Solid Lipid Nanoparticles in Lymph and Plasma After Duodenal Administration to Rats

Alessandro Bargoni,[1] Roberta Cavalli,[2] Otto Caputo,[2] Anna Fundarò,[3] Maria Rosa Gasco,[3,4] and Gian Paolo Zara[3]

Received September 26, 1997; accepted February 20, 1998

Purpose. To evaluate the uptake and transport of solid lipid nanoparticles (SLN), which have been proposed as alternative drug carriers, into the lymph and blood after duodenal administration in rats.
Methods. Single doses of two different concentrations of aqueous dispersions of unlabelled and labelled SLN (average diameter 80 nm) were administered intraduodenally to rats. At different times, samples of lymph were withdrawn by cannulating the thoracic duct and blood was sampled from the jugular vein. Monitoring continued for 45 and 180 minutes, for unlabelled and labelled SLN respectively. The biological samples were analysed by photon correlation spectroscopy (PCS), transmission electron microscopy (TEM) and gamma-counting.
Results. TEM analysis evidenced SLN in lymph and blood after duodenal administration to rats; the size of SLN in lymph did not change markedly compared to that before administration. The labelled SLN confirmed the presence of SLN in lymph and blood.
Conclusions. The uptake and transport of SLN in the lymph, and to a lesser extent in the blood, were evidenced. The in vivo physical stability of SLN may have important implications in designing drug-carrying SLN.

KEY WORDS: duodenal administration; solid lipid nanoparticles; lymphatic uptake; lymphatic transport.

INTRODUCTION

The gastrointestinal tract is probably the route of choice for drug delivery, although several factors, such as pH of the gastrointestinal tract, residence time and drug solubility, can affect this administration route. Several delivery systems have been proposed to overcome some of the problems of this route; many micro and nanoparticulate drug carriers have been developed to favour uptake and transport of orally-administered drugs. Increasing attention has been paid to their potential use as carriers both for biodegradable molecules, such as peptides and proteins, and for poorly water-soluble drugs, such as some cytostatics.

Numerous studies have examined the uptake of colloidal polymeric carriers, both biodegradable and otherwise, from the gastrointestinal tract after peroral administration, and the passage of these colloidal particles across the intestinal mucosa has been shown to be possible (1–8).

Many factors can affect the uptake of particulate carriers from the gut: these include particle size, nature of the particulate, hydrophobicity and surface charge (9).

Jani *et al.* (10) studied the oral absorption of fluorescent polystyrene nanospheres, of diameters ranging from 50 nm to 3 μm, administered orally to rats for 10 days, and found that uptake and traslocation were inversely size dependent.

Various researchers have studied solid lipid nanoparticles (SLN) as alternative colloidal carriers of drugs and diagnostics (11–13).

The main aim of this research was to study the gastrointestinal uptake and transport into lymphatic circulation of solid lipid nanoparticles, considering that chylomicrons, the physiological carriers that transport lipidic molecules to the lymph, are absorbed by the lymphatic system. We studied nanoparticles without incorporated drugs, in order to separate the transport of the carriers from the transport of a drug carried by them. SLN were administered as a single dose into the duodenal lumen of rats.

We obtained SLN by dispersing warm oil-in-water (o/w) microemulsions in a cold aqueous medium (14). It is possible to incorporate several lipophilic and hydrophilic drugs into SLN, including nifedipine, doxorubicine, pilocarpine and some peptides such as thymopentin and (TRP-6) LH-RH (15–19), provided that different approaches were used during the preparation process.

For this study, a warm o/w microemulsion consisting of bioacceptable substances (namely stearic acid, taurocholate sodium salt, water and phospholipids) was prepared: the composition was the same for all experiments. Two types of experiments were performed to characterise and quantify the uptake: in the first, we used unlabelled SLN to evaluate the transport into the lymph of the thoracic duct and blood by Trasmission Electron Microscopy (TEM) and Photon Correlation Spectroscopy (PCS); in the second, using labelled SLN, we verified the quantities transported into the lymph and blood over time, by gamma-counting. In the latter experiment, an iodine-labelled derivative with a structure like stearic acid, namely ^{131}I-17-iodoheptadecanoic acid, was used as tracer.

