Gastrointestinal Transit and Mucoadhesion of Colloidal Suspensions of *Lycopersicon esculentum L.* and *Lotus tetragonolobus* Lectin-PLA Microsphere Conjugates in Rats

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Received January 26, 2001; accepted March 7, 2001

Purpose. To investigate *in vivo* the fate and the behavior of lectin-particle conjugates after oral administration.
Methods. Two plant lectins were selected, namely *Lycopersicon esculentum L.* and *Lotus tetragonolobus* lectins, which have been reported to be specific for oligomers of *N*-acetyl-D-glucosamine and L-fucose, respectively, and conjugated to small poly(lactide) microspheres. Their intestinal transit was investigated in detail using radiolabeled particles. The transport and the distribution of the particles along the intestine, as well as their interactions with the intestinal mucosa, were determined after oral administration in rat.
Results. The overall transit of the particles was shown to be strongly delayed when the microspheres were conjugated to the lectins, mainly due to the gastric retention of the particles. A significant fraction of the conjugates adhered to the gastric and intestinal mucosae. No significant differences were observed after a preliminary incubation of lectin-microsphere conjugates with specific sugars.
Conclusion. Although specific interactions could not be excluded, especially in the stomach, it was likely that adhesion was predominantly due to nonspecific interactions. These results could be attributed both to unfavorable physicochemical characteristics of the conjugates and to premature adsorption of soluble mucin glycoproteins, preventing any further specific adhesion.

KEY WORDS: oral route; mucoadhesion; gastrointestinal transit; lectin-microsphere conjugates; *Lycopersicon esculentum L.*; *Lotus tetragonolobus* lectin.

INTRODUCTION

Numerous pharmacologically active molecules are poorly available when administered via the oral route. To circumvent this problem, it has been proposed to associate these drugs to bioadhesive dosage forms. The immobilization of the drug on the surface of the intestine for a prolonged period of time would allow simultaneously (1) the following: (i) an increase in the time interval available for drug absorption; (ii) a localized increase in the concentration gradient between the luminal and the serosal sides of the intestinal membrane; and (iii) possibly a localization and retention of the dosage form in a given area of the gastrointestinal tract specifically favorable to the absorption of a given drug. However, the complexity of the gastrointestinal tract makes the design of such dosage forms not an easy task.

In this context, due to their high specific surface, colloidal polymeric particulate systems (or small particles in the micrometer range in size) are of interest because the development of many interactions with the mucosal surface can be expected during their transit. The immobilization of the particles at the intestinal surface can be based either on the development of non-specific or specific interactions. On one hand, the intensity and the fraction of particles interacting would depend on the physico-chemical properties of the intestinal and the polymeric surfaces which are brought into contact (2,3). In this respect, different pharmacokinetic improvements (4–7) have been reported in animals when various poorly available drugs were associated to polymeric colloidal systems, including poly(alkylcyanoacrylate) and poly(lactide) (PLA). On the other hand, a biomimetic approach based on the use of specific ligands, such as lectins (8) or vitamin B12 (9) has been proposed. Lectins are proteins that bind specifically to sugars and, therefore, agglutinate cells, polysaccharides, and glycoconjugates. These sugars, which are present in the glycoproteins and the glycolipids of mammalian mucosae, either at the surface of the enterocytes or in the mucus layer, may act as receptors for lectins (10).

Attempts have been made to graft such ligands at the surface of polymeric nano- or micro-particles or polymers (11–14) to develop specific interactions with specific molecular structures existing at the intestinal surface. In this context, the aim of the present work was to investigate *in vivo* the fate and the behavior of lectin-particle conjugates after oral administration. Two plant lectins were selected, namely *Lycopersicon esculentum L.* and *Lotus tetragonolobus* lectin, specific for oligomers of *N*-acetyl-D-glucosamine and L-fucose, respectively, and conjugated to small PLA microspheres. The former has been extensively studied by several authors and was reported to be nontoxic and resistant to digestion in gastrointestinal tract (15). Additionally, to evaluate the influence of the nature of the sugar on microsphere-mucosa interactions, *Lotus tetragonolobus* lectin was selected. Their intestinal transit was investigated in detail using radiolabeled particles. The transport and the distribution of the particles along the intestine, as well as their interactions with the intestinal mucosa, were determined after oral administration in rat.

