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Polyalkylcyanoacrylate nanocapsules increase the intestinal absorption of a lipophilic drug

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Summary

The advantage of a polymeric drug carrier, poly-isobutylcyanoacrylate nanocapsules, in the intestinal absorption of a lipophilic drug (Lipiodol) has been investigated in the dog. Lipiodol, an iodized oil, was characterized by X-ray emission of iodine in a scanning electron microscope fitted with an X-ray microprobe analyser. Nanocapsules, administered in the jejunal lumen, increased the absorption of Lipiodol: the plasma level of iodine was maximal 45 min after the administration of Lipiodol emulsion (3 times basal value) and nanocapsules (3.5 times). Then iodemia decreased again but remained 2.5-fold higher than control values 105 min after the administration of nanocapsules. At the cellular level, nanocapsules accelerate, intensify and prolong the passage of iodine through the intestinal mucosa. These results could be explained by a direct transport of the drug by nanocapsules through the mucosa, or by increasing the intraluminal concentration of the drug close to absorptive cells.

Introduction

The use of polyalkylcyanoacrylate nanoparticles as a biodegradable drug carrier has been previously demonstrated (Leonard et al., 1966; Couvreur et al., 1979; Grislain et al., 1983; Lenaerts et al, 1984). This carrier, able to adsorb a great variety of chemicals, has been shown to enhance the activity of antimitotic drugs such as actinomycin D against an experimental subcutaneous sarcoma (Brasseur et al., 1980). Moreover, nanoparticles reduce the cardiac toxicity of antitumoral drugs such as doxorubicin (Couvreur et al., 1982). More recently, another type of polyalkylcyanoacrylate drug carrier has been developed (Al Khouri Fallouh et al., 1986). The latter, composed of submicroscopical capsules (nanocapsules) can be easily applied to lipophilic drugs which can be encapsulated. Indeed, these nanocapsules of less than 300 nm in diameter, are prepared by interfacial polymerization of the monomer around a lipidic phase within emulsion. The aim of this work was to determine the interest of these nanocapsules in the intestinal absorption of a lipophilic drug, Lipiodol, an iodized oil. This latter, used as a contrast material for tomography (Vermers et al., 1980) can be also considered as a tracer in electron microscopy.

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Materials and methods

Preparation of nanocapsules

Nanocapsules of Lipiodol were prepared by the method of Al Khouri Fallouh et al. (1986). A lipophilic phase, composed of 1 ml of Lipiodol (Laboratoire Guerbet, Aulnay-sous-Bois, France), and 0.25 ml of isobutyl-2-cyanoacrylate (Ethnor, Paris, France) was dissolved in 100 ml of absolute ethanol and added under mechanical stirring to 200 ml of an aqueous phase (pH 6) containing 0.5% of non-ionic surfactant (Poloxamer 188, ICI, Clamart, France). The nanocapsules are formed immediately by polymerization of the monomer around the Lipiodol droplets. The colloidal suspension obtained thus was concentrated by evaporation under vacuum and the final volume of 40 ml filtered through fritted glass filter (9–15 μm).

An emulsion of Lipiodol has been prepared according to the same procedure but in the absence of the monomer.

Characterization of nanocapsules

The size of nanocapsules was estimated by laser light scattering using a monochromatic laser ray diffusion counter (Nanosizer, Coultronics France SA, Margency, France). Nanocapsules have also been 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 dog

Six Beagle dogs were anaesthetized with sodium pentobarbital (20 mg/kg b. wt.). After laparotomy, two segments of the proximal jejunum, 10 cm in length each, were isolated by ligature from the digestive tract. Moreover, each segment was isolated at the vascular level by ligating the collaterals of the vascular arcad which irrigates this area (Fig. 1). A 1-ml suspension of encapsulated Lipiodol was injected in the intestinal lumen of one segment while the same amount of emulsion of Lipiodol was injected in the other segment. Samples of blood were collected at 15 min intervals during 105 min in the right mesenteric vein of each segment. Biopsies of the intestinal mucosa were performed periodically at the edges of both intestinal segments until 60 min in order to examine the iodine content at different sites of the mucosa.