MATERIALS AND METHODS

Materials

Stearic acid was from Fluka (Buchs, CH). Epikuron 200 (soya phosphatidylcholine 95%) was a kind gift from Lucas Mayer (Hamburg, G), taurocholate sodium salt was a kind gift from PCA (Basaluzzo, I), ketamine hydrochloride and droperidol were from Sigma (Milan, I), 17-iodoheptanoic acid was from Emka-Chemie (Markgröngingen, G) and ^{131}I-17-iodoheptanoic acid was a kind gift of Sorin RadioFarmaci S.r.l. (Saluggia, I).

Animals

Eighteen male albino rats of a Wistar derived strain (Charles River-Italy) were used. Before experimentation, they were kept in groups of three in cages (temperature 20°C) with food (purina chops) and water ad libitum. The study complied with the rules set forth in the NIH Guide for the Care and Use of Laboratory Animals

[1] Dipartimento di Fisiopatologia Clinica, Facoltà di Medicina, Università degli Studi di Torino.
[2] Dipartimento di Scienza e Tecnologia del Farmaco, Facoltà di Farmacia — Università degli Studi di Torino.
[3] Dipartimento di Anatomia, Farmacologia e Medicina Legale, Facoltà di Medicina, Università degli Studi di Torino.
[4] To whom correspondence should be addressed.

0724-8741/98/0500-0745$15.00/0 © 1998 Plenum Publishing Corporation

Preparation of Unlabelled SLN

The SLN were prepared from a warm o/w microemulsion containing stearic acid as internal phase (0.70 mM), Epikuron 200 as surfactant (0.14 mM), taurocholate sodium salt as cosurfactant (0.66 mM) and filtered water as continuous phase (111.11 mM). Epikuron 200 and warm filtered water were added to melted stearic acid at about 70°C. The cosurfactant was then added to the warm mixture, and a clear system was easily obtained under stirring. SLN were obtained by dispersing the warm microemulsion (about 70°C) in filtered cold water (2–3°C) at a ratio of 1:4 (microemulsion:water, v/v) under mechanical stirring. The nanoparticle dispersion was washed three times with equal volumes of distilled water by diaultrafiltration with a TCF2 system (Amicon, Grace, Danvers, USA) and then concentrated to the selected concentration. The washing waters and SLN were freeze-dried and then analyzed to determine the amount of taurocholate

Preparation of SLN incorporating unlabelled 17-iodoheptadecanoic acid was as follows: a warm o/w microemulsion was obtained, by first adding the iodine derivative to the melted stearic acid then dispersing the microemulsion in cold water. The amount of unlabelled 17- iodoheptadecanoic acid was 0.2% w/w calculated on the whole warm microemulsion. In order to determine the iodine derivative content, SLN and washing waters were freeze-dried after washing.

Preparation of Labelled SLN

^{131}I-17-iodoheptadecanoic acid was used as tracer; its activity was 9.75 mCi/mg; a solution was prepared adding 400 μl of chloroform.

Radiolabelled SLN were obtained by adding volumes (100 μl or 200 μl) of the chloroformic solution of ^{131}I-17-iodoheptadecanoic acid to the warm microemulsion, according to the number of rats used, the amounts of SLN/ml of dispersion and the time after the labelling. After the evaporation of chloroform, the microemulsion was dispersed in water and SLN were obtained as described above,

Characterization of Unlabelled SLN

Photon Correlation Spectroscpy

Particle size of SLN was determined by photon correlation spectroscopy using an N4 MD Coulter instrument at a fixed angle of 90° and at a temperature of 25°C. Both samples (40 mg/ml and 80 mg/ml) were diluted 1:40 (v/v) with filtered water. The same dilution was maintained for the lymph samples. The polydispersity index is a measure of the distribution of SLN population (20).

Transmission Electron Microscopy

Transmission electron microscopy (TEM) was performed using a Philips CM10 instrument.

All TEM samples, whether lymph or blood, were diluted 1:40 with saline solution (v/v) and were stained with a 2% solution of osmium tetraoxide before analysis.

Zeta Potential Analysis

The electrophoretic mobility and zeta potential measurements of SLN were determined using a DELSA 440 (Coulter, USA) instrument; SLN dispersions in water were diluted 1:40 with filtered water (v/v) before analysis.

