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Alginate-based oral drug delivery system for tuberculosis: pharmacokinetics and therapeutic effects

Qurrat-ul-Ain[1], Sadhna Sharma[1], G. K. Khuller[1]* and S. K. Garg[2]

Departments of [1]Biochemistry and [2]Pharmacology, Postgraduate Institute of Medical Education & Research, Chandigarh 160 012, India

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Alginate microparticles were developed as oral sustained delivery carriers for antitubercular drugs in order to improve patient compliance. In the present study, pharmacokinetics and therapeutic effects of alginate microparticle encapsulated antitubercular drugs, i.e. isoniazid, rifampicin and pyrazinamide were examined in guinea pigs. Alginate microparticles containing antitubercular drugs were evaluated for *in vitro* and *in vivo* release profiles. These microparticles exhibited sustained release of isoniazid, rifampicin and pyrazinamide for 3–5 days in plasma and up to 9 days in organs. Peak plasma concentration (C_{max}), T_{max}, elimination half-life ($t_{1/2e}$) and $AUC_{0-\infty}$ of alginate drugs were significantly higher than those of free drugs. The encapsulation of drug in alginate microparticles resulted in up to a nine-fold increase in relative bioavailability compared with free drugs. Chemotherapeutic efficacy of alginate drug microspheres against experimental tuberculosis showed no detectable cfu values at 1:100 and 1:1000 dilutions of spleen and lung homogenates. Histopathological studies further substantiated these observations, thus suggesting that application of alginate-encapsulated drugs could be useful in the effective treatment of tuberculosis.

Keywords: tuberculosis, pharmacokinetics, alginate, drug delivery, antitubercular drugs

Introduction

Poor patient compliance is the single most common reason for the failure of chemotherapy of tuberculosis.[1] One useful method to ensure compliance in tuberculosis patients is to supervise the administration of drugs, which is not always practical. An alternative approach is to administer the drugs in carriers/delivery systems that release drugs in a sustained manner at therapeutic concentrations over a period of time. This strategy helps to improve patient compliance in terms of reducing the dosage frequency, and may also minimize the risk of emergence of drug-resistant mutants and potential toxicity. During the last few years, various carrier systems like stealth liposomes, synthetic polymers such as poly (DL-lactide-co-glycolide) (PLG) microspheres, have been developed for the sustained delivery of antitubercular drugs in mice with better chemotherapeutic efficacy against tuberculosis.[2,3] However, these formulations have to be injected either subcutaneously or intravenously, which in general is not acceptable. Hence, there is a need to develop an oral drug delivery system that is convenient for patients. Various synthetic and natural polymers like alginate, chitosan and polyesters have been used to develop drug delivery systems for entrapping and delivering drugs orally.[4] Sodium alginate, a salt of alginic acid (brown algae), a linear copolymer of α-guluronic acid and α-mannuronic acid, has the ability to form a gel/meshwork in the presence of divalent cations such as $CaCl_2$. This gel shrinks at acidic pH and erodes at alkaline pH. Therefore, it can be used effectively to deliver drugs to the intestine, which has a pH of >6.7. Moreover, alginate is mucoadhesive and is likely to stick to intestinal mucosa for prolonged periods of time.[5] Various drugs and peptides like insulin have been effectively delivered orally in alginate microparticles to rats.[6] Alginate microspheres have been studied for development of oral drug delivery systems for

*Corresponding author. Tel: +91-172-747-585, ext. 5174; Fax: +91-172-744-401; E-mail: gkkhuller@yahoo.co.nic.in

entrapment and *in vitro* and *in vivo* studies of proteins and drugs.[7] However, reports are not available regarding the entrapment or delivery of antitubercular drugs.

The present study was planned to entrap antitubercular drugs isoniazid, rifampicin and pyrazinamide alone and in combination in alginate hydrogel microparticles and to evaluate their *in vivo* release profiles in guinea pigs. The formulation was also evaluated for chemotherapeutic potential against experimental tuberculosis.

Materials and methods

Chemicals and drugs

Sodium alginate of medium viscosity (~3500 cps), isoniazid, rifampicin and pyrazinamide were obtained from Sigma. All other reagents were of analytical grade and deionized water was used throughout the study.

Animals

Dunkin Hartley guinea pigs weighing 300–400 g obtained from the Central Animal House, PGIMER, Chandigarh or Haryana Agricultural University, Hissar, India were used in this study. The animals were housed in an appropriate animal house facility under natural light conditions. They were fed standard pellet diet and water *ad libitum*. The work was approved by the Institute Animal Ethics Committee.