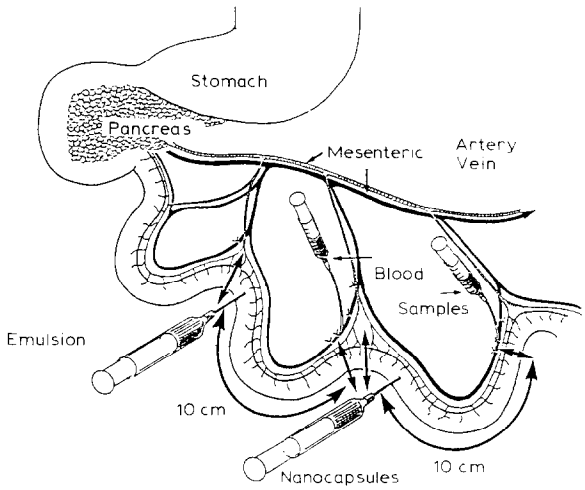

Fig. 1. Experimental procedure.

Electron scan and microanalytical analysis

Blood samples were centrifuged at 3000 *g* for 20 min. A drop of plasma was placed on a plastic cover slip, air-dried and carbon-coated before X-ray analysis (Schreiber and Wims, 1981). Samples of small intestinal mucosa were fixed in 0.2 M cacodylate-buffered 2% glutaraldehyde, pH 7.4 at 4°C. They were then dehydrated and embedded in paraffin. Sections of 5 μm thick were mounted onto amorphous cover slips, carbon-coated and analysed in a scanning electron microscope fitted with an energy-dispersive X-ray analyzer (Moreton, 1981). The analysis conditions were 20 kV and 40 μA from 20 to 1 μm of spot size. Data were acquired for 100 s on each area, analysed 10 times.

Results

Characterization of nanocapsules

Analysed by laser light scattering, the suspen-

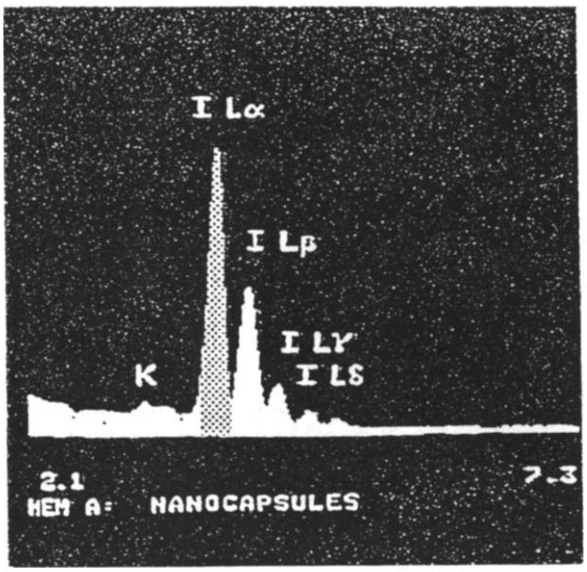

Fig. 2. X-ray analysis of a Lipiodol nanocapsule indicating the different L-lines (α, β, γ, δ) of iodine.

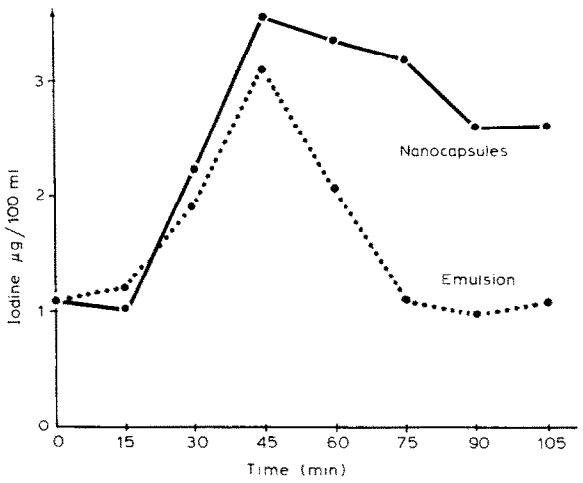

Fig. 3. Plasma iodine concentration after intrajejunal administration of Lipiodol emulsion and nanocapsules.

sion of nanocapsules showed an homogeneous distribution of particles, the mean size being 165 nm (S.D. 46 nm). The emulsion of Lipiodol was composed of droplets of 258 nm (S.D. 71 nm) in mean size.