Determination of 17-iodoheptadecanoic Acid Incorporated into SLN

The amounts of 17-iodoheptadecanoic acid present in the SLN and in washing waters were determined spectrophotometrically using a Lamba 2 spectrophotometer (Perkin Elmer). The freeze-dried samples were dissolved in methanol and analysed directly (λ_{max} = 254 nm).

Determination of Taurocholate Sodium Salt

The amount of sodium taurocholate present in the SLN and in washing waters was determined by HPLC (21), using a Perkin Elmer Binary LC Pump 250 liquid chromatograph and a Bio-Rad ODS column (25 cm × 4.6 mm). The eluent was acetonitrile:methanol:phosphate buffer 0.03 M pH 3.4 15:30:55 v/v. The analysis was run at a flow rate of 0.6 ml/min with the UV detector operating at 210 nm

Administration of SLN to Rats

SLN dispersions were administered directly into the duodenal lumen of fed rats under anesthesia, anesthesia being achieved by intraperitoneal injection of ketamine (8 mg/100 g body weight) and droperidol (0.5 mg/100 g of body weight).

The lymph was directed externally by cannulating the thoracic duct near its confluence with the cisterna chili, and a second catheter was positioned in the jugular vein to collect blood samples. All operations were performed with the help of a surgical microscope (Zeiss f 20 and 2.5 x lenses) using surgical techniques suitable for repetitive sampling (22).

SLN were then administered through a puncture in the first portion of the duodenum; the tubing was exteriorized through the main incision, which was closed in layers (muscle and skin).

Samples of both lymph and blood were then withdrawn from the rat; the advantage of this technique is that lymph and blood may be obtained from the same animal simultaneously and repeatedly. After collection of each lymph and blood sample, an equal volume of saline solution was injected i.v. in the rat to maintain body hydration.

The experiments with unlabelled SLN lasted for 45 minutes, administering a fixed volume (0.2 ml) of SLN dispersion at a concentration of either 40 mg/ml or 80 mg/ml. Rats weighing 450–500 g were used. Nine plasma and lymph samples (50 μl collected every five minutes after administration and diluted 1:40 v/v with saline) were analyzed by PCS and by TEM analysis.

The experiments with labelled SLN lasted for 180 minutes, administering a fixed volume (0.2 ml) of SLN dispersions at different concentrations to each animal; about 60 μCi (13 mg of SLN/kg body weight) and about 90 μCi (26 mg of SLN/kg body weight) were administered.; rats weighing 550–650 g were used.

Throughout the experiment, twelve lymph samples were continuously collected every 15 minutes, while six blood samples (0.3 ml) were collected every 30 minutes.

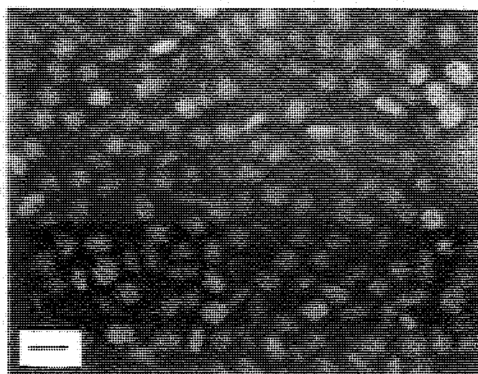

Fig. 1. Transmission electron microscopy micrographs of SLN water dispersion before administration to rats. Bar = 100 nm.

RESULTS

The unlabelled SLN dispersed in water had an average diameter of 80 (±3) nm, a polydispersity index of 0.2 (± 0.05) and a zeta potential of −47 (± 1) mV. The chemico-physical characteristics of SLN dispersion at the two concentrations considered were the same. The percentage of taurocholate eliminated with the washings was about 75% of the initial amount.

The average diameter of SLN, determined by laser light scattering before duodenal administration, showed a narrow size distribution with no particles larger than 100 nm; TEM analysis confirmed both narrow size distribution and spherical shape of SLN (Fig. 1).

A portion of unlabelled SLN were dispersed in lymph to obtain a reference sample used as control; the average diameter of SLN dispersed in lymph increased to 143 nm (Table I).