Culture

The culture of *Mycobacterium tuberculosis* H_{37}Rv was originally obtained from the National Collection of Type Cultures (NCTC-7416), London, UK and was maintained on Youman's modified medium and Lowenstein Jensen medium.

Preparation and characterization of alginate gel microparticles

Two millilitres of isoniazid, rifampicin or pyrazinamide solution (25 g/L) was added to 2 mL of 2% alginate aqueous solution. For entrapment of isoniazid, rifampicin and pyrazinamide in combination, 2 mL of each drug (at a concentration of 25 g/L) was added to 6 mL of 2% alginate. Solutions were mixed and vortexed thoroughly for 5–10 min at 1000 rpm. This mixture was dropped through a 26G needle into 0.15 M $CaCl_2$ solution, which was being continuously stirred. These droplets formed gel beads instantaneously,[8] which were washed 2–3 times with distilled water before drying at room temperature for 12 h. These dried hydrogel microparticles were sized microscopically. The particle size of 30–40 microparticles was measured with a micrometer and the mean particle size was determined as described by Takka & Acarturk.[9] Alginate and water content associated with beads were assayed by measuring uronic acid production[8] (degradation product of alginate) and the difference between the dry and wet weight of beads, respectively.[9]

Determination of drug content of hydrogel microparticles

The entrapment of drugs in alginate hydrogel microparticles was determined by lysing them in phosphate-buffered saline (pH 7.5). The drugs released in the supernatant were assayed by standard protocols. Isoniazid was estimated by the spectrofluorometric method of Scott & Wright[10] with a sensitivity of 0.1 mg/L, rifampicin was measured spectrophotometrically as described by Deol *et al.*[2] and pyrazinamide was estimated by a spectrophotometric method as described by Gurumurthy *et al.*[11] with a sensitivity of 5 mg/L.

In vitro dissolution studies

In vitro release studies of drugs from the alginate microparticles were carried out at room temperature (27–30°C) for up to 30 days by suspending 20 mg of drug-loaded alginate beads in simulated gastric (0.1 M HCl buffer, pH 1.2) and intestinal fluids (phosphate buffer, pH 7.4) prepared according to the US Pharmacopeia.[9] Samples (5 mL) at appropriate intervals were withdrawn and an equal volume of dissolution medium was added to maintain constant volume. Drugs were assayed in the fluids by similar methods to those employed for determination of drug content in hydrogel microparticles.

In vivo drug disposition studies from alginate hydrogel microparticles

Guinea pigs were orally administered free drugs/drug-loaded alginate microparticles at doses of 10, 12 and 25 mg/kg body weight of isoniazid, rifampicin and pyrazinamide, respectively, through oral feeding cannula.

Guinea pigs were bled and killed at different time points post-administration of free drugs/drug-laden alginate hydrogel microparticles. Organ homogenates (20%) were prepared from lungs, liver, spleen and intestine in 0.9% NaCl. Isoniazid and pyrazinamide were estimated as reported earlier, whereas rifampicin concentration in plasma and tissue homogenates was determined by microbiological assay[12] using *Bacillus subtilis* with a sensitivity of 0.01 mg/L. Results were expressed as concentration of drugs in mg/L obtained in plasma/tissue homogenates at various time intervals.

Pharmacokinetic analysis

C_{max} (peak plasma concentration) and T_{max} (time to reach peak concentration) were calculated from the actual time–concentration curve of plasma. The elimination rate constant (k_{el}) was calculated by least square regression analysis.

Elimination half-life ($t_{1/2e}$) was calculated using the formula $0.693/k_{el}$. The absorption rate constant (k_a) was calculated by the residual method and absorption half-life ($t_{1/2a}$) was determined using the formula $0.694/k_a$. The area under the concentration–time curve $(AUC)_{t-\infty}$ was calculated by the trapezoidal rule and the $AUC_{0-\infty}$ by dividing the last concentration of drug observed in plasma by the respective k_{el}. The $AUC_{0-\infty}$ is the sum of AUC_{0-t} and $AUC_{t-\infty}$. Relative bioavailability was calculated by dividing the $AUC_{0-\infty}$ of alginate encapsulated drug by the $AUC_{0-\infty}$ of free drug given by the same route.

Chemotherapeutic studies

Guinea pigs were infected with 1.0 mg of cells harvested from a 3 week culture of *M. tuberculosis* $H_{37}Rv$ in a volume of 0.1 mL of 0.9% sterile NaCl solution via the intramuscular route in the left thigh muscle.[13] After 15–20 days, infection was confirmed by Ziehl-Neelsen staining of tissue smears of spleen after the killing of two animals. The animals were then divided into three groups and each group had at least eight animals. Dosages of isoniazid, rifampicin and pyrazinamide used in this study were 10, 12 and 25 mg/kg body weight.