Observed in a scanning electron microscope, nanocapsules of Lipiodol consisted of spheric, homogeneous smooth particles which can be characterized by their strong X-ray emission of iodine as illustrated in Fig. 2. Indeed, the 4 L-lines of X-ray emission of iodine (α, β, γ, δ) can be easily distinguished.

Intestinal absorption of encapsulated and non-encapsulated Lipiodol

After the administration of a 1-ml emulsion of Lipiodol in the jejunal lumen, iodine plasma level increased from the 15th minute in the mesenteric vein and reached its maximal level (3 times the basal level) after 45 min. Further, iodemia decreased again and reached basal values after 75 min (Fig. 3). When the same amount of encapsulated Lipiodol was administered in the jejunal lumen, the plasma iodine level increased also progressively during the first 45 min, reaching 3.5 times the basal value at this period. However, iodemia remained elevated until 105 min and did not return to basal values.

In order to define further the pathway of

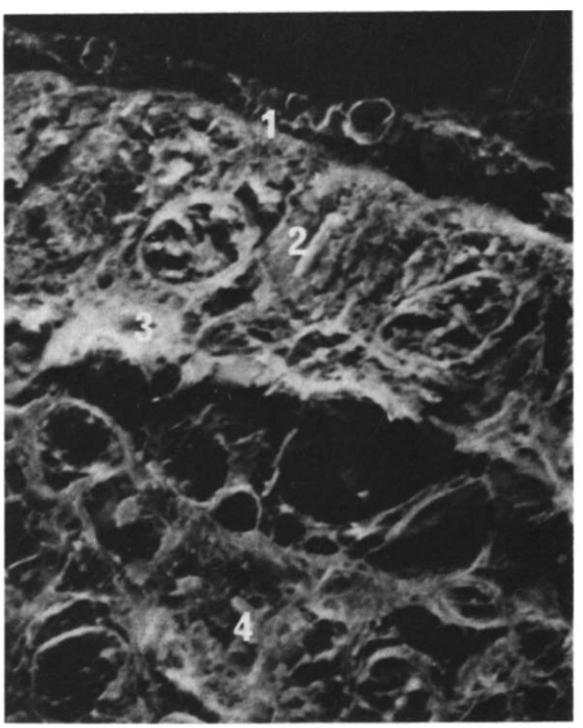

Fig. 4. Scanning electron microscopy of a section through the intestinal mucosa indicating the various sites of microanalysis. 1 = microvilli; 2 = apex of the absorptive cell; 3 = base of the absorptive cell; 4 = lamina propria. (×2500).

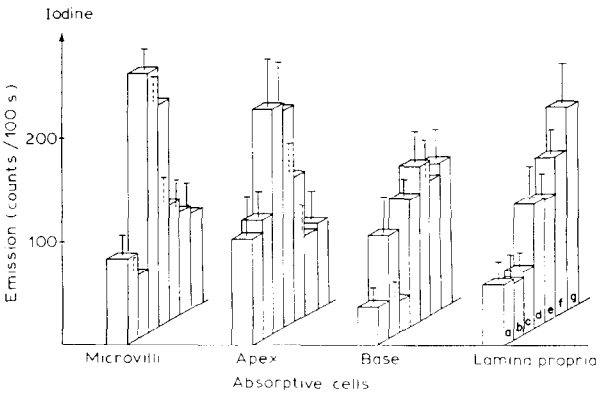

Fig. 5. Effect of an intrajejunal administration of Lipiodol emulsion on the X-ray emission of iodine at various sites of the intestinal mucosa as a function of time (a = 2 min 30 s; b = 5 min; c = 7 min 30 s; d = 10 min; e = 15 min; f = 30 min; g = 60 min).

