH was significantly increased in PLGA encapsulated form as compared to free form. $C_{max}$, $T_{max}$ and $AUC_{o-\alpha}$ were enhanced significantly in encapsulated form when given alone or in combination as compared to free form. The increased

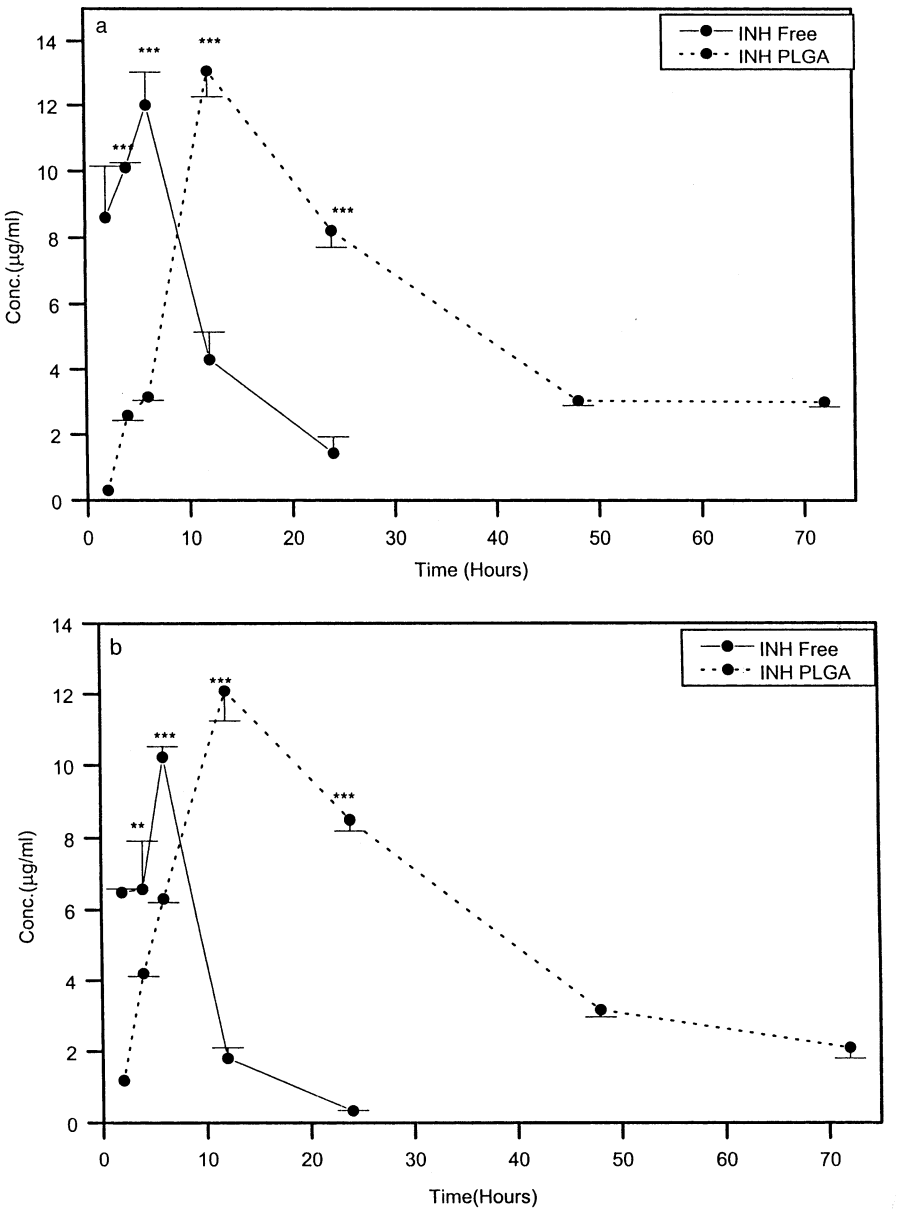


Fig. 2. Plasma levels of free and encapsulated Isoniazid (INH) at different time points. (a) Alone; (b) in combination with RIF and PZA. All values are ± S.E.M. of five animals. Plasma levels of PLG encapsulated drugs as compared to free drugs were statistically significant at ** $P < 0.01$ and *** $P < 0.001$.

