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International Journal of Pharmaceutics 290 (2005) 55–62
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Biodisposition of PEG-coated lipid microspheres of indomethacin in arthritic rats

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Received 13 July 2003; received in revised form 17 November 2004; accepted 17 November 2004
Available online 7 January 2005

Abstract

Conventional lipid microspheres (LM) were prepared using soybean oil and lipid at a 5.5:1 weight ratio with lipid phase consisting of PC (phosphatidyl choline):CH (cholesterol) (1:0.5) by molar ratio. The average diameter of the particles was 150 nm. Long-circulating microspheres (S-LM) were also prepared similarly but the lipid phase consisted of PC:CH:DSPE-PEG (phosphatidyl choline:cholesterol:distearoyl phosphatidyl ethanolamine-polyethylene glycol) 1:0.5:0.16 by molar ratio. A comparative biodistribution study was conducted between free indomethacin and lipo-indomethacin (LM and S-LM) in the arthritic rats by administering the formulations at a dose equivalent to 12 mg of indomethacin/kg. It was observed that the free drug as well as the encapsulated drug followed biphasic clearance from the blood. Pharmacokinetic parameters, such as $AUC_{0\text{-}t}$, terminal half-life, MRT increased significantly when the drug was used in encapsulated form ($p < 0.05$). Clearance of the drug was reduced 1.4 times with the conventional lipid microspheres and was reduced three-fold when encapsulated in polyethylene glycol-coated lipid microspheres. The overall drug targeting efficiency (T_e) with the PEG-coated lipid microspheres was 7.5-fold higher than the conventional lipid microspheres. The high accumulation of the drug in arthritic paw with S-LM system may be accounted for by the reduced uptake by RES cells, and thereby, availability for extravascularization in the inflammatory tissues.

Keywords: Lipid microspheres; Adjuvant arthritis; Long-circulating lipid microspheres; Polyethyleneglycol-2000; Reticuloendothelial system; Pharmacokinetics

1. Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs), such as indomethacin show a wide variety of side effects, especially when used for a long period of time. Often, patients suffering from chronic inflammatory

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doi:10.1016/j.ijpharm.2004.11.017

conditions (e.g. arthritis) discontinue the NSAID therapy because of the severity of the associated side effects (Insel, 1996). One of the ways of improving the therapeutic efficacy of these drugs could be encapsulation in a target oriented carrier system (Shoji et al., 1986). Though many drug carrier systems, such as microspheres and liposomes have been used for targeted delivery, they suffer from poor loading capacity for lipophilic drugs. Hence, lipid microspheres (LM), a phospholipid based carrier system comprising of an internal oil phase surrounded by a monolayer of phospholipids was assumed to be a better choice (Takino et al., 1994). Lipid microspheres are also commercially available as complete parenteral nutrient system (Deitel et al., 1992). The preparations in which drug is incorporated into lipid microspheres are called lipo-preparations. Presently, three lipo-preparations, lipo-steroid (Narisada et al., 1988), lipo-prostaglandin E (Hashimoto and Sakuma, 1987), lipo-non-steroidal anti-inflammatory drug (Mizushima et al., 1982) are now available for clinical use and many more drugs are under investigation to deliver in this form. It is well known that lipophilic carriers are rapidly cleared from circulation because of uptake by the reticuloendothelial (RES) system (Klibanov et al., 1990; Klang and Benita, 1998; Klang et al., 1998). Therefore, efforts have been made to manipulate the surface properties of the lipid microspheres for improving the stability and to achieve modified delivery characteristics (Klang et al., 1994; Kurihara et al., 1996; Yang and Benita, 2000; Del Curto et al., 2003; Fukui et al., 2003; Medda et al., 2003; Abrol et al., 2004; Akahori et al., 2004). Our laboratory has been involved in the comparative in vitro and in vivo evaluation of the phospholipid-based drug delivery systems, such as lipid microspheres and liposomes to develop an optimal delivery system for lipophilic drugs. Earlier, pharmacodynamic studies in our laboratory have revealed that at 30% edema inhibitory dose, lipid microspheres of indomethacin were about 1.5 times more potent than free indomethacin indicating the possible localization of LM at the inflammatory sites (Srinath et al., 1998). However, one of the serious obstacles in using LM is their rapid uptake by reticuloendothelial system (RES), thus interfering with the targeting pathway of lipid microspheres. It has been reported earlier that any attempt which confers a net increase in hydrophilicity on the colloidal carrier surface, will substantially reduce their RES uptake and increase the circulation time (Klibanov et al., 1990; Allen, 1994). In the present study, amphipathic polyethyleneglycol-2000 coated lipid microspheres were prepared and their pharmacokinetics and targetability were studied in arthritic rats.