Group A. Served as controls (no therapy was given to them).
Group B. Orally administered drug-loaded alginate hydrogel microparticles (containing isoniazid, rifampicin and pyrazinamide) weekly for 8 weeks.
Group C. Orally administered free isoniazid, rifampicin and pyrazinamide in combination daily for 8 weeks.

Assessment of the number of viable bacilli

After 8 weeks of chemotherapy, guinea pigs were killed 6 days after the administration of the last therapeutic dose. One quarter of spleen tissue or caudal left lung lobe was homogenized in 5 mL of sterile normal saline and different dilutions were plated for cfu enumeration. One quarter of the liver was placed in 10% neutral buffered formalin for histopathological examination. Duplicate Lowenstein Jensen slopes were inoculated with 0.1 mL of diluted homogenates in normal saline, incubated at 37°C and after 4 weeks colonies were counted. Colony forming units counted were expressed as $\log_{10}$ cfu/mL.

Histopathology

Formalin fixed tissues embedded in paraffin and sectioned at 5 μm were mounted on glass slides and stained with haematoxylin–eosin and acid fast stains.

Toxicity studies

The activities of alkaline phosphatase and alanine transaminase were determined in plasma samples of guinea pigs (on the seventh day post-administration of last therapeutic dose) using Boehringer-Mannheim kits, and results were expressed as U/L.

Statistical analysis

The data were analysed by Student's unpaired *t*-test. Free drugs and alginate drug treatment groups were compared with untreated groups.

Results

Characterization of alginate gel microparticles

The alginate microspheres were almost spherical particles. The mean particle size of all formulations was between 90 and 100 μm. The entrapment of isoniazid, rifampicin and pyrazinamide was found to be 25–35%, 40–70% and 33–43%, respectively (the percentages given are of the initial drug concentrations); 70% water content and 10.5% uronic acid content (degradation product of alginate) were found to be associated with 100 mg of drug-loaded alginate microparticles.

In vitro release profiles

Release of isoniazid, rifampicin and pyrazinamide from drug-loaded alginate microparticles was studied in simulated intestinal fluid (SIF), pH 7.4 and simulated gastric medium (SGM), pH 1.2. All the drugs showed sustained release for 25 days in SIF, whereas limited drug release was observed in SGM. Approximately 27–36% of the drugs were released in gastric fluid over a period of 25 days, whereas 85–90% of the drugs were released in simulated intestinal fluid over a period of 20 days. This is because at acidic pH, alginate microparticles shrink due to tightening of gel meshwork, whereas at alkaline pH, alginate erodes and releases the contents in a sustained manner.[14,15]

In vivo drug disposition studies from alginate hydrogel microparticles

In vivo drug disposition studies were carried out in guinea pigs by oral administration of a single dose of alginate microparticles containing individual or a combination of drugs. Subsequently, the plasma and organ levels of isoniazid, rifampicin and pyrazinamide were monitored at different time intervals. Figure 1(a) depicts the plasma concentration of free isoniazid and alginate entrapped isoniazid at different time intervals. Both the formulations exhibited comparable peak concentration (C_{max}), but the time to reach peak level was 6 h for free isoniazid whereas isoniazid in alginate peaked later. However, entrapped isoniazid was detected up to 96 h whereas free isoniazid disappeared from plasma after 20 h.