MATERIALS AND METHODS

Materials

Poly(D,L-lactide) (PLA) Resomer® 206 was supplied by Boehringer Ingelheim (Germany). Poly(vinyl alcohol) (PVA) (Mowiol 4-88) was a gift from Hoechst (Paris, France). *Lotus tetragonolobus* lectin (LT), *Lycopersicon esculentum L.* lectin (LE), α-L-fucose, chitin, glutaraldehyde (25% aqueous solution), and crude pig gastric mucin were purchased from Sigma (St Quentin Fallavier, France). Oleic acid 1-^{14}C was purchased from NEN Life Science Products (Paris, France). Na^{125}I was supplied by Amersham (France). Hionic-Fluor® and Ultima-Gold® (Packard, Rungis, France) were used as scintillating cocktails. Tissues were solubilized with Soluene-

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350® (Packard, Rungis, France). Ultrapure water was obtained from a Millipore purification system (Milli-Q plus, Millipore, St Quentin en Yvelines, France). Other chemicals were analytical grade.

Methods

Preparation of Radiolabeled PLA Microspheres

Radiolabeled PLA microspheres were prepared by encapsulating 1-^{14}C oleic acid by a solvent emulsification/evaporation technique. Briefly, the commercially available ethanolic solution of 1-^{14}C oleic acid (1,850 kBq) was evaporated under nitrogen. The residue was then dissolved in 1 ml of dichloromethane containing 52 mg of PLA. Further, this organic phase was added to 20 ml of a an aqueous solution of PVA (5% w/v) and emulsified with a mechanical stirrer (Ultra-turrax, Janke and Kunkel IKA Labortechnic, Germany) operated at 24,000 rpm for 3 min. The emulsion was stirred on a magnetic stirrer. Finally, the microspheres were centrifuged (3,300 g, 15 min) and washed six times with 40 ml of water and resuspended in NaCl 0.9% before use. The specific activity of the microspheres was determined on an aliquot of the suspension.

The weight yield of the preparation was determined by weighing the lyophilization residue of a 1.5-ml aliquot of a suspension obtained after preparing nonradiolabeled microspheres with cold oleic acid accordingly to the same protocol. The size distribution was also determined on this preparation by an electrical sensing method (Coulter Multisizer II, Coultronics, Margency, France). The mean diameter of unmodified-PLA microspheres was of 1.6 μm (90% of the particles were under 4.4 μm).

Preparation of Lectin-Poly(Lactide) Microsphere Conjugates

Lectins were covalently bound to the hydroxyl groups of the PVA chains on the surface of the particles.

Activation of Radiolabeled Microspheres. Fifty milligrams of particles were washed in milli-Q water by centrifugation (3,300 g, 10 min). The pellet was resuspended by vortexing in 750 μl of water to which was added 1 ml of glutaraldehyde and 250 μl of H_2SO_4 0.3 M (16). The mixture was then shaken gently for 1 h at 30°C to activate the hydroxyl groups.

Fixation of Lectins. Once the microspheres were activated, the suspension was centrifuged to remove unreacted glutaraldehyde and further washed 4 times in phosphate buffer saline (PBS; 10 mM, pH 7.4) to remove any remaining traces of glutaraldehyde which might otherwise cross-link the lectin molecules. Then, 900 μl of PBS containing 250 μg of lectin (LT or LE) were added and the linkage was made by incubation overnight at room temperature. The conjugates were centrifuged to remove free lectin and incubated 1 h with 1 ml of ethanolamine (0.1 M) to block unreacted groups on the particles. The ethanolamine was eliminated and the microspheres were washed by three centrifugations. The lectin-microsphere conjugates were finally resuspended in 1 ml of PBS and stored at 4°C.

Determination of the Amount of Fixed Lectins. The amount of lectin bound to the microspheres was calculated as the difference between the lectin added initially and the lectin recovered in solution after incubation with the microspheres. The amount of lectins in the supernatant were determined by the Lowry method, using a commercialized test kit (Bio Rad protein assay, Ivry sur Seine, France) and using bovine serum albumin (BSA) for calibration.