2. Materials and methods

2.1. Materials

Phosphatidyl choline from egg yolk (PC), phosphatidyl ethanolamine (PE) and cholesterol (CH) were purchased from Sigma chemical Co., USA. DSPE-PEG (polyethylene glycol conjugated distearoyl phosphatidyl ethanolamine) was synthesized as described in a pervious report (Klibanov et al., 1990). Indomethacin was a gift sample from Astra IDL Ltd., Bangalore, India. Acetonitrile, methanol, water and acetic acid were of HPLC grade (Qualigens, India) and all other reagents were of analytical grade.

2.2. Preparation of lipid microspheres

Soybean oil (2 g) was taken in a 50 mL beaker and ethanol was added (20% by weight of the oil phase) and indomethacin (50 mg) was dissolved. The lipid phase (360 mg) containing PC:CH (1:0.5 by molar ratio) was added to the above solution and emulsified at 15,000 rpm for 10 min. Normal saline (20 mL) along with glycerin (500 mg) was added to the lipid phase and was homogenized at 15,000 rpm for 20 min. It was followed by sonication for 1 min at 100% duty cycle (Branson Ultrasonifier, Canada). Long-circulating lipid microspheres were prepared with the same composition as conventional lipospheres except that the lipid phase consisted of PC:CH:DSPE-PEG (1:0.5:0.16) by molar ratio.

2.3. Characterization of lipid microspheres

2.3.1. Size

The size of the lipid microspheres was analyzed by laser particle size analyzer (Malvern Instruments, Master sizer 2000, UK). The shape of the lipid microspheres was observed under scanning electron microscope (Hitachi, Japan) after lyophilization of the preparation.

2.3.2. Loading efficiency

A volume of 1 mL of lipo preparation was taken in a glass tube and centrifuged at 100,000 × *g* for 15 min, the supernatant was injected to HPLC system to determine the amount of the drug present in the aqueous phase. The amount of drug present in the internal phase was calculated by subtracting the amount of the drug present in the aqueous phase from the amount of drug added initially.

2.3.3. Proportion of vesicle formation during emulsification

To determine the percent of liposome formation during emulsification process, liposomes and lipid microspheres were prepared with the same composition (as mentioned before) containing 5,6-carboxy fluorescein (5,6-CF) as a vesicle marker at a concentration of 100 mM. The preparations were then passed through sephadex G-50 (1.5 cm × 30 cm) and about 40 elute fractions were collected, and thus the free CF was separated from the particulate fractions. The fluorescence entrapped in the vesicles was released with 0.5% Triton X-100 and measured with a spectrofluorometer (Hitachi, Japan) with extension and emission wavelength of 490 and 518 nm respectively.

From the lipid content of the liposome preparation and fluorescence data of liposomes and lipid microspheres, the fraction of the lipid-forming vesicles in the lipid microspheres formulation was calculated. It was found that the amount of lipid in the form of vesicles in the microsphere system was less than 1%.

2.4. Adjuvant-induced arthritis

Male wistar rats weighing 200 ± 10 g were given a single intradermal injection of 0.25 mL of Freund's complete adjuvant into the sub-planter area of the right hind paw (Graeme et al., 1966). Arthritis was assessed by measuring the difference in paw volume and the mean thickness between the right and left paws. Three weeks after induction of arthritis, the difference in thickness of the right and left paws was around 5.33 ± 0.57 mm. These rats were used for tissue distribution studies of lipid microspheres.