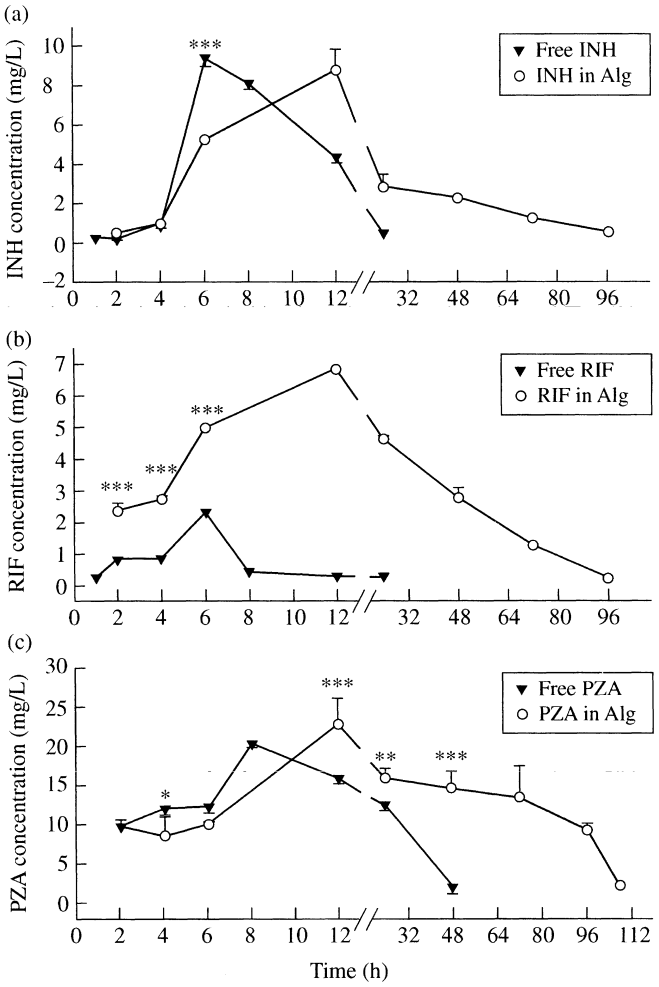

Figure 1. Plasma levels of free and encapsulated isoniazid (a), rifampicin (b) and pyrazinamide (c) at different time points. All values are means ± S.E.M. of eight animals. Plasma levels of alginate-encapsulated drugs compared with free drugs were statistically significant at $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$.

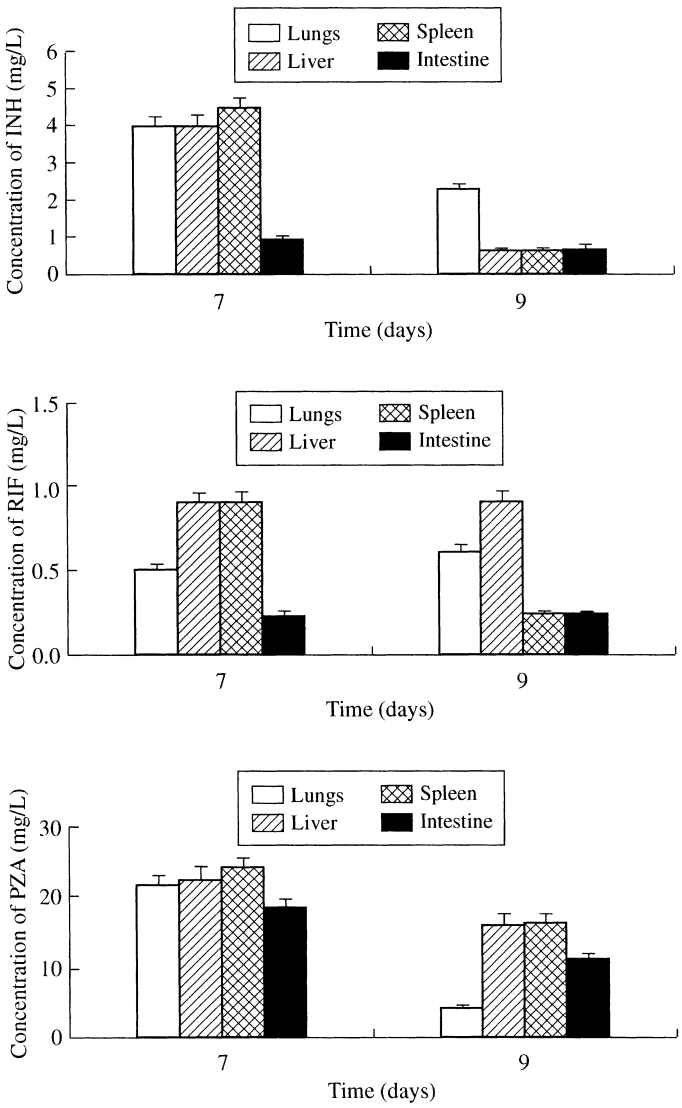

Figure 2. Tissue levels of alginate-encapsulated isoniazid, rifampicin and pyrazinamide (in combination) when administered orally to guinea pigs. Values are means ± S.D. of 7–8 animals.

Figure 1(b) shows the plasma concentration of free rifampicin and entrapped rifampicin when administered alone to guinea pigs. Entrapped rifampicin had a significantly higher concentration ($P < 0.001$) at 2, 4 and 6 h compared with free rifampicin. Moreover, free rifampicin was not detected after 18 h and had a T_{max} of 6 h, whereas entrapped rifampicin levels were observed until 96 h and peaked later than 6 h. Free rifampicin peaked at 6 h and drug levels were not detected after 20 h. However, the rifampicin levels in entrapped rifampicin formulation could be detected up to 72 h.