In Vitro *Activity and Specificity of the Conjugates.* Pig gastric mucin was used for determining the activity *in vitro* of the grafted lectins. Mucin was radiolabeled with ^{125}I by the chloramine T method. A solution of mucin (1 mg/ml in PBS 10 mM, pH 7.4) was prepared and filtered (Low proteic adsorption filters, porosity 0.45 micron) before use. One-hundred microliters of a solution containing 0.5 mCi of Na^{125}I and 20 μg of chloramine T was added to 100 μl of mucin solution, and incubated 1 min. Radiolabeled mucin was then separated from the excess of free iodine on a G10 sephadex column. The radiolabeled mucin was recovered in two fractions of 500 μl.

Thirty microliters of ^{125}I mucin (approximately 2.4 μg) were incubated for 60 min with aliquots of the conjugates corresponding to 40 μg of bound lectins (200 μl). Free ^{125}I mucin was eliminated by centrifuging the microspheres three times (7,950 g, 4 min). Finally, the radioactivity of the microspheres was determined with a gamma counter.

The preservation of the specificity of the lectins after coupling was determined by incubating the conjugates in ^{125}I mucin and in the presence of the lectin-specific sugars. α-L-Fucose (200 μl, 50 mM) and a saturated solution of chitin (a polymer of *N*-acetyl-D-glucosamine) were used for blocking the LT and LE lectins, respectively. Non-conjugated microspheres were used as controls.

Oral Administration in Rats

Radiolabeled microsphere suspensions were administered by intragastric gavage (cannula, Nemco, France) to male Wistar rats (mean weight 220 g) (Charles River, France). The rats were stabilized in metabolic cages and fasted for 16 h before administration. They had free access to water. During this time, it was possible that the rats ate their feces. Because the presence of material in the stomach could modify the particles transit, these rats were excluded from the study.

The rats were sacrificed 1 h after gavage. Taking into account the following, i) the fast transit of particles observed in previous experiments (2 h after oral administration, all PLA microspheres were present in the distal part of intestine [data not shown]) and ii) the mucus turnover, which has been estimated to be between 47 and 270 min (17), a short time (1 h) was chosen to give favorable conditions for observing the interactions of particles with gastrointestinal tract.

Administration of PLA Microspheres

Two-milliliter suspensions of PLA microspheres were administered to rats. The microsphere concentration was 1 mg/ml and corresponded to 59.2 kBq total activity.

Administration of Lectin-PLA Microsphere Conjugates

Two milliters of the different suspensions of lectin-microsphere conjugates (1 mg/ml and 59.2 kBq total activity) were administered to rats. Additionally, control suspensions were prepared by incubating the conjugates with their specific

sugars to block their lectin activity. LT-PLA microsphere conjugates were incubated overnight with a 50 mM solution of α-L-fucose and, similarly, the LE-PLA microsphere conjugates were incubated overnight with a saturated solution of chitin.

Distribution of the Microspheres in the Body and the Gastrointestinal Tract

The abdominal cavity was immediately opened, and 2 ml of blood was taken from the portal vein. It was assumed that total blood volume was equivalent to 6.4% of the body weight. The liver, spleen, lungs, and gastrointestinal tract were removed. Urine was collected 1 h after administration, and there were no feces. The intestine was cut in 10 or 11 segments (10 cm approximately), which were opened lengthwise along the mesentery. They were rinsed carefully with 25 to 40 ml of 0.9% NaCl to eliminate the nonadherent particles. Rinsing liquids were collected and counted by liquid scintillation.

The opened mucosal segments were cut into 2-cm pieces. The Peyer's patches were separated out. The tissues were digested overnight in 1 ml of Soluene-350® at 50°C. The stomach and the caecum were divided into six pieces and digested in 2 ml of Soluene-350® at 50°C. The rinsing solutions from stomach, intestine, and caecum were centrifuged. Accurately weighed pellets (0.5 ml) were mixed with 1 ml of Soluene-350® and digested overnight at 50°C. The rest of the organs (liver, spleen, lungs) were weighed, and two aliquots weighing 60 mg were dissolved in 1 ml of Soluene-350® at 50°C overnight. Two aliquots of 100 μl of blood were added to 1 ml of a mixture of Soluene-350®/isopropanol (1/1) at 50°C for 30 min.