2.5. Biodistribution studies

Biodistribution studies were performed in arthritic male wistar rats as described previously (Srinath et al., 1998). Briefly, free indomethacin was given at a dose of 12 mg/kg (a solution in ethanol:propylene glycol:water, 2:1:5) and lipid microsphere formulation was given at the same dose intravenously via tail vein. Following the treatment, blood samples were collected at various time intervals by retro-orbit puncture, rats were sacrificed, and various organs such as liver, kidney, spleen, paw, heart, lung and brain were removed, dried, weighed and stored at −20 °C until further analysis. To each organ, required amount of methanol was added and homogenized for few minutes. The homogenate was centrifuged at 4000 rpm for 5 min, the supernatant was collected and assayed for indomethacin by HPLC method as described by Taro et al. (1989) with some modifications. Recovery of the drug (as percent of administered dose) in blood was determined by assuming that the total volume of the blood is 7.5% of the body weight (Wu et al., 1992). Pharmacokinetic parameters such as $AUC_{0\text{-}t}$ (by trapezoidal), elimination half-life (non-compartmental model), clearance (dose/AUC), MRT (AUMC/AUC) were calculated by using NCOMP-A windows-based computer program for non-compartmental analysis of pharmacokinetic data designed by Laub and Gallo (1996). Statistical significance of the data was analyzed using ANOVA and $p < 0.05$ was considered significant.

3. Results and discussion

Conventional lipid microspheres were prepared with soybean oil:lipid (5.5:1 by weight ratio) with ethanol as 20% of the oil phase and the lipid phase consisting of PC:CH (1:0.5 molar ratio). The above composition of the lipid microspheres was optimized based on size, loading efficiency and the release rate profile (Srinath et al., 1998). The loading efficiency of the lipid microspheres was 95%. Long-circulating lipid microspheres were also prepared with the same composition as that of the conventional lipid microspheres except that the lipid phase consisted of PC:CH:DSPE-PEG (1:0.5:0.16). No significant difference was observed in loading efficiency between conventional lipid microspheres and the PEG-coated lipid microspheres (92%). The size of the lipid microspheres was determined by laser particle size analyzer and was found that the average diameter was 150 nm (Fig. 1).

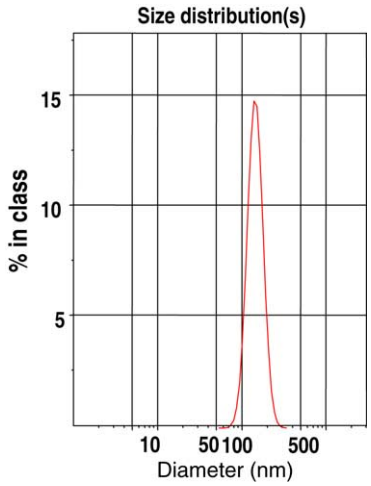

Fig. 1. Size distribution analysis of PEG-coated lipid microspheres of indomethacin using laser particle size analyzer.

The biodisposition of a drug is expected to markedly change when the drug is encapsulated in a carrier because it is affected by the drug release profile from the carrier as well as the fate of the carrier itself. The targeting potential of a carrier is restricted by 'opsonization' mediated by RES-rich organs such as liver and spleen. Needham et al. (1992) have performed X-ray diffraction studies to characterize the surface structure that promotes steric stability of PEG-grafted lipid vesicles and concluded that the membrane-bound PEGs can exert a significant interbilayer repulsion, thereby inhibiting mutual aggregation and possibly reducing the interaction with plasma proteins and phagocytic cells reducing the RES uptake and disintegration. Therefore, an attempt was made to reduce the RES interference by grafting PEG-2000 to the lipid microsphere surface. DSPE conjugated with PEG was included in the lipid composition to get a PEG coated liposphere surface.

A comparative biodistribution study was performed between the three formulations, namely free indomethacin (FI), lipo-indomethacin (LI), PEG-coated LM (S-LM) by measuring the drug levels in various organs up to 24 h after the administration. About 32% of the administered dose was recovered in blood 1 h after administration of the conventional lipid microspheres. The plasma clearance of the drug was very rapid with the free drug formulation. Only about 2.8% of administered dose was estimated 24 h after administration and significantly high concentrations of the drug (three-fold high) were detected with the lipid microsphere system even after 24 h. The clearance of the drug with PEG-coated lipid microspheres was slow and about 23% of the administered dose was estimated 24 h after administration. The slow clearance of the drug with PEG-coated lipid microspheres may be attributed for the possible RES avoidance. In addition, polyethylene glycol coating might have imparted a physical barrier property to the microsphere surface, thus resulting in slow release of the drug from coated microspheres. Therefore, the alteration in in vivo release profile of the drug was expected to bring about marked changes in the pharmacokinetic parameters of the drug when encapsulated in PEG-coated microspheres.