Although free pyrazinamide levels were higher at 2, 4, 6 and 8 h, it could only be detected up to 48 h. Entrapped pyrazinamide had a T_{max} of later than 6 h, had significantly higher drug levels at 12, 24 and 48 h, and remained in circulation for 108 h (Figure 1c). Thus, it is clear that all three drugs when administered orally in alginate hydrogel particles exhibited a sustained release pattern of drugs in plasma for up to 3–5 days. Similar observations were made when alginate-encapsulated isoniazid, rifampicin and pyrazinamide were administered in combination.

When the tissue disposition of drugs was examined, sustained release of isoniazid, rifampicin and pyrazinamide was detected in all the organs up to 9 days (Figure 2). The concentrations of isoniazid, rifampicin and pyrazinamide obtained at all the time points were found to be higher than the MICs of respective drugs. When the same dose of free drugs was administered to guinea pigs, isoniazid, rifampicin and pyrazinamide levels were seen in only liver and spleen until 48 h.

The relative bioavailability of isoniazid was significantly increased in the alginate-encapsulated form compared with the free form (Table 1). T_{max} and $AUC_{0-\infty}$ were significantly enhanced in the encapsulated form (when given alone or in

Table 1. Pharmacokinetics of free/alginate-entrapped isoniazid after oral administration to guinea pigs

	Free INH (10 mg/kg body weight)		Alg-entrapped INH (10 mg/kg body weight)	
	alone	in combination	alone	in combination
C_{max} (μg/mL)	9.30 ± 0.40	10.30 ± 1.56[a]	8.76 ± 0.72[d]	12.30 ± 1.38[b,c]
T_{max} (h)	6.00	6.00	12.00	12.00
$t_{1/2e}$ (h)	3.98 ± 0.40	3.96 ± 0.06[a]	22.30 ± 1.03[a***]	21.60 ± 0.69[b,c***]
$t_{1/2a}$ (h)	3.50 ± 0.26	2.38 ± 0.03[a*]	2.22 ± 0.44[d***]	8.80 ± 0.43[b***,c]
$AUC_{0-\infty}$ (μg·h/mL)	18.34 ± 0.44	21.33 ± 0.36[a***]	103.66 ± 0.83[d***]	199.20 ± 3.08[b***,c***]

All values are expressed as means ± S.E.M.; no. of animals = 8.
[a]Depicts comparison of free drug alone versus free drug in combination.
[b]Depicts comparison of entrapped drug alone versus entrapped drug in combination.
[c]Depicts comparison of free drug in combination versus entrapped drug in combination.
[d]Depicts comparison of free drug alone versus entrapped drug alone.
*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$; values without asterisks are non-significant.

Table 2. Pharmacokinetics of free/alginate-entrapped rifampicin after oral administration to guinea pigs

	Free RIF (12 mg/kg body weight)		Alg-entrapped RIF (12 mg/kg body weight)	
	alone	in combination	alone	in combination
C_{max} (μg/mL)	2.30 ± 0.03	1.27 ± 0.03[a**]	6.82 ± 0.07[d***]	2.65 ± 0.30[b***,c***]
T_{max} (h)	6.00	6.00	12.00	12.00
$t_{1/2e}$ (h)	10.53 ± 0.51	13.86 ± 0.06[a***]	19.46 ± 0.44[d***]	23.00 ± 0.42[b**,c***]
$t_{1/2a}$ (h)	3.08 ± 0.12	1.24 ± 0.08[a***]	5.46 ± 0.40[*d]	3.42 ± 0.30[b*,c*]
$AUC_{0-\infty}$ (μg·h/mL)	18.60 ± 0.14	12.60 ± 0.14[a***]	125.08 ± 0.04[d***]	46.33 ± 0.04[b***,c***]

All values are expressed as means ± S.E.M.; no. of animals = 8.
[a]Depicts comparison of free drug alone versus free drug in combination.
[b]Depicts comparison of entrapped drug alone versus entrapped drug in combination.
[c]Depicts comparison of free drug in combination versus entrapped drug in combination.
[d]Depicts comparison of free drug alone versus entrapped drug alone.
*$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$; values without asterisks are non-significant.

combination) compared with the free form. The increased bioavailability of isoniazid in the encapsulated form is substantiated by the prolonged elimination half-life of drugs in entrapped form, thereby indicating that alginate-encapsulated isoniazid has a sustained release property and the therapeutic effect remained above the MIC for a longer period of time.

Table 2 shows kinetic parameters of rifampicin when given alone or in combination in the free or encapsulated form. Significant C_{max}, T_{max}, $AUC_{0-\infty}$, relative bioavailability and prolonged half-life elimination ($t_{1/2e}$) of entrapped rifampicin showed that rifampicin bioavailability was increased and exhibited its sustained release property. Alginate-entrapped rifampicin (alone/in combination) had enhanced half-life absorption compared with free rifampicin, thereby depicting that absorption is slower in the entrapped form.