Lipiodol from the intestinal lumen to the mesenteric blood, we analysed, on 5-μm-thick sections of the intestinal mucosa, the content in iodine at following sites: (1) in microvilli which represent the luminal part of absorptive cells, (2) in the apical and basal part of these cells and (3) in the axis of intestinal villi (lamina propria) composed of connective, vascular tissues and lymph ducts (Fig. 4). The content in iodine in these different tissue compartments is represented in Figs. 5 and 6.

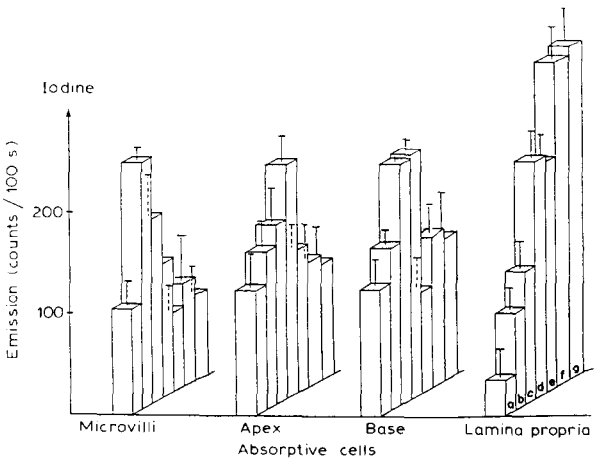

Fig. 6. Effect of an intrajejunal administration of Lipiodol nanocapsules on the X-ray emission of iodine at various sites of the intestinal mucosa in function of time (a = 2 min 30 s; b = 5 min; c = 7 min 30 s; d = 10 min; e = 15 min; f = 30 min; g = 60 min).

After intraluminal injection of the emulsion of Lipiodol (Fig. 5), iodine penetrated very fastly in the absorptive cell, its maximal concentration in microvilli being attained within 7.30 min. Then, the tracer moved in the cell and reached its basal part 10 min after the administration. Finally, iodine was accumulated in the lamina propria where its maximal concentration could be noted after 60 min.

After intraluminal administration of nanocapsules of Lipiodol, the kinetics of absorption of iodine were accelerated (Fig. 6); the maximal concentration of the tracer was observed from 5 min in microvilli and since 7.30 min in the basal part of the absorptive cell. In the lamina propria, it was two-fold higher than after the administration of Lipiodol emulsion and was maximal at 10 min.

Thus it appeared that nanocapsules of poly-isobutylcyanoacrylate accelerate and intensify the passage of Lipiodol across the intestinal mucosa.

Discussion

This study indicates for the first time the advantage of the use of a colloidal polymeric drug delivery system in the intestinal absorption of a lipophilic tracer, Lipiodol, an iodized oil. Generally, it is admitted that lipids are solubilized by biliary salts in the intestinal lumen after the action of pancreatic lipase on the emulsion. The so-formed micelles move toward the microvilli where absorption occurs by transmembrane diffusion. Thus the size of lipid droplets does not interact with the rate of absorption. In the absorptive cell, lipids are transported to the Golgi complex via the endoplasmic reticulum. Thus chylomicrons are formed and excreted by the cell at the level of the basolateral membranes; then they gain in part the lymph ducts and in part the blood stream. The jejunal absorption of the emulsion of Lipiodol seems to follow this scheme (Cardell et al, 1967; Gangl and Ockner, 1975). Indeed, the concentration–time profile of iodine at different sites of the intestinal mucosa shows the passage of iodine from microvilli to the apical, then the basal part of

the absorptive cell and, finally, to the lamina propria. If it is assumed that iodine is absorbed together with lipids on which it is fixed, the profile of iodemia in the mesenteric vein and the kinetics of iodine in the intestinal mucosa reflect the absorption of Lipiodol.

Encapsulation of Lipiodol in poly-isobutylcyanoacrylate nanocapsules improved markedly this process. In blood, the maximal level of plasma iodine was slightly enhanced and remained elevated during the whole experiment while it returned to basal values after administration of non-encapsulated Lipiodol. This suggests that nanocapsules intensify the absorption of Lipiodol and prolong it markedly. Moreover, a more rapid passage of iodine through the absorptive cell has been found; this passage was also more intense since iodine concentration doubled in the lamina propria.

