J. Pharm. Pharmacol. 2000, 52: 1049–1056
Received March 8, 2000
Accepted May 11, 2000

Ileal Uptake of Polyalkylcyanoacrylate Nanocapsules in the Rat

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Abstract

The ileal uptake of polyalkylcyanoacrylate nanocapsules (less than 300 nm in diameter) has been investigated in the rat. Iodised oil (Lipiodol) was used as the tracer for X-ray microprobe analysis in scanning electron microscopy.

Lipiodol nanocapsules, or an emulsion of Lipiodol, were administered in the lumen of an isolated ileal loop of rat. Lipiodol nanocapsules improved the absorption of the tracer as indicated by increased concentrations of iodine in the mesenteric blood (+27%, $P < 0{\cdot}01$, compared with Lipiodol emulsion). Intestinal biopsies were taken at different time points and the samples underwent cryofixation and freeze-drying. The nanocapsules were characterized by their strong iodine emission, and electron microscopy of the biopsy samples revealed nanocapsules in the intraluminal mucus of the non-follicular epithelium, then in the intercellular spaces between enterocytes, and finally the nanocapsules were found within intravillus capillaries. However, nanocapsules were most abundant in the Peyer's patches, where the intestinal epithelium had been crossed by way of the specialized epithelial cells, designated membranous cells, or M cells, and their adjacent absorptive cells. These observations were confirmed quantitatively by measuring iodine concentrations in the various tissue compartments. Ten minutes after the intraluminal administration of Lipiodol nanocapsules, the emission of iodine peaked in the mucus (+77%, $P < 0{\cdot}01$), in M cells (+366%, $P < 0{\cdot}001$), in enterocytes adjacent to M cells (+70%, $P < 0{\cdot}05$) and in lymph vessels (+59%, $P < 0{\cdot}05$).

Polyalkylcyanoacrylate nanocapsules were able to pass through the ileal mucosa of the rat via a paracellular pathway in the non-follicular epithelium, and most predominantly, via M cells and adjacent enterocytes in Peyer's patches.

Polyalkylcyanoacrylate nanoparticles and nanocapsules have been developed as biocompatible drug carriers able to adsorb or encapsulate a great variety of chemicals. Nanoparticles are full polymeric structures while nanocapsules are composed of a thin polymeric wall that encounters a lipophilic material. Nanoparticles, administered subcutaneously, have been shown to enhance the activity of antimitotic drugs such as actinomycin D against an experimental subcutaneous sarcoma (Brasseur et al 1980). The cardiac toxicity of the antitumoral drug doxorubicin was reduced when administered as nanoparticles (Couvreur et al 1982). The main advantage of these colloidal drug carriers is their biodegradability, the rate of degradation being a function of the length of the alkyl-chain (Leonard et al 1966; Lenaerts et al 1984). Generally, they have been administered parenterally and may be useful as sustained-release products. Administered orally, these drug carriers have been shown to protect a peptidic drug against proteolytic degradation by gastrointestinal enzymes and to facilitate its intestinal absorption. Previously, we reported that insulin, a 51 amino acid peptide, associated to polyalkylcyanoacrylate nanocapsules or nanoparticles reduced glycaemia for a prolonged time after oral administration in diabetic rats (Damgé et al 1988, 1990, 1995, 1997b). However, the intensity and duration of that effect depended on the site of administration of the insulin-loaded nanocapsules along the gastrointestinal tract. The ileum was the most potent site of absorption, followed by the stomach, then the duodenum and the jejunum and finally the colon (Michel et al 1991). This study was designed to elucidate the route of absorption of nanocapsules

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through the rat ileum by means of biochemical and morphological analysis. Lipiodol, an iodised oil generally considered as a contrast material for tomography (Vermers et al 1980), was used as the tracer for electron microscopy.

Materials and Methods

Preparation and characterization of nanocapsules
Nanocapsules were prepared by interfacial emulsion polymerization of isobutyl cyanoacrylate (Al Khouri Fallouh et al 1986). A lipophilic phase (composed of 1 mL Lipiodol (Laboratoire Guerbet, Aulnay-sous-Bois, France) and 0·25 mL isobutyl-2-cyanoacrylate (Ethnor, Paris, France) was dissolved in 100 mL absolute ethanol and added under mechanical stirring to 200 mL of an aqueous phase containing 0·5% nonionic surfactant (Poloxamer 188, ICI, Clamart, France). The nanocapsules were formed immediately by polymerization of the monomer around the Lipiodol droplets. The obtained colloidal suspension was then concentrated by evaporation under vacuum and the final volume filtered through fritted glass filters (9–15 μm).