TEM analysis of samples of rat lymph and plasma showed that SLN were present in the two biological fluids after duodenal administration. In the lymph, the nanoparticles were already present 5 minutes after administration: they maintained colloidal size and spherical shape in both lymph and plasma, as shown in Fig. 2. The photomicrographs show the difference in SLN population between samples of lymph and plasma after administration of 0. 2 ml of SLN dispersion, containing either 8 mg (Fig. 2 A and B) or 16 mg (Fig. 2 C and D) of SLN.

Table I. Average Diameter and Polydispersity Index of SLN Before and After Oral Administration

	Average diameter (nm)	Polydispersity index
Before administration		
in water	80	0.20
in lymph	143	0.14
After administration		
5 minutes	134	0.22
10 minutes	134	0.20
15 minutes	133	0.26
20 minutes	132	0.20
25 minutes	132	0.20
30 minutes	127	0.27
35 minutes	119	0.36
40 minutes	115	0.39
45 minutes	113	0.35

Note: Administered concentration: 8 mg of SLN in 0.2 ml.

The average diameter of the SLN in lymph determined by laser light scattering (see Table I), was not significantly different from that obtained directly placing SLN in a lymph sample, and remained constant over time.

The average diameter and polydispersity index of SLN incorporating unlabelled 17-iodoheptadecanoic acid did not differ from that of SLN without the iodine derivative. Spectrophotometric analysis of the washing waters showed that no iodine derivative was present.

The percentages of total radioactivity of the dose administered/g of lymph or blood over time are reported in Figure 3. The graph (Fig. 3A) shows that the amount of 26 mg of SLN/kg of body weight produced a peak that was higher and shifted later over time than did 13 mg of SLN/kg of b. w.; with the lower amount, SLN in the lymph began to decrease earlier. The rate of appearence of SLN in the lymph was similar.

The percentage of radioactivity found in the blood samples was not so markedly increased, although it was higher for the higher amount (Fig. 2B).

DISCUSSION

Research reported in literature on lymphatic uptake relates to micro- and nanoparticles constituted of polymers; we were not able to find data on solid lipid nanoparticles. Consequently, we comment here on research regarding polymeric particulates.

In our study, unlabelled and labelled SLN, constituted of bioacceptable and biodegradable components such as stearic acid and phosphatidylcholine, were evidenced in lymph and blood after duodenal administration to rats.

The small average diameters of SLN, which remained practically constant after duodenal administration as seen in both lymph (Table I) and plasma, may facilitate their uptake by the lymphatics. Indeed, the value obtained in lymph in vitro is quite considerably higher than the average diameter values of SLN measured in lymph in vivo.

Using a size range of polymeric particles, it has been shown (10,23) that particle size is a critical determinant of the fate of microparticles administered orally; larger particles may be retained for longer periods in the Peyer's patches, while smaller particles are transported to the thoracic duct.

The fairly spherical shape maintained in the two biological fluids is also an aspect to be considered, showing that SLN have a certain physical stability in the gut lumen and during lymphatic uptake and transport. Considering SLN as carriers of therapeutic agents, the maintainance of the shape showed ensure at least a partial protection of the incorporated drugs.

The composition of SLN can also play a role in relation to the uptake pathway, most of all because of their hydrophobicity. The extent of accumulation of polymeric particles in the Peyer's patches has been shown to be dependent on the hydrophobicity of the polymeric material (23); indeed, the accumulation of poly(lactide-co-glycolide) microparticles was found to be lower than that of polystyrene nanoparticles. It has also been shown that the uptake of polystyrene nanoparticles was reduced when polystyrene latex surfaces are coated with hydrophilic poloxamers (9,24). Besides, lipid liquid vehicles,