Similarly, the pharmacokinetics of pyrazinamide given alone or in combination in the free or entrapped form revealed significantly higher C_{max}, T_{max}, relative bioavailability, $t_{1/2e}$ and $AUC_{0-\infty}$ compared with free drugs (Table 3).

Pharmacokinetic analysis thus revealed that entrapped formulations have a higher time of residence and therefore exhibit increased bioavailability.

Therapeutic efficacy

The chemotherapeutic efficacy of free/alginate-encapsulated isoniazid (10 mg/kg body weight), rifampicin (12 mg/kg body weight) and pyrazinamide (25 mg/kg body weight) in the lungs and spleens of guinea pigs infected with *M. tuberculosis* was evaluated (data not shown). Free drugs were administered daily for 8 weeks, whereas alginate-encapsulated drugs were given weekly for the same time period. Treatment in all the groups resulted in no detectable bacilli in spleens and

Table 3. Pharmacokinetics of free/alginate-entrapped pyrazinamide after oral administration to guinea pigs

	Free PZA (25 mg/kg body weight)		Alg-entrapped PZA (25 mg/kg body weight)	
	alone	in combination	alone	in combination
C_{max} (μg/mL)	19.98 ± 1.27	28.89 ± 1.35[a]***	22.60 ± 2.25[d]***	38.40 ± 1.64[b]***,[c]
T_{max} (h)	8.00	8.00	12.00	12.00
$t_{1/2e}$ (h)	13.50 ± 0.80	13.58 ± 0.23[a]	19.60 ± 0.63[d]***	16.90 ± 0.40[b,c]***
$t_{1/2a}$ (h)	8.66 ± 0.40	4.30 ± 0.34[a]***	4.40 ± 0.58	4.95 ± 0.41[b,c]***
$AUC_{0-\infty}$ (μg·h/mL)	245.40 ± 3.76	287.00 ± 10.43[a]***	225.90 ± 10.3[d]	423.00 ± 3.44[b]***,[c]***

All values are expressed as means ± S.E.M.; no. of animals = 8.
[a]Depicts comparison of free drug alone versus free drug in combination.
[b]Depicts comparison of entrapped drug alone versus entrapped drug in combination.
[c]Depicts comparison of free drug in combination versus entrapped drug in combination.
[d]Depicts comparison of free drug alone versus entrapped drug alone.
*P < 0.05; **P < 0.01, ***P < 0.001; values without asterisks are non-significant.

lungs at 1:100 and 1:1000 dilutions of infected tissue homogenates. At the same dilutions, controls exhibited 5.1 log units of *M. tuberculosis* in lungs and 5.0 log units in spleens. Daily therapy with isoniazid, rifampicin and pyrazinamide exhibited the same results as that of 8 weeks of alginate-encapsulated drugs administered weekly.

Evaluation of the in vivo toxicity of the drugs

In vivo hepatotoxicity induced (in terms of alkaline phosphatase and alanine transaminase) by the antitubercular drugs given in free/alginate-entrapped form was evaluated on day 7 after the last therapeutic dose. Free drugs were capable of inducing toxicity in infected guinea pigs as reflected by the increased activity of alanine aminotransaminase (152.0 ± 39.5 U/L), whereas encapsulated drugs did not show apparent change in activity of this enzyme (46.5 ± 5.7 U/L) compared with normal animals (45.2 ± 1.2 U/L). Alkaline phosphatase levels were almost normal in untreated (58.7 ± 5.6 U/L), free drug (71.6 ± 6.4 U/L) and encapsulated drug treated groups (61.5 ± 15.3 U/L).

Histopathological studies

Histopathology of liver in the control group showed multiple granulomas in portal tract, Langhan's type of giant cell infiltration and microscopic foci of necrosis. The free drug treatment group revealed no granulomas in liver, very little inflammation in portal tract and no infiltration in portal veins but fibrosis was observed in the tissues. Fatty steatosis was also seen in the portal veins. The alginate-encapsulated drug treatment group had the same histopathological findings as that of the free drug treated group but calcification was observed in portal tract and liver tissue. Furthermore, rod-shaped acid fast bacilli were seen in liver tissue in controls, whereas no bacilli were seen in liver of treated groups.