For control experiments, an emulsion of Lipiodol was prepared according to the same procedure but in the absence of the monomer.

Characterization of nanocapsules
The size of the nanocapsules was estimated by laser light scattering using a monochromatic laser ray diffusion counter (Autosizer 2C, Malvern Instruments, Les Ulis, France). Nanocapsules were examined in a scanning electron microscope (Philips 501 B, Philips, Bobigny, France) after coating with carbon, and characterized by their X-ray emission of iodine using an energy dispersive X-ray spectrometer (Link Systems 860 type II, Link Systems, Evry, France).

Experimental study in the rat
Adult male Wistar rats (250 g; Iffa Credo, L'Arbresle, France) were housed in air-conditioned quarters under a 12-h light–dark photoperiod schedule. Standard laboratory chow diet (UAR, Villemoisson-sur-Orge, France) and tap water were freely available. All treatments were carried out between 09 00 and 10 00 h after an overnight fast. Experimental and control groups were formed by random assignment.

Eighty-four rats were anaesthetized with ether. After laparotomy, a 40-cm ileal loop, situated above the ileocaecal valve, was isolated from the digestive tract by a ligature at each side. The rats were divided into two groups of seven rats each. One group received a 1-mL suspension of encapsulated Lipiodol injected into the intestinal lumen of this ileal segment. The second group (control group) received an injection of 1 mL Lipiodol emulsion into the intestinal lumen. Samples of blood from the mesenteric vein, which irrigates this intestinal segment, were collected at 10-min intervals up to 30 min, then at 45, 60 and 90 min after the luminal injection of non-encapsulated (emulsion) or encapsulated Lipiodol. Biopsies of the non-follicular intestinal mucosa and follicular mucosa (Peyer's patches) were performed at the same time as the blood sampling to examine the iodine content at different sites of the mucosa. Peyer's patches were easily identified at the anti-mesenteric site of the intestine as they appeared as oblong structures measuring between 0·5–1 cm in diameter, and constituted 10–20 nodules of 1–2 mm in diameter. They were mainly located in the distal part of the ileum.

Scanning electron microscopy and microanalytical analysis
Blood samples were mixed with an equal part of Triton 10% and then homogenized (Polytron, Kinematica, Bioblock, Strasbourg, France). A sample was placed on a plastic cover slip, air dried and carbon-coated before X-ray analysis (Schreiber & Wims 1981). A scanning electron microscope (Philips 501 B) fitted with an X-ray analyser (Link system 860 type II) was used to quantify the iodine contained in the blood samples. The analysis conditions were 20 kV, 40 μÅ and 2000 Å spot size. The intensity of the emission corresponded to six measurements of 100 s analysed 10 times. The intensity of the iodine emission, expressed in counts s^{-1}, was calculated in g/100 mL blood using Heptan (Aguettant Laboratories, Lyon, France) as a standard containing 3·80 μg/100 mL iodine.

Samples of small intestine and Peyer's patches were quickly removed and thrown without a cryoprotectant into a mixture of liquid propane (80%) and isopentane (20%), cooled down to −196°C by liquid nitrogen (Jehl et al 1981). The samples remained there for storage. When required, the samples were fractured and transferred to a vacuum device under liquid nitrogen and freeze-dried (Terracio & Schwabe 1981) for 48h at −90°C and 10 mPa. Samples were mounted on aluminium stubs, carbon-coated and stored in a dry atmosphere. Samples were examined and analysed in a scanning electron microscope with an electron probe at 20 kV, 100 μÅ and 1000 Å spot size. Data were collected for 100 s on each area, analysed six times.