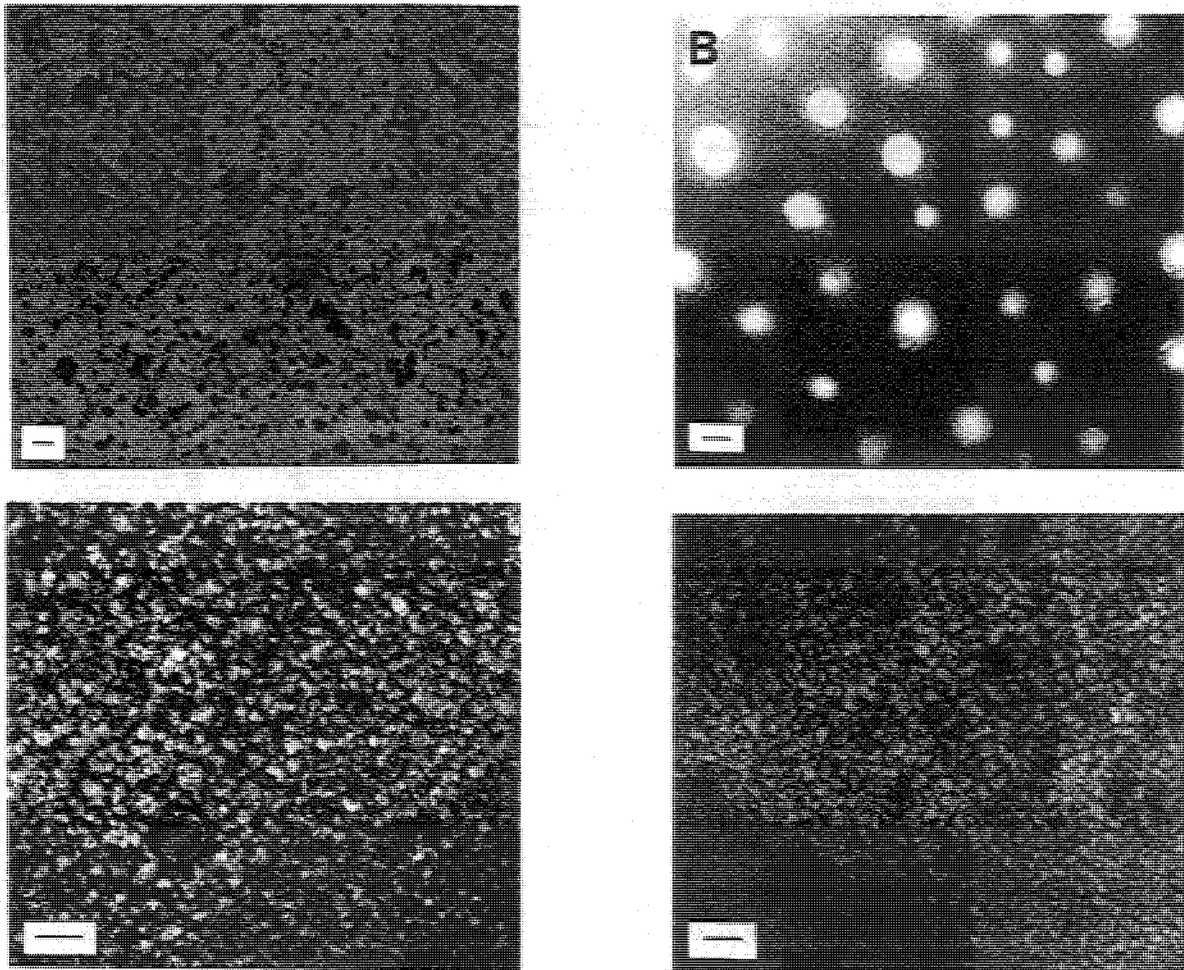

Fig. 2. Trasmission electron microscopy micrographs of SLN after 30 minutes from the administration to rats: in lymph (administered concentration: A, 40 mg/ml and B, 80 mg/ml) and in plasma (C, 40 mg/ml and D, 80 mg/ml). Bar = 100 nm.