As shown in Table 1, a 2.7-fold increase in AUC_{0-t} was noted when indomethacin was administered in S-LM form (1253 μg/mL/h) as compared to free indomethacin (460 μg/mL/h), and the increase was 2.6-fold to that of the conventional LM. Elimination half-life ($t_{1/2}$) of the drug with S-LM was significantly higher than the conventional microspheres. Mean residence time (MRT) of the drug increased 2.4-fold when administered as S-LM form (26 h) and two-fold as compared to LM form (22 h). Clearance of the drug was reduced three-fold when encapsulated in S-LM (1.02 mL/h) when compared with LM (2.97 mL/h). From the data it may be assumed that the slow rate of

Table 1
Pharmacokinetic parameters of free indomethacin (FI), lipid microspheres (LM) and PEG coated lipid microspheres (S-LM) of indomethacin following intravenous administration in arthritic rats (dose: 12 mg/kg)

Parameter	FI	LM	S-LM
AUC_{0-t} (μg/mL/h)	460.98 ± 27.14	472.03 ± 19.85	1253.37 ± 52.55
Terminal half-life (h)	9.14 ± 1.22	16.25 ± 0.84	18.60 ± 0.41
Clearance (mL/h)	4.07 ± 0.26	2.97 ± 0.20	1.02 ± 0.58
MRT (h)	10.89 ± 0.88	22.33 ± 1.20	26.68 ± 0.58

Table 2
Recovery of indomethacin (as percent of administered dose) in various organs at different time intervals following i.v. administration of free indomethacin in arthritic rats (dose: 12 mg/kg)

Organ	1 h	2 h	4 h	8 h	24 h
Liver	10.02 ± 0.85	7.85 ± 1.10	6.78 ± 0.74	6.47 ± 0.60	4.30 ± 0.86
Spleen	0.22 ± 0.04	0.30 ± 0.08	0.26 ± 0.04	0.18 ± 0.05	0.11 ± 0.04
Kidney	2.13 ± 0.13	1.61 ± 0.08	1.20 ± 0.18	0.84 ± 0.12	0.55 ± 0.12
Brain	0.08 ± 0.03	0.06 ± 0.01	0.06 ± 0.02	0.06 ± 0.01	0.04 ± 0.01
Paw	0.007 ± 0.003	0.006 ± 0.002	0.004 ± 0.001	0.0003 ± 0.001	0.001 ± 0.002

distribution of S-LM to RES-rich and other organs and slow release of the drug from these PEG-coated lipid microspheres could be the possible reason for the significant changes observed in various pharmacokinetic parameters as presented in Table 1.

It has been well established that biodistribution of any colloidal system depends on the size of the particles (Liu and Liu, 1996). In the present work, the average size of the lipid microspheres was found to be 150 nm. This is in the range of the particle size where their in vivo circulation properties are dependent only on their surface properties (Liu and Liu, 1995).

Tissue distribution data with the free drug, LM and S-LM preparations are presented in Tables 2–4. Within 1 h after administration, the microspheres distributed mainly in blood and liver. Less than 5% of the injected dose was found in other organs except the liver and spleen. About 61% of the injected dose was found in liver after 4 h with LM preparation whereas it was 33% of the injected dose when lipid microspheres grafted with polyethyleneglycol-2000 were used (Tables 2–4). Thus, microsphere uptake by liver was significantly reduced when they were coated with polyethyleneglycol-2000 ($p < 0.01$).

With S-LM, 24 h after administration there was still about 48% of the injected dose circulating in blood whereas it was only 28 and 38% with conventional lipid microspheres and free drug, respectively (Fig. 2). Increase in drug concentrations in liver were accompanied by clearance of the lipid microspheres from the blood, thereby lower serum concentrations of the drug were observed. Concentration of the drug in liver reached the peak in 4 h (226 μg/g of the tissue) and decreased gradually to 63 μg/g of the tissue after 24 h. However, grafting the microsphere surface with amphipathic polyethyleneglycol-2000 did not alter t_{max} in

Table 3
Recovery of indomethacin (as percent of administered dose) in various organs at different time intervals following i.v. administration of lipid microspheres indomethacin in arthritic rats (dose: 12 mg/kg)