Discussion

Sustained release of microspheres after oral administration offers several potential advantages, including: (i) higher patient compliance; (ii) better drug bioavailability; and (iii) reduction of systemic side effects by decreasing the total dose and frequency of drug administration compared with conventional preparations.[16]

Alginate microparticles have been examined for *in vitro* studies of proteins and oral delivery of drugs.[6] Insulin has been entrapped to 70% and delivered effectively to mice to control hypoglycaemia. In our study, we observed 25–70% entrapment efficiencies of antitubercular drugs in alginate microspheres of size 90–100 μm, whereas with PLG microspheres, the maximum antitubercular drug loading was from 8% to 18% as reported earlier,[3] thus indicating that alginate hydrogel microparticles have better drug loading capacity than PLG polymers. Furthermore, alginate microspheres containing antitubercular drugs exhibited sustained release in simulated intestinal fluid and limited release in the gastric medium, thereby surviving the harsh gastric environment and providing an excellent system for delivering drugs to the intestine where they are absorbed in the usual manner.[15]

When drug-loaded alginate microparticles/free drugs were administered orally to guinea pigs, higher C_{max}, T_{max}, delayed elimination rate and higher bioavailability were observed compared with free drugs. Previously, these drugs in PLG microspheres exhibited five-fold increased relative bioavailability compared with free drugs;[16] however, with alginate microspheres the relative bioavailability observed was increased nine-fold. Also, alginate has been found to have bio-adhesive properties that help in delaying the intestinal transit time of the encapsulated compound.[5] Therefore, it is suggested that alginate microspheres probably adhere to the

intestine mucosa for a prolonged period where they release drug in a sustained manner before being eroded off. In this study, $AUC_{0-\infty}$ was increased significantly for alginate-encapsulated isoniazid; however, the gain in the AUC with rifampicin was small (45–125 μg·h/mL). In contrast, substantially higher AUC for rifampicin (367–386 μg·h/mL) have been reported in man following the same dose per kg;[17] this difference may reflect differences in absorption between species.

The chemotherapeutic efficacy of alginate (encapsulated isoniazid, rifampicin and pyrazinamide) microparticles showed no apparent bacilli in lungs and spleens of guinea pigs after 8 weeks of weekly administration. Comparable clearance of bacilli was observed in the organs of guinea pigs treated with alginate-entrapped/free drugs; however, free drugs were administered daily for 8 weeks and alginate-entrapped drugs were given weekly for the same time. In our earlier reports, the chemotherapeutic potential of liposomal drugs[2] and PLG-encapsulated antitubercular drugs[18] has been evaluated in mice where the bacterial load in organs was reduced only by 1–2 log units, whereas in the present study we were able to achieve an apparent sterilization in lungs and spleens of guinea pigs with 8 weeks of therapy with alginate-entrapped drugs administered weekly. Entrapped drug groups showed normal liver morphology, whereas untreated animals exhibited characteristic histopathological changes in the liver tissue. Few reports of complete clearance of bacilli from organs of mice after treatment with isoniazid, rifabutin and pyrazinamide are available, which is probably due to pyrazinamide, a potent sterilizing agent.[19,20] Our results clearly demonstrate that 57 doses of free drugs can be replaced with eight doses of alginate-entrapped drugs to achieve almost complete clearance of bacilli within 8 weeks in guinea pigs. Earlier reports[21–23] indicated that isoniazid in slow release preparations when administered at higher doses gave adverse side effects. In our study we did not observe toxicity with alginate-encapsulated isoniazid when administered in combination with rifampicin and pyrazinamide at therapeutic doses; however, free drugs at the same dose indicated biochemical toxicity in guinea pigs.

In brief, alginate-entrapped antitubercular drugs can be delivered orally weekly to improve antimycobacterial therapy, thus resulting in improved compliance by the patients.

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References

1. Fox, W. (1983). Compliance of patients and physicians: experiences and lessons from tuberculosis—II. *British Medical Journal* **287**, 101–5.

2. Deol, P., Khuller, G. K. & Joshi, K. (1997). Therapeutic efficacies of isoniazid and rifampicin encapsulated in lung specific stealth liposomes against *Mycobacterium tuberculosis* infection induced in mice. *Antimicrobial Agents and Chemotherapy* **41**, 1211–4.

3. Dutt, M. & Khuller, G. K. (2000). Poly (DL-lactide-co-glycolide) microparticles as carriers for antimycobacterial drug rifampicin. *Indian Journal of Experimental Biology* **38**, 887–94.

4. Longer, M. A., Ching, H. S. & Robinson, J. R. (1985). Bioadhesive polymers as platforms for oral controlled drug delivery III: oral delivery of chlorothiazide using a bioadhesive polymer. *Journal of Pharmaceutical Sciences* **74**, 406–11.