extent of bioavailability of INH in encapsulated form is further confirmed by prolonged elimination half-life of the drug in entrapped form, indicating thereby that the PLG encapsulated INH has a sustained release property and the therapeutic effect remained above MIC for a longer period of time. Table 2 shows the kinetic parameters of RIF when given alone or in combination in free or encapsulated form. The extent of bioavailability of RIF was significantly increased in encapsu-

lated form as evident from enhanced $C_{max}$ and $AUC_{o-a}$. No difference was observed in $T_{max}$ of free and entrapped drug when given alone or in combination indicating that the encapsulation of drug does not alter the rate of bioavailability of RIF although the absorption half-life of drug was significantly reduced in encapsulated form. Prolonged elimination of RIF after encapsulation confirms its sustained release property. Table 3 shows pharmacokinetics of PZA when given alone

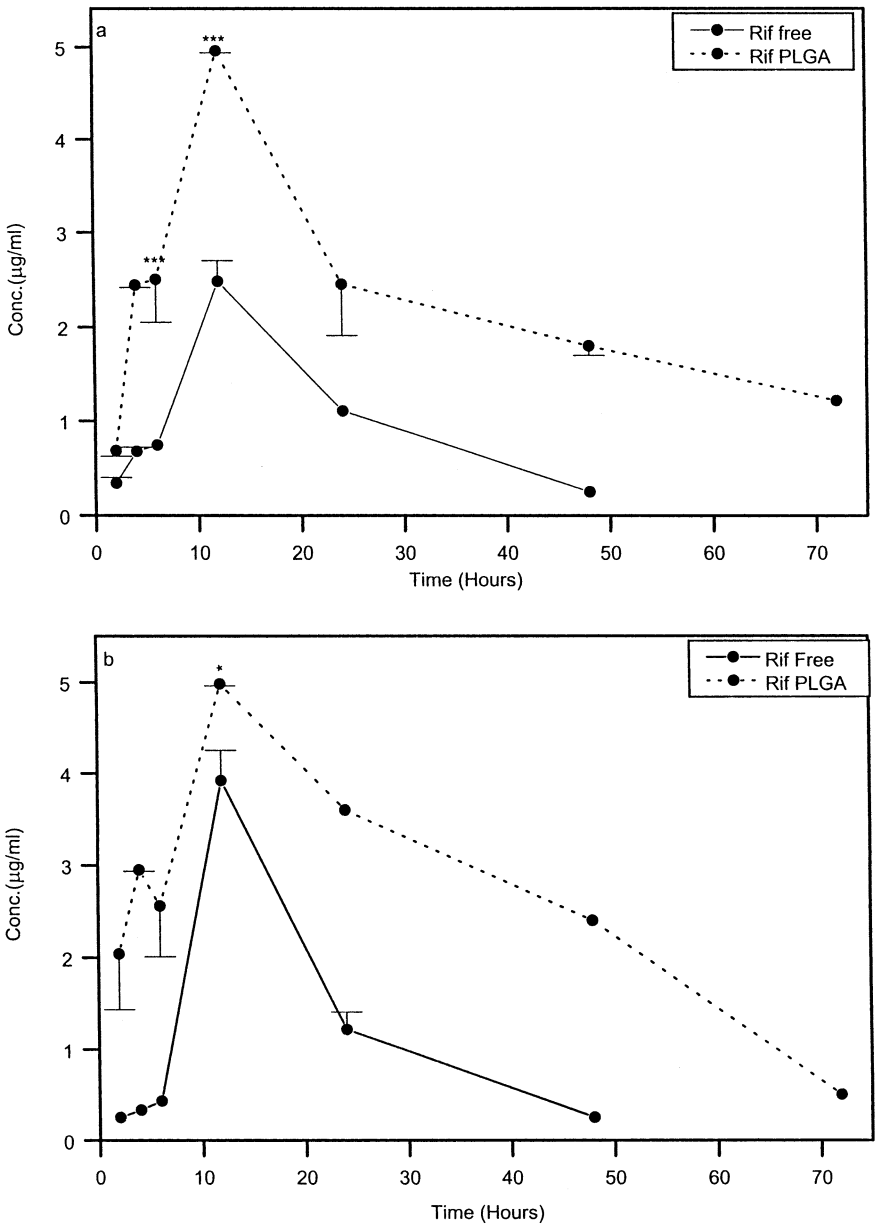


Fig. 3. Plasma levels of free and encapsulated Rifampicin (RIF) at different time points. (a) Alone; (b) in combination with INH and PZA. All values are ± S.E.M. of five animals. Plasma levels of PLG encapsulated drugs as compared to free drugs were statistically significant at * $P < 0.05$ and *** $P < 0.001$