Statistical analysis

Results were expressed as means ± s.e.m. of the mean values obtained from each rat. There were seven rats in each group. Comparisons between rats treated with encapsulated and non-encapsulated Lipiodol were assessed using a one-way analysis of variance followed by a parametric Student's unpaired *t*-test when Barlett's test gave homogeneity of variance (for mesenteric blood samples). Instat 2.00 Macintosh software (Graph Pad Software, San Diego, CA) was used. The difference was considered as significant when $P < 0{\cdot}05$.

Results

Structure and characterization of Lipiodol nanocapsules and emulsion

Lipiodol nanocapsules and emulsion showed homogeneous distribution of particles, the mean size being 287 and 367 nm, respectively, as analysed by laser light scattering.

Scanning electron microscopy revealed that the Lipiodol nanocapsules consisted of round, homogeneous smooth particles, characterized by their strong X-ray emission of iodine. The spectrum of iodine was composed of four L lines (α, β, γ, δ) of energy levels.

Ileal absorption of Lipiodol nanocapsules and emulsion

Concentration of iodine in the mesenteric blood. After the administration of Lipiodol emulsion (1 mL) in the ileal lumen of rats, the iodine concentration in mesenteric blood increased by 27% of the basal value ($P < 0{\cdot}05$) after 10 min. This level was maintained until 45 min, and then the blood iodine concentration decreased reaching basal value after 75 min (Figure 1).

When Lipiodol nanocapsules (1 mL) were administered into the ileal lumen of rats, the blood

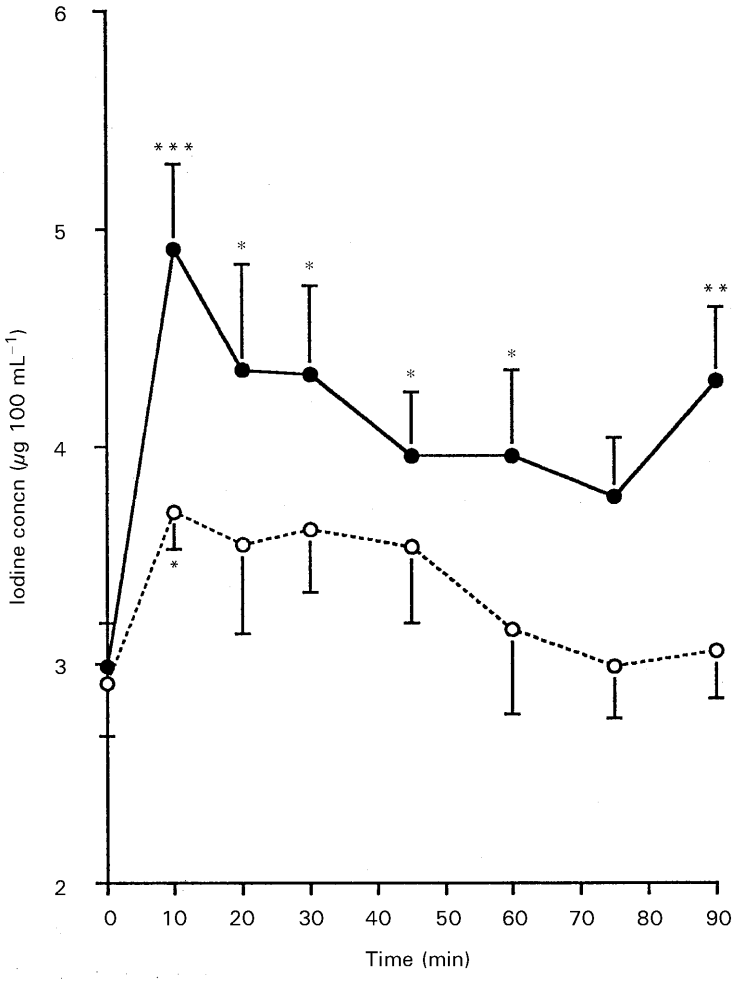

Figure 1. Plasma iodine concentration after intra-ileal administration of Lipiodol either as an emulsion (○) or as nanocapsules (●). Data are means ± s.e.m. from seven animals. $*P < 0{\cdot}05$, $**P < 0{\cdot}01$, $***P < 0{\cdot}001$, compared with controls.

iodine concentration increased immediately too. However, this increase was greatest at 10 min (64% higher than the basal value, $P < 0{\cdot}001$), then iodinaemia slowly decreased but never returned to basal values. Indeed, it always remained significantly higher than control values ($P < 0{\cdot}05$), and even at 75 and 90 min respectively iodinaemia was 26% ($P < 0{\cdot}05$) and 40% ($P < 0{\cdot}01$) higher than values observed in control animals receiving an emulsion of Lipiodol (Figure 1).