Organ	1 h	2 h	4 h	8 h	24 h
Liver	19.20 ± 1.38	48.95 ± 5.11	61.05 ± 4.04	22.29 ± 1.66	17.27 ± 1.83
Spleen	6.70 ± 1.15	9.34 ± 0.94	16.86 ± 1.75	10.18 ± 2.01	6.41 ± 1.01
Kidney	0.80 ± 0.25	1.32 ± 0.17	1.55 ± 0.39	0.97 ± 0.29	0.49 ± 0.13
Brain	0.04 ± 0.01	0.04 ± 0.02	0.03 ± 0.02	0.02 ± 0.01	0.01 ± 0.005
Paw	0.021 ± 0.008	0.028 ± 0.007	0.022 ± 0.006	0.017 ± 0.001	0.009 ± 0.0004

Table 4
Recovery of indomethacin (as percent of administered dose) in various organs at different time intervals following i.v. administration of PEG-coated lipid microspheres of indomethacin in arthritic rats (dose: 12 mg/kg)

Organ	1 h	2 h	4 h	8 h	24 h
Liver	10.58 ± 1.21	19.44 ± 1.78	32.66 ± 3.39	13.45 ± 1.77	8.89 ± 1.46
Spleen	3.61 ± 0.42	5.01 ± 1.18	8.43 ± 1.56	4.98 ± 0.55	3.08 ± 0.86
Kidney	0.44 ± 0.17	0.59 ± 0.10	0.79 ± 0.26	0.61 ± 0.07	0.31 ± 0.12
Brain	0.04 ± 0.01	0.03 ± 0.02	0.024 ± 0.02	0.015 ± 0.007	0.009 ± 0.005
Paw	0.051 ± 0.006	0.048 ± 0.016	0.043 ± 0.010	0.039 ± 0.012	0.029 ± 0.009

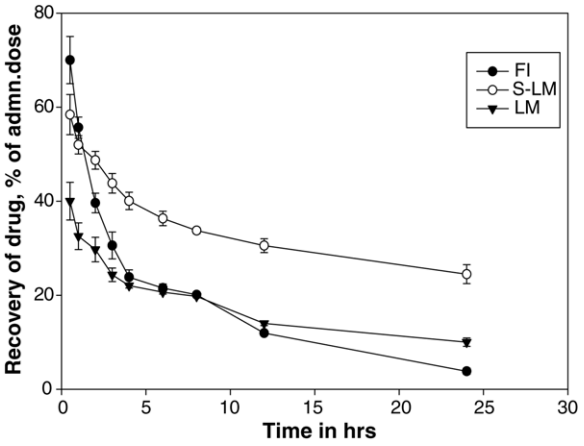

Fig. 2. Recovery of indomethacin in blood as percent of administered dose following i.v. administration of free indomethacin (FI), lipid microspheres (LM) and PEG-coated lipid microspheres (S-LM) of indomethacin in arthritic rats (dose: 12 mg/kg).

liver but C_{max} was low (1121 μg/g of the tissue). This data clearly indicates that PEG-2000 has significantly reduced the uptake by the RES cells of the liver, and thereby, contributed for the prolonging the circulation time of the lipid microspheres.

After 1 h of administration, spleen displayed a 1.7-fold high concentration of the drug with lipid microsphere system (6.7% of the admn. dose) as compared to S-LM system (3.61% of the admn. dose). C_{max} in kidney (1.6% of the admn. dose) was found at 2 h with LM whereas C_{max} with S-LM was achieved in 4 h (0.79% of the admn. dose). Peak drug concentration in the paw was achieved in 1 h with free drug and with S-LM, whereas it was 2 h with LM system. C_{max} in paw was 0.47, 0.26 and 0.064 μg/g of the tissue with S-LM, LM and FI formulations, respectively. After 24 h, concentration of indomethacin in the paw with S-LM was three times greater than the conventional lipid microspheres and 20 times greater than the free drug.

From the drug distribution data in the inflammatory paw with various preparations studied above, it is evident that indomethacin is available at significantly high concentrations with both LM and S-LM preparations and localization of the drug with S-LM system was higher than the LM system. The high accumulation of the drug in the inflammatory tissue with LM may be attributed for the extravascularization of the lipid microspheres through the leaky vasculature and their possible uptake by the circulating monocytes, which would subsequently be concentrated in the rheumatic joints. The localization of the drug in the inflammatory areas was improved by grafting the PEG derivative to the microsphere surface. The higher accumulation of the drug in arthritic paw with S-LM system (compared to LM) may be accounted for by the reduced uptake of the lipid microspheres by RES cells, and thereby, the availability for the high extravascularization.