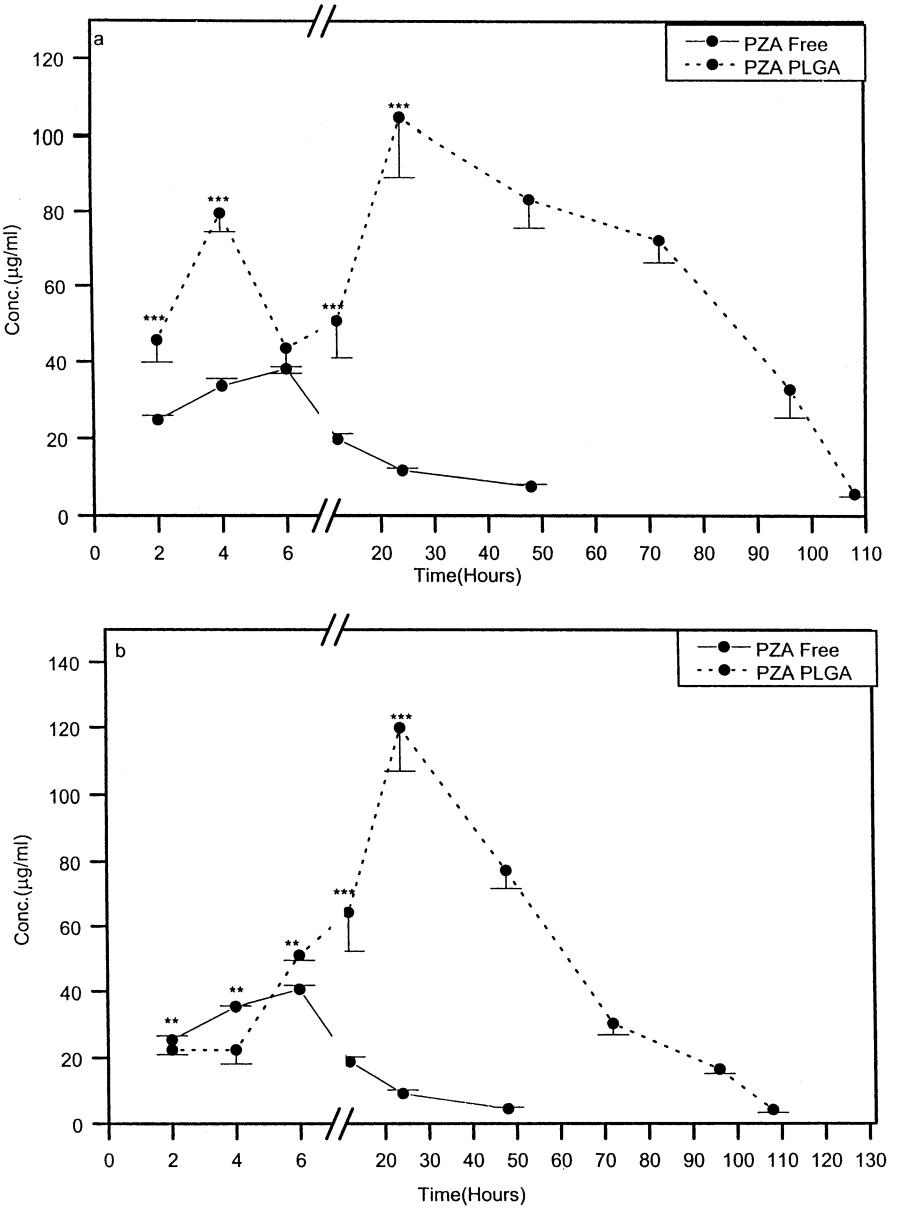


Fig. 4. Plasma levels of free and encapsulated pyrazinamide (PZA) at different time points. (a) Alone; (b) in combination with RIF and INH. All values are ± S.E.M. of five animals. Plasma levels of PLG encapsulated drugs as compared to free drugs were statistically significant at ** $P < 0.01$ and *** $P < 0.001$

or in combination in free or encapsulated form. The kinetic parameter results were similar to those found with entrapped INH or RIF. Pharmacokinetic analysis of the three drugs shows that peak plasma concentrations are higher in encapsulated drug, which lead to higher residence time of the drugs in the body and increased bioavailability.

PLG polymers have been used as implants (Gangadharam et al., 1989) and microspheres for

delivering antituberculous drugs subcutaneously (Dutt and Khuller, 2001a,b) for their sustained release. However, PLG microspheres containing anti TB drugs have not been used as oral drug delivery system against tuberculosis, although, they have been earlier employed to deliver orally protein antigens and hormones (Okada et al., 1994). The observed sustained release of drugs in plasma is probably due to the retention of PLG microparticles in the intestine as this polymer has a bio-adhesive property. Moreover, it has been demonstrated that microparticles do maintain contact with intestinal mucosa for an extended period of time before they are absorbed or release their contents. It is being supported by an earlier study in which Rhodamine-B-labeled PLG has been shown to cross intestinal mucosa in rats, when administered orally (Damge et al., 1996).