Ultrastructural aspect of the non-follicular intestinal mucosa and Peyer's patches. After rapid freezing and freeze-drying, scanning electron microscopy revealed that the intestinal mucosa was quite different from that observed after a classical fixation with glutaraldehyde. In particular, the intraluminal mucus was well preserved.

As illustrated in Figure 2, the ileal villi were surrounded by an abundant layer of mucus, forming a continuous lamellar meshwork. At a higher magnification, this mucus appeared reticulated and in close contact with the microvilli of absorptive cells. The epithelium was composed mainly of enterocytes; some goblet cells, characterized by their numerous mucin droplets, were also recognized (Figure 2A). Due to freezing, the cytoplasm appeared reticulated, thus no intracellular organelles could be identified. The lamina propria displayed a large whorled pattern in which blood capillaries were easily recognized by the presence of red cells in their lumen and fenestrated endothelial cells. In the core of the villus, lymph ducts appeared as dilated spaces filled with a lamellar material similar to intraluminal mucus. However, the connective tissue was not well preserved by cryofixation followed by freeze-drying.

As illustrated in Figure 3 (A and C) at the level of Peyer's patches, the follicular epithelium was mainly composed of two types of cells: epithelial cells similar to the non-follicular epithelial cells and membranous epithelial cells also called "M cells". The microvilli of the M cells were shorter and less regular than the microvilli of the adjacent enterocytes (Figure 3C, 3D). Their plasma membrane showed numerous digitations, leading to large intercellular spaces occupied by round lymphocytes (Figure 3A, C). Thus, the M cells extended between the adjacent epithelial cells and separated the lymphocytes from the intestinal lumen. Lymph ducts were more numerous than in the non-follicular ileal epithelium. Capillaries were rare and not easily identified.

Lipiodol nanocapsules in the non-follicular intestinal mucosa and Peyer's patches: qualitative observations. Observed using scanning electron microscopy, Lipiodol nanocapsules displayed a round shape 100–300 nm in diameter. They were characterized by their strong X-ray emission of

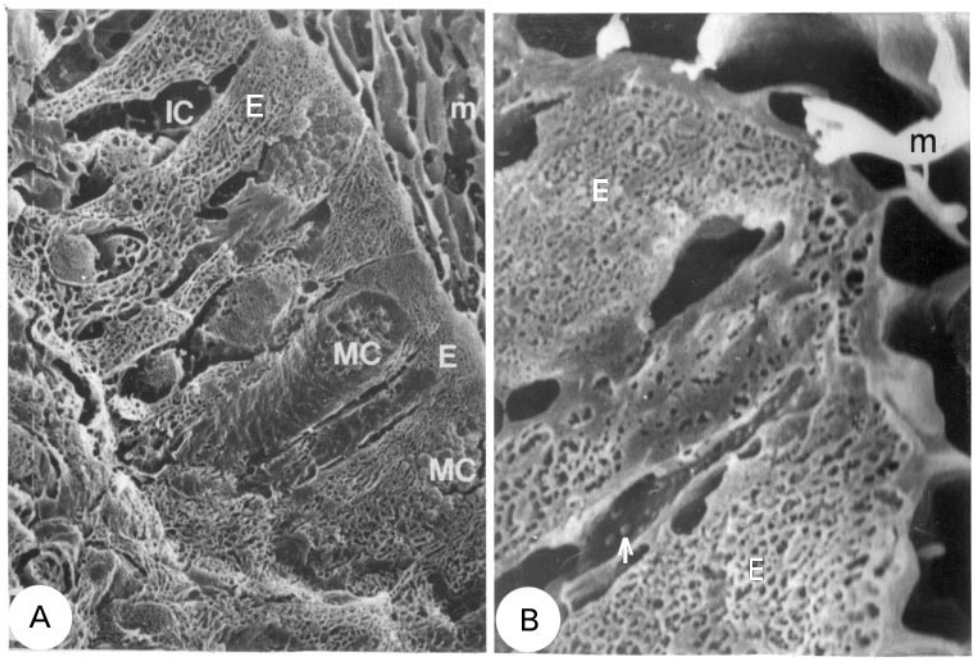

Figure 2. Scanning electron microscopy of the ileal mucosa after the intraluminal administration of Lipiodol-loaded nanocapsules in an isolated ileal segment. Nanocapsules (arrows) were in close contact with the intraluminal mucus (m), then they appeared in intercellular spaces (IC) between two enterocytes (E). MC, mucous cell. Magnification: A, ×3000; B, ×6000.