5. Chickering, D. E., Jacob, J. S., Desia, T. A., Harrison, M., Harris, W. P., Morell, C. N. *et al.* (1997). Bioadhesive polymers III. An *in vivo* transit and bioavailability study of drug loaded alginate and poly (fumaric-co-sebacic anhydride) microspheres. *Journal of Controlled Release* **48**, 35–46.

6. Ramadas, M., Paul, W., Dileep, K. J., Anitha, Y. & Sharma, C. P. (2000). Lipoinsulin encapsulated alginate-chitosan capsules. Intestinal delivery in diabetic rats. *Journal of Microencapsulation* **4**, 405–11.

7. Hari, P. R., Chandy, T. & Sharma, C. P. (1996). Chitosan-calcium alginate micro capsules for intestinal delivery of insulin. *Journal of Microencapsulation* **13**, 319–29.

8. Takagi, I., Shimazu, H. & Yotsunagi, T. (1996). Application of alginate gel as a vehicle for liposomes I. Factors affecting the loading of drug-containing liposomes and drug release. *Chemical and Pharmaceutical Bulletin* **44**, 1941–7.

9. Takka, S. & Acarturk, F. (1999). Calcium alginate microparticles for oral administration. The effect of sodium alginate type on drug release and drug entrapment efficiency. *Journal of Microencapsulation* **16**, 275–90.

10. Scott, E. H. & Wright, R. C. (1967). Fluorimetric determination of isonicotinic acid hydrazide in serum. *Journal of Laboratory and Clinical Medicine* **70**, 355–60.

11. Gurumurthy, P., Nair, N. G. K. & Sarma, G. R. (1980). Methods for the estimation of pyrazinamide and pyrazinoic acid in body fluids. *Indian Journal of Laboratory and Clinical Medicine* **71**, 129–34.

12. Saito, H. & Tomoika, H. (1989). Therapeutic efficacy of liposome-entrapped rifampicin against *M. avium* complex infections induced in mice. *Antimicrobial Agents and Chemotherapy* **33**, 429–33.

13. Naganathan, N., Mahadev, B., Challu, V. K., Rajlakshmi, R. & Jones, B. (1986). Virulence of tubercle bacilli isolated from patients with tuberculosis in Bangalore, India. *Tubercle* **67**, 261–7.

14. Yotsuyanagi, T., Oskubo, T., Ohhashi, T. & Ikeda, K. (1986). Calcium induced gelation of alginic acid and pH re-swelling of dried gels. *Chemical and Pharmaceutical Bulletin* **35**, 1555–63.

15. Nascimento, A., Laranjeira, M. C. M., Favere, V. T. & Josue, A. (2001). Impregnation and release of aspirin from chitosan/poly-

(acrylic acid) graft copolymer microspheres. *Journal of Microencapsulation* **18**, 679–84.

16. Ain, Q., Sharma, S., Garg, S. K. & Khuller, G. K. (2002). Role of poly (DL-lactide-co-glycolide) in development of a sustained oral delivery system for antitubercular drug(s). *International Journal of Pharmaceutics* **238**, 123–32.

17. Keung, A., Eller, M. G., Mekenzie, K. A. & Weir, S. J. (1999). Single and multiple dose pharmacokinetics of rifapentine in man: Part II. *International Journal of Lung Diseases* **5**, 437–44.

18. Dutt, M. & Khuller, G. K. (2001). Chemotherapy of mycobacterial infections in mice by a combination of antitubercular drugs entrapped in poly (DL-lactide-co-glycolide) microparticles. *Journal of Antimicrobial Chemotherapy* **47**, 829–35.

19. Dhillon, J., Dickinson, J. M., Sole, K. & Mitchison, D. A. (1996). Preventive chemotherapy of tuberculosis in Cornell model mice with combinations of rifampin, isoniazid, and pyrazinamide. *Antimicrobial Agents and Chemotherapy* **40**, 552–5.

20. Lenaert, A. M., Chase, S. E., Chmielewski, A. J. & Cynamon, M. H. (1999). Evaluation of rifapentine in long-term treatment regimens for tuberculosis in mice. *Antimicrobial Agents and Chemotherapy* **43**, 2356–60.

21. Tripathy, S. P. (1976). A slow release preparation of isoniazid: therapeutic efficacy and adverse side effects. *Bulletin of the International Union against Tuberculosis* **51**, 133–41.

22. Polansky, F., Viznerova, S., Vodrazkova, A. & Scott, H. (1976). Clinical experience of acceptability of slow release matrix isoniazid. *Bulletin of the International Union against Tuberculosis* **51**, 155–8.

23. Ellard, G. A. (1976). A slow release preparation of isoniazid: pharmacological aspects. *Bulletin of the International Union against Tuberculosis* **51**, 143–54.