These results could be explained by two mechanisms. At first, nanocapsules could prolong the duration of contact between Lipiodol and the microvilli, the first step of intestinal absorption. Secondly, nanocapsules could also transport the drug through the intestinal mucosa.

The analysis by laser light scattering of the size and distribution of nanocapsules showed that this polymeric carrier consists of spheric, homogeneous capsules, 100–300 nm in diameter. It seems less probable that these nanocapsules could pass through the apical cell membrane even by pinocytosis. Indeed, ultra-thin sections of jejunum after intraluminal administration of nanocapsules of Lipiodol never showed these nanocapsules in the cytoplasm nor in another subcellular compartment of absorptive cells. However, nanocapsules have been observed in intercellular spaces of the mucosa (unpublished data) suggesting a paracellular pathway for absorption.

In conclusion, it is suggested that nanocapsules increase and prolong the absorption of Lipiodol (1) by increasing the intraluminal concentration of the drug close to absorptive cells and (2) by carrying the drug directly from the intestinal lumen to the mesenteric blood, most probably via a paracellular pathway.

Acknowledgements

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References

Al Khouri Fallouh, N., Roblot-Treupel, L., Fessi, H., Devissaguet, J.P. and Puisieux, F., Development of a new process for the manufacture of polyisobutylcyanoacrylate nanocapsules. *Int. J. Pharm.*, 28 (1986) 125–132.

Brasseur, F., Couvreur, P., Kante B., Deckers-Passau, L., Roland, M., Deckers, C. and Speiser, P., Actinomycin D adsorbed on polymethylcyanoacrylate nanoparticles: increased efficiency against an experimental tumor. *Eur. J. Cancer*, 16 (1980) 1441–1445.

Cardell Jr, R.R., Badenhausen, S. and Porter, K.P., Intestinal triglyceride absorption in the rat. An electron microscopical study. *J. Cell Biol.*, 34 (1967) 123–155.

Couvreur, P., Kante, B., Roland, M., Guiot, P., Baudhuin, P. and Speiser, P., Polycyanoacrylate nanocapsules as potential lysosomotropic carriers: preparation, morphological and sorptive properties. *J. Pharm. Pharmacol.*, 31 (1979) 331–332.

Couvreur, P., Kante, B., Grislain, L., Roland, M. and Speiser, P., Toxicity of polyalkylcyanoacrylate nanoparticles. II. Doxorubicin loaded nanoparticles. *J. Pharm. Sci.*, 71 (1982) 790–792.

Gangl, A. and Ockner, R.K., Intestinal metabolism of lipids and lipoproteins. *Gastroenterology*, 68 (1975) 167–186.

Grislain, L., Couvreur, P., Lenaerts, V., Roland, M., Deprez-Decampaneere, D. and Speiser, P., Pharmacokinetics and distribution of a biodegradable drug-carrier. *Int. J. Pharm.*, 15 (1983) 335–345.

Lenaerts, V., Couvreur, P., Christiaens-Leyh, D., Joiris, E., Roland, M., Rollman, B. and Speiser, P., Degradation of poly (isobutylcyanoacrylate) nanoparticles. *Biomaterials*, 5 (1984) 65–68.

Leonard, F., Kulkarni, R.K., Brandes, G., Nelson, J. and Cameron J., Synthesis and degradation of poly (alkylcyanoacrylates). *J. Appl. Polym. Sci.*, 19 (1966) 259–272.

Moreton, R.B., Electron-probe X-ray microanalysis: techniques and recent applications in biology. *Biol. Rev.*, 56 (1981) 409–461.

Schreiber, T.P. and Wims, A.M., A quantitative X-ray microanalysis thin film method using K,L,M-lines. *Ultramicroscopy*, 6 (1981) 323–334.

Vermers, M., Doppman, J.L., Sugarbaker, P., Fisher, R.J. Chatterji, D.C., Luetzerler, J., Grimer, G., Girton, M. and Adamson, R.H., Clinical trials with a new intravenous liposoluble contrast material for computed tomography of the liver and spleen. *Radiology*, 137 (1980) 217–222.