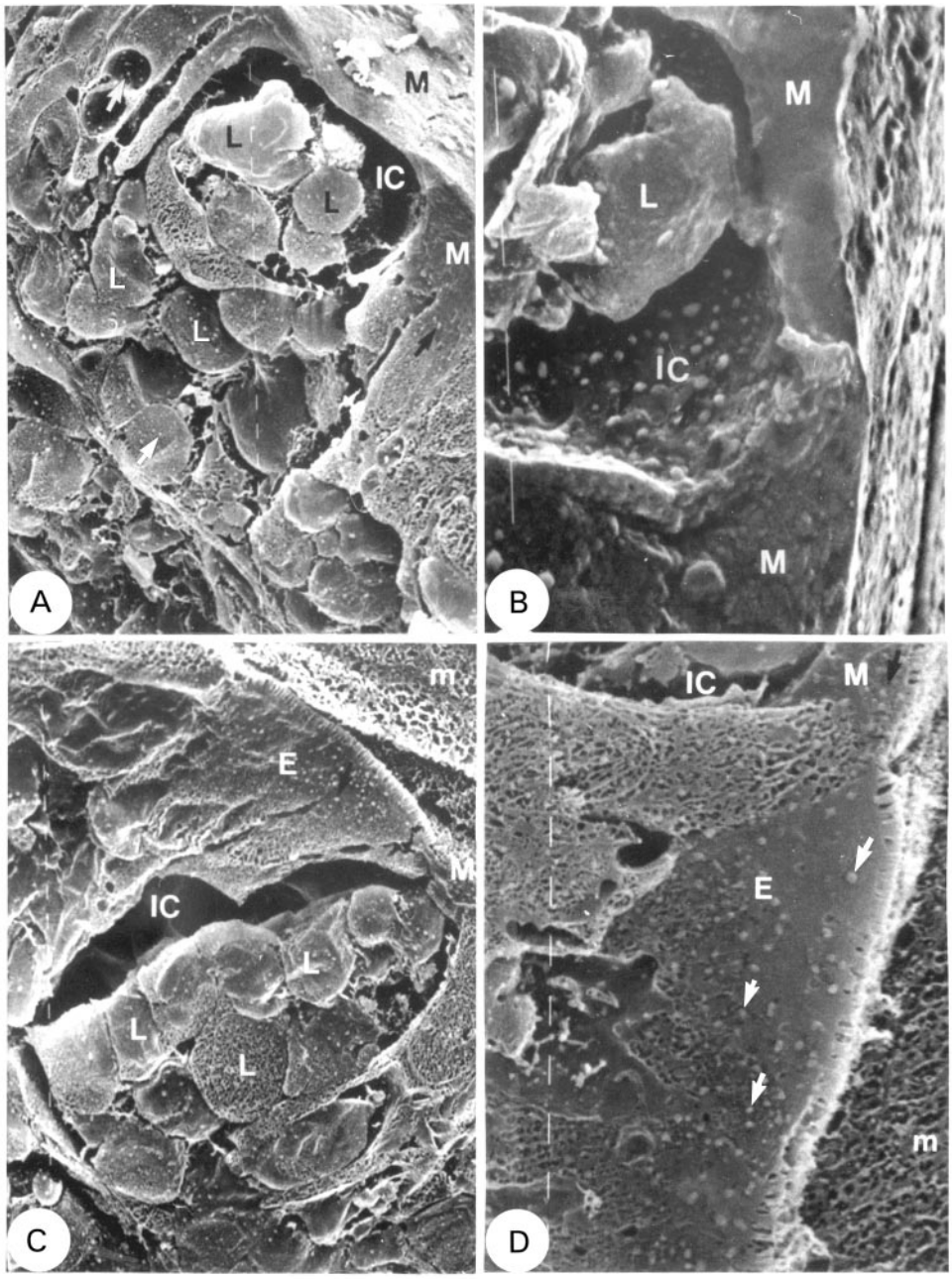

Figure 3. Scanning electron microscopy of Peyer's patches after the intraluminal administration of Lipiodol-loaded nanocapsules in an isolated ileal segment. M cells separate the lymph cells (L) from the intraluminal mucus (m). Nanocapsules (arrows) at the surface of lymph cells (A, B, C) were also found in intercellular spaces (IC) between M cells and lymph cells (B). Note the abundant nanocapsules in enterocytes (E) adjacent to M cells (C, D). Magnification: A and C, ×3000; B, ×12000; D, ×6000.

iodine (700–900 counts $100\,s^{-1}$). These nanocapsules were looked for at various sites of the non-follicular intestinal mucosa and Peyer's patches as a function of time after their intraluminal administration.

Ten minutes after their intraluminal administration, nanocapsules were observed in close contact with the intraluminal mucus in the non-follicular epithelium (Figure 2A). Some nanocapsules were found in the intercellular spaces between two enterocytes (Figure 2B) but never in the cytoplasm of enterocytes.

At the site of Peyer's patches, nanocapsules were very abundant. Ten minutes after their intra-ileal administration, they were found in M cells (Figure 3D) and appeared in the intercellular spaces adjacent to M cells (Figure 3B). In these spaces, nanocapsules were found in close contact with lymph cells (Figure 3B, C). In addition, it was very interesting to find numerous nanocapsules within