C_{max} in kidney with FI (2.13% of admn. dose) was achieved in 1 h after administration whereas C_{max} with LM was 1.6% of admn. dose and was achieved in 4 h. Pegylation of the microspheres (coating microspheres with PEG) reduced the C_{max} (0.8% of admn. dose) in the kidney and was achieved in 4 h. The significant reduction ($p < 0.01$) in accumulation of indomethacin in kidney by using S-LM system is assumed to considerably reduce the toxicity symptoms associated with kidney during the long-term indomethacin therapy for rheumatoid arthritis.

About 25% of the patients under indomethacin therapy suffer from severe and frequent CNS effects. This may be due to high accumulation of the drug in brain with the conventional formulations. When indomethacin was administered in lipid microsphere form, concentration of the drug in brain (C_{max}) drastically reduced from 1.73 (with FI) to 0.69 μg/g and with S-LM, it further reduced to 0.58 μg/g of the tissue. The lower accumulation of the drug in kidney as well as in brain with lipid microspheres could be because of the reduced availability of the free drug in the blood.

The disposition kinetics of the drug in various organs following intravenous administration of FI, LM and S-LM preparations is shown in Tables 2–4. From the $AUC_{0-\infty}$ data of each tissue, time-averaged relative drug exposure (r_e) was calculated using the equation, $r_e = (AUC_{0-\infty})$ with Lipid microspheres/$(AUC_{0-\infty})$ with free indomethacin (Gallo et al., 1989; Gupta and Hung, 1989). From the r_e data in Table 5 it was observed that, except in the arthritic paw tissue, the relative extent of the drug exposure to all other non-target tissues was higher with conventional lipid microspheres in comparison to long-circulating lipid microspheres. Although r_e-value provides a good indication of about the relative efficacy of the two delivery systems with reference to one tissue, it does not provide any information regarding the efficacy of a given carrier system in terms of target versus non-target tissue distribution of the drug. However, drug targeting efficiency (t_e) as calculated

Table 5
Total area under indomethacin concentration–time curve ($AUC_{0-\infty}$) in various tissues of arthritic rats following i.v. administration of free indomethacin (FI), lipid microspheres (LM) and PEG-coated lipid microspheres (S-LM) of indomethacin

Tissue	$AUC_{0-\infty}$ (μg/g/h)			r_e 1	r_e 2
	FI	LM	S-LM		
Liver	979.85	3647.91	1883.96	3.72	1.92
Spleen	141.7	10360.97	4984.65	73.11	35.18
Kidney	856.16	502.52	250.13	0.59	0.29
Lung	254.70	502.26	370.59	1.97	1.46
Brain	35.16	12.89	8.79	0.37	0.25
Paw	0.82	5.54	20.67	6.73	25.12

r_e = ($AUC_{0-\infty}$) with lipid microspheres/($AUC_{0-\infty}$) with free indomethacin.

Table 6
(a) Drug targeting efficiency (t_e) and (b) overall targeting efficiency (T_e) of free indomethacin (FI), lipid microspheres (LM) and PEG-coated lipid microspheres (S-LM) of indomethacin in arthritic rats

(a)
Drug targeting efficiency (t_e = ($AUC_{0-\infty}$) target tissue/($AUC_{0-\infty}$) non-target tissue)

Tissue	FI	LM	S-LM
Liver	0.0008	0.0005	0.011
Spleen	0.006	0.0002	0.003
Kidney	0.0009	0.004	0.080
Lung	0.003	0.004	0.053
Brain	0.23	0.14	2.26

(b)
Overall drug targeting efficiency (T_e = ($AUC_{0-\infty}$) target tissue/$\sum_{i=1}^{n}$($AUC_{0-\infty}$) non-target tissue)