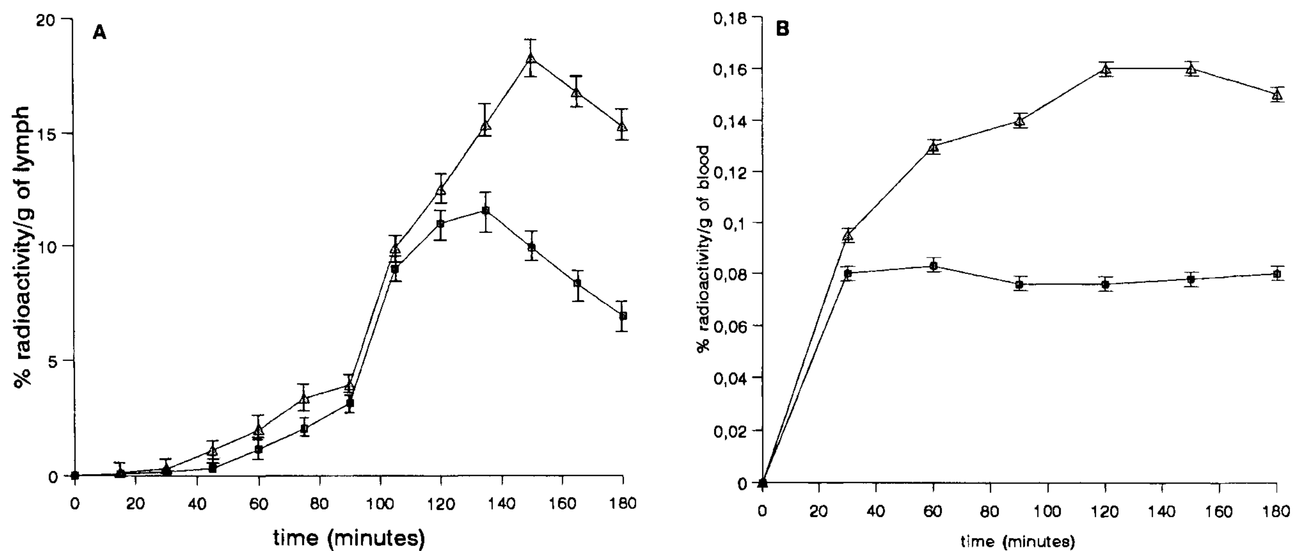

Fig. 3. Means ± SE of percentage of radioactivity of the administered dose per gram of tissue (A lymph and B blood) determined over time by gamma-counting. Administered doses: about 60 μCi (13 mg of SLN/kg body weight) and about 90 μCi (26 mg of SLN/kg of body weight).

constituted of unsaturated fatty acids and tryglycerides, can favour intestinal lymphatic absorption of drugs administered orally (25).

In spite of the washing, some taurocholate may be present on the surface of SLN, about 75% of the initial amount being eliminated. Like other biliar salts, taurocholate may act as an enhancer of the permeability of the epithelial membranes (26), even if in the case of orally administered cyclosporin it has been shown that the presence of bile did not affect its lymphatic absorption (27). Moreover, the absorption promoting effect of taurocholate is lower than other bile salts, as it has been shown for the trihydroxy derivatives of bile salts (28).

Our nanospheres were prepared from an o/w microemulsion containing phosphatidylcholine (Epikuron 200) as surfactant; as a consequence of their preparation methods, a certain amount of the phospholipid remains on the SLN surface, thus favouring the passage through the intestinal wall. Lecithins have been seen to promote oral absorption of drug; the positive contribution of phospholipids in promoting absorption of orally administered cyclosporin has already been shown. Indeed, liquid lipid microspheres containing egg lecithin and vegetable oils permitted 45 times more cyclosporin to be transported in the thoracic duct compared to a conventional preparation (29).

The negative charge on the surface of the SLN does not appear to affect their uptake, although it has been reported in the literature that a negative surface charge on polymeric nanoparticles reduced their uptake (12).

Labelled SLN were used to evaluate the extent of lymphatic uptake. The radioactivity data confirmed the transport of SLN in the lymph after duodenal administration to rats, already evidenced by TEM analysis (Fig.2).

In order to investigate the effect of the SLN concentration on their lymphatic uptake, equal volumes of SLN dispersions containing different amounts of nanospheres were administered. The SLN concentration vs time in lymph was not proportional to the amounts administered, but was higher for the higher concentration of SLN.

A low radioactivity was detected in the blood, showing that direct transport of SLN into the blood is low, confirming lymphatic transport. To explain this low value in blood, it must be considered that Desai and coworkers (30) showed that nanoparticles of polylactic polyglycolic acid (50:50) copolymer were uptaken in prevalence at the level of ileum tissue, and in our acute animal model, a surgical paralitic ileus may affect the physiological transit and absorption of the administered SLN along the small bowel.

Moreover, in our experimental conditions, the passage of SLN from lymph to blood was almost completely inhibited by the external diversion of the lymph.