Table 1
Pharmacokinetics of isoniazid

| | Free drug | | PLGA encapsulated drug | |
|---|---|---|---|---|
| | Alone | In combination with RIF and PZA | Alone | In combination with RIF and PZA |
| $C_{max}$ (μg/ml) | $12.33 \pm 1.12$ | $10.22 \pm 0.30^{a}$ | $13.06 \pm 0.84^{d}$ | $12.11 \pm 0.86^{b,c}$ |
| $T_{max}$ (h) | $6.00 \pm 0.00$ | $6.00 \pm 0.00$ | $12.00 \pm 0.00$ | $12.00 \pm 0.00$ |
| $T_{1/2}$ e (h) | $5.54 \pm 0.77$ | $3.82 \pm 0.22^{a,*}$ | $28.00 \pm 1.21^{d,***}$ | $23.10 \pm 0.69^{b,*,c,***}$ |
| $T_{1/2}$ a (h) | $2.56 \pm 0.25$ | $2.23 \pm 0.41^{a}$ | $8.05 \pm 1.06^{d,***}$ | $4.07 \pm 0.49^{b,***,c,*}$ |
| $AUC_{0-\alpha}$ (μg/ml $h^{-1}$) | $47.88 \pm 1.52$ | $27.66 \pm 0.16^{a,***}$ | $257.00 \pm 3.26^{d,***}$ | $212.00 \pm 17.15^{b,***,c,**}$ |

All values are expressed as mean ± S.E.M.; $n = 5$. Superscripts without stars show non-significant values.
[a] Depicts comparison of free drug alone vs. free drug in combination.
[b] Depicts comparison of entrapped drug alone vs. entrapped drug in combination.
[c] Depicts comparison of free drug alone vs. entrapped drug alone.
[d] Depicts comparison of free drug in combination vs. entrapped drug in combination.
* $P < 0.05$;
** $P < 0.001$;
*** $P < 0.0001$.