FI	LM	S-LM
0.000368	0.000369	0.002749

by the equation, t_e = ($AUC_{0-\infty}$) target tissue/($AUC_{0-\infty}$) non-target tissue refers to the drug targeting efficiency of a delivery system against a particular non-target tissue implying that the higher the t_e-value, greater the target tissue selectivity. A better indicator for targeting efficiency may be assessed by calculating the overall drug targeting efficiency, T_e (T_e = $AUC_{0-\infty}$) target tissue/$\sum_{i=0}^{n}$($AUC_{0-\infty}$) non-target tissue). T_e refers to the drug accumulation in the target tissue in comparison to cumulative amount of the drug accumulated in all the tissues. The t_e and T_e-values with lipid microspheres and PEG-coated lipid microspheres is presented in Table 6a and b. Though the 'r_e' value with conventional lipid microspheres was higher than the free drug due to preferential localization in non-target issues such as liver and spleen, high $AUC_{0-\infty}$-values were obtained and therefore, the overall targeting efficiency (T_e) with conventional microspheres was comparable to that of the free drug. However, the targeting efficiency of long-circulating lipid microspheres (0.002749) was 7.5-fold higher than the conventional lipid microspheres and the free drug. As discussed earlier, the targeting efficiency was calculated by dividing AUC_{0-t} in the target tissue by ($AUC_{0-\infty}$) non-target tissues. In case of conventional lipid microspheres, ($AUC_{0-\infty}$) non-target tissues value was very high because of preferential and avid uptake by RES-rich organs. Therefore, though 'r_e' (time averaged relative drug exposure)-values in paw with LM were found higher than the free drug, T_e-value with LM was observed to be comparable to free drug.

Thus, it may be concluded that S-LM demonstrated better overall targeting efficiency (T_e) and the time-averaged relative drug exposure (r_e) values. Therefore, S-LM system holds promise for efficacious, safe and target delivery of anti-inflammatory agents negotiating better pharmacodynamic effects while side effects may be curtailed down to the minimum levels.

Acknowledgement

The authors thank CSIR, New Delhi for granting Senior Research Fellowship to SP for supporting the project.

References

Abrol, S.S., Trehan, A., Katare, O.P., 2004. Formulation, characterization and in vitro evaluation of Silymarin-loaded lipid microspheres. Drug Deliv. 11, 185–191.

Akahori, H., Takamura, T., Hayakawa, T., Ando, H., Yamashita, H., Kobayashi, K., 2004. Prostaglandin E1 in lipid microspheres ameliorates diabetic peripheral neuropathy: clinical usefulness of Semmes–Weinstein monofilaments for evaluating diabetic sensory abnormality. Diabetes Res. Clin. Pract. 64, 153–159.

Allen, T.M., 1994. The use of glycolipids and hydrophilic polymers in avoiding rapid uptake of liposomes by the mononuclear phagocyte system. Adv. Drug Deliv. Rev. 13, 285–309.

Deitel, M., Friedman, K.L., Cunnane, S., Lea, P.J., Chaiet, A., Chong, J., Almeida, B., 1992. Emulsion stability in a total nutrient admixture for total parenteral nutrition. J. Am. Coll. Nutr. 11, 5–10.

Del Curto, M.D., Chicco, D., D'Antonio, M., Ciolli, V., Dannan, H., D'Urso, S., Neuteboom, B., Pompili, S., Schesaro, S., Esposito, P., 2003. Lipid microparticles as sustained release system for a GnRH antagonist (Antide). J. Control. Release 89, 297–310.

Fukui, H., Koike, T., Saheki, A., Sonoke, S., Seki, J., 2003. A novel delivery system for amphotericin B with lipid nano-sphere (LNS). Int. J. Pharm. 265, 37–45.

Gallo, J.M., Hung, C.T., Gupta, P.K., Perrier, D.G., 1989. Physiological pharmacokinetic model of adriamycin delivered via magnetic albumin microspheres in the rat. J. Pharmacokinet. Biopharm. 17, 305–326.

Graeme, M.L., Fabry, E., Sigg, E.B., 1966. Mycobacterial adjuvant periarthritis in rodents and its modification by anti-inflammatory agents. J. Pharmacol. Exp. Ther. 153, 373–380.

Gupta, P.K., Hung, C.T., 1989. Quantitative evaluation of targeted drug delivery systems. Int. J. Pharm. 46, 618–638.

Hashimoto, H., Sakuma, A., 1987. A multicenter double blind controlled study of lipo-PGE1, PGE1 incorporated in lipid microspheres, in peripheral vascular disease secondary to connective tissue disorders. J. Rheumatol. 14, 97–101.

Insel, P.A., 1996. Analgesic-anti-pyretic and anti-inflammatory agents and drugs employed in the treatment of gout. In: The Pharmacological Basis of Therapeutics by Alfred Goodman Gilman, ninth ed. McGraw Hill, pp. 617.