The hypothesis that, as observed for polymeric nanoparticles, the main SLN uptake pathway in rats may be via the M-cells overlying the lymphoid follicles and the Peyer's patches and non patches, remains to be confirmed in a future study.

From a pharmaceutical point of view, the intestinal lymphatic transport of particulate matter may offer some potential advantages; indeed, direct lymphatic transport bypasses the liver first passage, and may enable the oral route to be used for some agents whose administration is otherwise limited to the parenteral route. In addition, particulates carrying drugs afford the possibility of targeting drugs to lymph, with a potential application in the treatment of lymphatic cancers.

The physical stability of SLN carrying drugs may be expected to reduce in vivo the local adverse effect of some drugs on the gastrointestinal tract, and to protect biodegradable molecules, such as peptides and small proteins, administered orally.

Future research will monitor SLN in the blood circulation after oral administration, and also its biodistribution, without drawing the lymph.

ACKNOWLEDGMENTS

We thank Sorin Radiofarmaci S.r.l (Saluggia, Italy) for help in labelling ^{131}I-17- iodoeptadecanoic acid, Mr. Vittorio Pasteris for his assistance in experiments with radiolabelled solid lipid nanoparticles and Dr. Roberto Canaparo and Ms. Elisabetta Muntoni for technical support.

The work has been supported by a 40% MURST grant.

REFERENCES

1. W. A. Walker, R. Cornell, and L. M. Davenport, K. G. Isselbaker. Progress in gastroenterology uptake and transport of macromolecules by the intestine. Possible role in clinical disorders. *Gastroenterology* **67**:531–550 (1974).
2. C. Damgé, M. Aprahamian, G. Balboni, A. Hoeltzel, V. Andrieu, J. and P. Devissaguet. Polyalkylcyanoacrilate nanocapsules increase the intestinal absorption of a lipophilic drug. *Int. J. Pharm.* **36**:121–125 (1987).
3. J. Kreuter, U. Muller, and K. Munz. Quantitative and microautoradiographic study on mouse intestinal distribution of polyalkylcyanoacrylate nanoparticles. *Int. J. Pharm.* **55**:39–45 (1989).
4. P. Maincent, R. Le Verge, P. Sado, P. Couvreur, J. and P. Devissaguet. Disposition kinetics and oral bioavailability of vincamine-loaded polyalkylcyanoacrylate nanoparticles. *J. Pharm. Sci.* **75**:955–958 (1986).
5. M. E. LeFevre, J. W. Vanderhoff, J. A. Laissue, D. and D. Joel. Accumulation of 2 μm latex particles in mouse Peyer's patches during chronic latex feeding. *Experientia* **34**:120– 122 (1978).
6. P. Couvreur, L. Grislain, V. Lenaerts, F. Brasseur, P. Guiot, and A. Biernacki. (1986) Biodegradable polymeric nanoparticles as drug carrier for antitumor agent. In: P. Guiot and P. Couvreur (Eds.), *Polymeric Nanoparticles and Microspheres,* CRC Press, Boca Raton, FL, pp. 27–93.
7. C. Damgé, C. Michel, M. Aprahamian, P. Couvreur, and J. P. Devissaguet. Nanocapsules as carriers for oral peptide delivery. *J. Controll. Rel.* **13**:233–239 (1990).
8. J. Kreuter. Peroral administration of nanoparticles. *Adv. Drug Del. Rev.* **7**:71–86 (1991).
9. A. T. Florence, A. M. Hillery, N. Hussain, and P. U. Jani. Nanoparticles as carriers for oral peptide absorption: studies on particles uptake and fate. *J. Control. Rel.* **36**:39–46 (1995).
10. P. U. Jani, G. W. Halbert, J. Langridge, and A. T. Florence. Uptake and translocation of latex nanospheres and microspheres after oral administration to rats. *J. Pharm. Pharmacol.* **41**:809–812 (1989).
11. R. H. Müller, W. Mehenert, J. S. Lucks, C. Schwarz, C. Freitas, and D. Ruhl. Solid lipid nanoparticles (SLN). An alternative colloidal carrier system for controlled drug delivery. *Eur. J. Pharm. Biopharm.* **41**:62–69 (1995).
12. A. J. Domb. Long-acting injectable oxytetracycline- liposphere formulation. *Int. J. Pharm.* **124**:271–278 (1995).
13. K. Westesen, B. Siekmann, and M. H. J. Koch. Investigation on the physical state of lipid nanoparticles by synchroton radiation X-ray diffraction. *Int. J. Pharm.* **93**:189–199 (1993).
14. R. Cavalli, M. R. Gasco, and S. Morel. Behaviour of timolol incorporated in lipospheres in the presence of a series of phosphate esters. *S.T.P. Pharma Science* **2**:514–518 (1992).
15. R. Cavalli, D. Aquilano, M. E. Carlotti, and M. R. Gasco. Study by X-ray powder diffraction and differential scanning calorimetry of two model drugs, phenothiazune and nifedipine incorporated into lipid nanoparticles *Eur. J. Pharm. Biopharm.* **41**:329–333 (1995).