Table 2
Pharmacokinetics of rifampicin

| | Free drug | | Entrapped drug in PLGA | |
|---|---|---|---|---|
| | Alone | In combination with INH and PZA | Alone | In combination with INH and PZA |
| $C_{max}$ (μg/ml) | $2.48 \pm 0.02$ | $3.92 \pm 0.67^{a}$ | $4.96 \pm 0.04^{d,*}$ | $4.98 \pm 0.02^{b,c}$ |
| $T_{max}$ (h) | $12.00 \pm 0.00$ | $12.00 \pm 0.00$ | $12.00 \pm 0.00$ | $12.00 \pm 0.00$ |
| $T_{1/2}$ e (h) | $10.99 \pm 0.15$ | $9.97 \pm 0.80^{a}$ | $32.04 \pm 1.55^{d,*}$ | $19.25 \pm 0.00^{b,*,c,*}$ |
| $T_{1/2}$ a (h) | $7.00 \pm 0.14$ | $8.05 \pm 0.45^{a}$ | $3.82 \pm 1.45^{d,*}$ | $2.25 \pm 0.00^{b,c,*}$ |
| $AUC_{0-\alpha}$ (μg/ml $h^{-1}$) | $18.93 \pm 0.19$ | $29.11 \pm 0.29^{a}$ | $99.9 \pm 6.84^{d,*}$ | $91.5 \pm 2.92^{b,c,*}$ |

All values are expressed as mean ± S.E.M.; $n = 5$. Superscripts without stars show non-significant values.
[a] Depicts comparison of free drug alone vs. free drug in combination.
[b] Depicts comparison of entrapped drug alone vs. entrapped drug in combination.
[c] Depicts comparison of free drug alone vs. entrapped drug alone.
[d] Depicts comparison of free drug in combination vs. entrapped drug in combination.
* $P < 0.0001$.

Table 3
Pharmacokinetics of pyrazinamide

| | Free drug | | PLGA encapsulated drug | |
|---|---|---|---|---|
| | Alone | In combination with INH and RIF | Alone | In combination with INH and RIF |
| $C_{max}$ (μg/ml) | $38.02 \pm 0.55$ | $40.65 \pm 2.43^{a}$ | $104.7 \pm 15.60^{d,**}$ | $119.00 \pm 12.95^{b,*,c,**}$ |
| $T_{max}$ (h) | $6.00 \pm 0.00$ | $6.00 \pm 0.00$ | $24.00 \pm 0.00$ | $24.00 \pm 0.00$ |
| $T_{1/2}$ e (h) | $19.80 \pm 1.20$ | $14.43 \pm 0.80^{a,**}$ | $24.65 \pm 1.95^{d,**}$ | $19.25 \pm 1.11^{b,**,c,**}$ |
| $T_{1/2}$ a (h) | $1.43 \pm 1.00$ | $4.20 \pm 1.00^{a,**}$ | $7.80 \pm 0.00^{d,**}$ | $7.20 \pm 0.00^{b,c,**}$ |
| $AUC_{0-\alpha}$ (μg/ml $h^{-1}$) | $514.00 \pm 2.92$ | $397.00 \pm 14.20^{a,**}$ | $3137.00 \pm 26.90^{d,**}$ | $3368.00 \pm 25.10^{b,**,c,**}$ |

All values are expressed as mean ± S.E.M.; $n = 5$. Superscripts without stars show non-significant values.
[a] Depicts comparison of free drug alone vs. free drug in combination.
[b] Depicts comparison of entrapped drug alone vs. entrapped drug in combination.
[c] Depicts comparison of free drug alone vs. entrapped drug alone.
[d] Depicts comparison of free drug in combination vs. entrapped drug in combination.
* $P < 0.001$;
** $P < 0.0001$.

This is first report indicating the potential application of poly-lactide-co-glycolide polymers for oral delivery of antituberculous drugs. Microsphere based oral drug delivery of antituberculous drugs will provide a sound basis for predicting once-weekly treatment for tuberculosis. Chemotherapeutic potential of these particles is under study and we believe that this approach will improve the management of drug therapy in tuberculous patients.

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