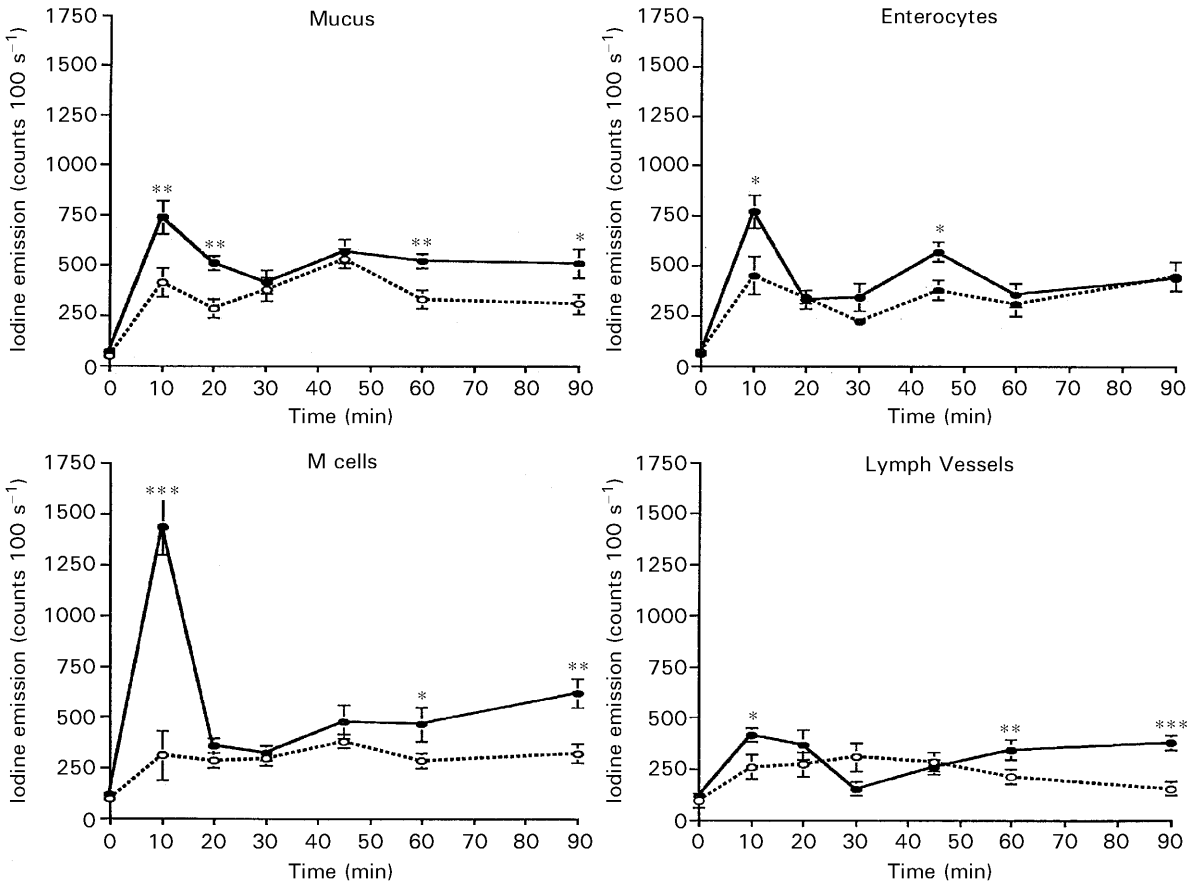

Figure 4. Iodine emission at various sites of Peyer's patches (intraluminal mucus, M cells, enterocytes adjacent to M cells and lymph vessels) as a function of time after an intra-ileal administration of either an emulsion of Lipiodol (○) or Lipiodol nanocapsules (●). Data are means ± s.e.m. from seven animals. $*P < 0{\cdot}05$, $**P < 0{\cdot}01$, $***P < 0{\cdot}001$ compared with controls.

the cytoplasm of the epithelial cells adjacent to M cells (Figure 3C, D). These cells probably possess particular properties allowing the phagocytosis of nanocapsules, a process that was never observed for enterocytes of the non-follicular epithelium. Finally, nanocapsules were seen in the lymph ducts underlying the Peyer's patches.

The massive presence of nanocapsules in Peyer's patches, though often observed, is not a general phenomenon. While nanocapsules could be abundant in some Peyer's patches, they were less abundant in others. However, after the intraluminal administration of the Lipiodol emulsion round particles rich in iodine were never observed.

Lipiodol nanocapsules in the non-follicular intestinal mucosa and Peyer's patches: quantitative observations. Whatever the period after administration, nanocapsules were always more numerous in Peyer's patches than in the non-follicular ileal mucosa. To follow the passage of nanocapsules through these Peyer's patches, the emission of iodine was counted at various sites of this mucosa: the intraluminal mucus, M cells, enterocytes just adjacent to Peyer's patches, and lymph ducts.