16. R. Cavalli, O. Caputo, and M. R. Gasco. Solid lipospheres of doxorubicin and idarubicin. *Int. J. Pharm.* **89**:R9–R12 (1993).
17. R. Cavalli, S. Morel, M. R. Gasco, P. Chetoni, and M. F. Saettone. Preparation and evaluation in vitro of colloidal lipospheres containing pilocarpine as ion pair. *Int. J. Pharm.* **117**:243–245 (1994).
18. S. Morel, E. Ugazio, R. Cavalli, and M. R. Gasco. Thymopentin in solid lipid nanoparticles. *Int. J. Pharm.* **132**:259–261 (1996).
19. S. Morel, M. R. Gasco, and R. Cavalli. Incorporation in lipospheres of D-Trp-6 LHRH *Int. J. Pharm.* **105**:R1–R5 (1994).
20. D. E. Koppel. Analysis of macromolecular polydispersity in intensity correlation spectroscopy: the methods of cumulants. *J. Chem. Phys.* **57**:4814–4816 (1972).
21. F. Nakayama and H. Nakagaki. Quantitative determination of bile acids in bile with reversed phase high performance liquid chromatography. *J. Chromatogr.* **183**:287–293 (1980).
22. R. G. Girardet. Surgical techniques for long-term studies of thoracic duct circulation in the rats. *J. Applied Phys.* **39**:682–688 (1975).
23. J. H. Eldridge, C. J. Hammond, J. A. Meulbroek, J. K. Staas, R. M. Gilley, and T. M. Tice. Controlled vaccine release in the gut. I. Orally administered biodegradable microspheres target the Peyer's patches associated lymphoid tissues. *J. Control. Rel.* **11**:205–214 (1990).
24. A. T. Florence. The oral absorbtion of micro-nanopartirculates: neither exceptional nor unusual *Pharm. Res.* **14**:259–266 (1997).
25. W. N. A. Charman and V. J. Stella. Transport of liphophilic molecules by the intestinal lymphatic system. *Adv. Drug. Del. Rev.* **7**:1–14 (1991).
26. N. Ukida, Y. Maitani, Y. Machida, M. Nakagaki, and T. Nagai. Influence of bile salts on the permeability through the nasal mucosa of rabbits of insulin in comparison with dextran derivatives. *Drug. Dev. Ind. Pharm.* **17**:1625–1634 (1991).
27. K. Takada, F. Yoshihiro, Y. Hiroshi, and S. Muranishi. Biological and pharmaceutical factors affecting the absorption and lymphatic delivery of Cyclosporin A from gastrointestinal tract. *J. Pharmacobio-din.* **11**:80–87 (1988).
28. T. Murakami, Y. Sasaki, K Yamajo, and N. Yata. Effect of bile salts on the rectal absorption of sodium ampicillin in rats. *Chem. Pharm. Bull.* **32**:1948–1955 (1984).
29. A. Yanagawa, T. Iwayama, T. Saotome, Y. Shoji, K. Takano, H. Oka, and Y. Mizushima. J. Microencapsulation **6**:161–165 (1989).
30. P. Desai, V. Labhasetwar, G. L. Amidon, and R. J. Levy. Gastrointestinal uptake of biodegradable microparticles.: effect of particles size. *Pharm. Res.* **13**:1838–1845 (1996).