Klang, S., Benita, S., 1998. Design and evaluation of submicron emulsions as colloid drug carriers for intravenous administration. In: Benita, S. (Ed.), Submicron Emulsions in Drug Targeting and Delivery. Harwood Academic Publishers, pp. 119–152.

Klang, S., Frucht-Pery, J., Hoffman, A., Benita, S., 1994. Physicochemical and acute toxicity evaluation of a positively charged submicron emulsion vehicle. J. Pharm. Pharmacol. 46, 986–993.

Klang, S.H., Parnas, M., Benita, S., 1998. Emulsions as drug carriers – possibilities and limitations and future perspectives. In: Muller, R.H., Benita, S., Bohm, B. (Eds.), Emulsions and Nanosuspensions for the Formulation of Poorly Soluble Drugs. Medpharm Scientific Publishers, Stuttgart, pp. 31–65.

Klibanov, A.L., Maruyama, K., Torchillin, V.P., Huang, L., 1990. Amphipathic polyethylene glycols effectively prolong the circulation time of liposomes. FEBS Lett. 268, 235–237.

Kurihara, A., Shibayama, Y., Yasuno, A., Ikeda, M., Hisaoka, M., 1996. Lipid emulsions of palmitoylrhizoxin: effects of particle size on blood disposition of emulsion lipid and incorporated compound in rats. Biopharm. Drug Dispos. 17, 343–353.

Laub, P.B., Gallo, J.M., 1996. NCOMP – a windows based computer programme for non-compartmental analysis of pharmacokinetic data. J. Pharm. Sci. 85, 393–395.

Liu, F., Liu, D., 1995. Long-circulating emulsions (oil in water) as carriers for lipophilic drugs. Pharm. Res. 12, 1060–1064.

Liu, F., Liu, D., 1996. Serum independent liposome uptake by mouse liver. Biochim. Biophys. Acta 1278, 5–11.

Medda, S., Jaishankar, P., Manna, R.K., Pal, B., Giri, V.S., Basu, M.K., 2003. Phospholipid microspheres: a novel delivery mode for targeting antileishmanial agent in experimental leishmaniasis. J. Drug Targeting 11 (2), 123–128.

Mizushima, Y., Hamano, T., Yokoyama, K., 1982. Emulsion stability in a total nutrient admixture for total parenteral nutrition. Ann. Rheum. Dis. 41, 263–267.

Narisada, M., Ohtani, M., Watanabe, F., Uchida, K., Arita, H., Doleuchin, M., Hanasaki, K., Kakushi, H., Otani, K., Hara, S., 1988. Synthesis and in vitro activity of various derivatives of a novel thromboxane receptor antagonist (+/−)-(5Z)-7-[3-endo-[(phenylsulfonyl)amino]bicyclo[2.2.1]hept-2-exo-yl]heptenoicacid. J. Med. Chem. 21, 1847–1854.

Needham, D., Mc Intosh, J.J., Lasic, D.D., 1992. Repulsive interactions and mechanical stability of polymer-grafted lipid membranes. Biochim. Biophys. Acta 1108, 40–48.

Srinath, P., Diwan, P.V., Pharmacodynamic, 1998. Pharmacokinetic evaluation of lipid microspheres of indomethacin. Pharm. Acta Helv. T3, 199–203.

Shoji, Y., Mizushima, Y., Yanagawa, A., Shiba, T., Takei, H., Fuji, M., Amino, M., 1986. Enhancement of anti-inflammatory effects of biphenylacetic acid by its incorporation into lipid microspheres. J. Pharm. Pharmacol. 38, 118–121.

Takino, T., Konishi, K., Takakura, Y., Hashida, M., 1994. Long-circulating emulsion carrier systems for highly lipophilic drugs. Biol. Pharm. Bull. 17, 121–125.

Taro, O., Yoshimasa, I., Masahiro, I., Hidehiko, A., 1989. A Pharmacokinetic model for the percutaneous absorption of indomethacin and the prediction of drug disposition kinetics. J. Pharm. Sci. 78, 319–323.

Wu, M.S., Robbins, J.C., Bugianesi, R.L., Poupipom, N.M., Shen, T.Y., 1992. Modified in-vivo behavior of liposomes containing synthetic glycolipids. Biochim. Biophys. Acta 1104, 95–101.

Yang, S.C., Benita, S., 2000. Enhanced absorption and drug targeting by positively charged submicron emulsions. Drug Dev. Res. 50, 476–486.