Figure 4 illustrates the emission of iodine at different sites of Peyer's patches after the intra-ileal administration of Lipiodol nanocapsules and Lipiodol emulsion, considered as the control. Ten minutes after the administration of Lipiodol nanocapsules, the emission of iodine increased significantly in the mucus (77%, $P < 0{\cdot}01$), in M cells (366%, $P < 0{\cdot}001$), in enterocytes adjacent to these M cells (70%, $P < 0{\cdot}05$%) and finally in lymph vessels (59%, $P < 0{\cdot}05$%). The emission of iodine then decreased, reaching values not significantly different from control values after 30 min. Finally, after 60 and 90 min, the emission of iodine increased again. The increase was only slight but it was significant, especially in the intraluminal mucus (57%, $P < 0{\cdot}01$, and 66%, $P < 0{\cdot}05$, respectively), M cells (63%, $P < 0{\cdot}05$ and 92%, $P < 0{\cdot}01$, respectively) and lymph vessels (61%, $P < 0{\cdot}01$ and 147%, $P < 0{\cdot}001$, respectively). From

these quantitative data it could be suggested that nanocapsules improved the passage of iodine through Peyer's patches principally by way of M cells, but adjacent enterocytes would also be implicated in this phenomenon. Finally, iodine was recovered within lymph vessels underlining these Peyer's patches. This phenomenon was fast, the peak of absorption being observed from 10 min after the intraluminal administration of Lipiodol nanocapsules.

Discussion

Particles are generally administered orally or intragastrically. Thus, it is difficult to evaluate correctly the kinetics of absorption of particles through the intestinal mucosa because of the variations of transit time between animals (from 5 to 24 h in the rat). Previously we reported (Michel et al 1991) that after the administration of insulin nanocapsules along the gastrointestinal tract in diabetic rats, the ileum was the most potent site of administration regarding the intensity and duration of the biological action of insulin. Therefore, in this study, we administered the nanocapsules directly into the lumen of the rat ileum after laparotomy.

Our data indicated that nanocapsules administered in the ileal lumen of the rat improved the intestinal absorption of Lipiodol by approximately 37% when compared with an emulsion of Lipiodol. The effect was fast, and peaked at 10 min. Our results agree with those of Damgé et al (1988, 1990, 1995, 1997a, b) who associated two peptides, insulin and octreotide, to polyisobutylcyanoacrylate nanocapsules and nanospheres. When administered orally in the rat, the octreotide nanocapsules were only effective for 2 h (Damgé et al 1997a), but the insulin nanocapsules and nanospheres were biologically active for several weeks (Damgé et al 1988, 1990, 1995, 1997b). Cyanoacrylate nanoparticles have also been shown to enhance the oral bioavailability of vincamine (Maincent et al 1986), indomethacin (Ammoury et al 1991) and avarol (Beck et al 1994). The improvement in peptide bioavailability after oral administration could be due, in part, to the protective effect of the nanocapsules, preventing degradation of the peptides by proteolytic enzymes in the digestive tract, as demonstrated in-vitro (Damgé et al 1990, 1997b; Michel et al 1991; Lowe & Temple 1994; Scherer et al 1994). However, it could also be due to the intraluminal liberation of peptides from nanocapsules followed by their intestinal absorption or by the direct uptake and translocation of nanocapsules through the intestinal mucosa.

Our electron microscopical study of the Lipiodol nanocapsules indicated that the nanocapsules were able to pass through the non-follicular ileal mucosa using a paracellular pathway. Ten minutes after their intra-ileal administration, nanocapsules were recovered in intercellular spaces between absorptive cells and finally were found in intravillus capillaries. These results agree with our previous findings that demonstrated a paracellular uptake of nanocapsules in the jejunal mucosa of the rat (Aprahamian et al 1987). Our qualitative observations also indicated an abundant passage of nanocapsules through the follicular epithelium in the ileum. Ten minutes after their intraluminal administration numerous nanocapsules were observed in the cytoplasm of absorptive cells adjacent to M cells, as well as in M cells, suggesting a rapid translocation. Nanocapsules were then found in the intercellular spaces under M cells where they were in close contact with lymphoid cells. The uptake of nano- and microparticles by M cells in Peyer's patches has been well documented (Jani et al 1989; Pappo & Ermak 1989; Kreuter 1991; Scherer et al 1993). However, this is the first report of their uptake by follicular enterocytes. Thus, these cells seem to have particular absorptive properties in contrast with the absorptive cells of the non-follicular epithelium. Indeed, neither in this study nor in our previous study (Damgé et al 1988) were nanocapsules observed in the cytoplasm of non-follicular enterocytes in the ileum or in the jejunum. Systematic histochemical and immunochemical studies of these cell surfaces have not been reported. However, cationized ferritin and the lectin, ricin, which binds to D-galactose and *N*-acetyl-glucosamine residues of cell surface glycoconjugates adhere to the apical surface of absorptive cells and M cells overlying ileal Peyer's patches (Neutra et al 1982). This suggests similar surface properties for both types of cells, which are the main cellular populations of the follicular epithelium of Peyer's patches.

Our quantitative ultrastructural data agreed with those observations. A peak of iodine emission was observed 10 min after the intraluminal administration of Lipiodol nanocapsules in M cells and in adjacent enterocytes. At 60 and 90 min there was another increase of iodine emission, albeit lower, in the mucus, M cells and lymph vessel, suggesting a later uptake of nanocapsules or their contents. These results are also consistent with our mesenteric plasma levels of iodine. Indeed, the level of iodine was maximal 10 min after the intra-ileal administration of nanocapsules but remained

increased over the whole experiment, although to a lesser extent.

Taken together, our data suggest that nanocapsules improved the ileal absorption of Lipiodol, the preferential way of absorption being the M cells and the adjacent enterocytes of Peyer's patches. These results could also explain why insulin nanocapsules administered in the rat ileum exerted the best biological effect compared with intragastric, intraduodenal, intrajejunal and intracolic administrations.

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