After digestion, the samples were discolored with 0.2–0.4 ml of a 30% H_2O_2 solution and mixed with 10 ml of a scintillating cocktail (Hionic-Fluor®, Packard, France). Two aliquots (0.5 ml) of the urine were directly counted after mixing with 10 ml of a scintillating cocktail (Ultima-Gold®, Packard, France). Samples were counted on a Liquid scintillation counter (Model LS 6000 TA, Beckman, France).

Gastric-Emptying Ratio

A gastric-emptying ratio was calculated for any experimental suspensions by dividing the total radioactivity (luminal and adherent particles) recovered in the entire intestine, including caecum and colon, by the total radioactivity recovered in the stomach plus the entire intestine.

Intestinal Distribution Profiles

Different transit profiles, including the overall, the luminal content, and the mucosal adhering-particles profiles, were built up from the radioactivity counting. These profiles were expressed either as differential or cumulated distributions of the radioactivity as a function of the gastrointestinal or intestinal length. The lengths arbitrarily attributed to the duodenum, the jejunum, and the ileum were 10, 35, and 55–65 cm, respectively. The profiles were characterized by their geometric center, which corresponded to the arithmetic mean of the distribution (18,19), calculated as follows:

$$\text{geometric center} = \Sigma\, R_i \cdot l_i / \Sigma\, R_i$$

where R_i and l_i stand for the radioactivity in the i^{th} intestinal segment and l_i for the median length of the i^{th} intestinal segment.

Data Analyses

The statistical significance was tested by Student's *t* test.

RESULTS

PLA Microsphere and Lectin-PLA Microsphere Conjugates

The mean diameter of the lectin-conjugated radiolabelled PLA microspheres was 3.9 and 5.8 μm for the LT- and LE-conjugates, respectively (90% of the particles were under 10.6 μm and 9.5 μm, respectively). These conjugates were obtained with a sufficiently high specific activity (31.3 kBq/mg). The leaching of radioactivity was evaluated on a suspension of PLA microspheres and was shown to be less than 2% after 2 months at 4°C.

The main characteristics of lectin-PLA microspheres are presented in Table I. The amount of fixed lectin was independent of the nature of lectin and was around 1 mg/m^2 for both lectins.

To evaluate the activity of fixed lectins, the affinity of the conjugates for pig gastric mucin (PGM) was measured. The conjugates showed 4-fold more interactions with PGM than the non-conjugated particles. A reduction (approximately of 45%) of the interactions of the conjugates with PGM was observed when the test was performed in presence of the corresponding specific competing sugars.

Biodistribution of PLA Microspheres and Lectin-PLA Microsphere Conjugates

The distribution of the different lectin-PLA microsphere conjugates has been determined 1 h after oral dosing (Table II). About 98% of the recovered radioactivity was localized in the gastrointestinal tract. The analysis of the gastrointestinal content showed that the amount of radioactivity due to particle adhesion on the whole mucosal surface, including stomach, small intestine, caecum, and colon, ranged between 28

Table I. Characteristics of Lectin-PLA Microspheres: Amounts of Lectin Associated to Particles and Activity of Conjugated Lectins

Lectin	Lectin associated to microspheres (μg of lectin/mg of particles)[a]	Interaction of conjugates with mucin[b]	Interaction of conjugates with mucin in presence of specific sugars[b]
LT	3.0 ± 0.8	4.5 ± 0.1	2.6 ± 0.2
LE	4.0 ± 0.2	3.8 ± 0.3	2.0 ± 0.9

Note: The activity was determined by measure of the interactions of the conjugates with pig gastric mucin in the absence or presence of specific competing sugars (α-L fucose and chitin, a polymer of N-acetyl-D-glucosamine to LT and LE respectively) (mean ± sd, n = 3).

[a] The amounts of lectin were expressed as BSA-equivalents.

[b] The data were normalized by reference to the non-conjugated PLA microspheres (by dividing the amount of the associated mucin to the conjugates by the one associated to the non-conjugated particles).

Table II. Body Distribution in Rat 1 h After Intragastric Administration (2 ml of a 1 mg/ml Particle Suspension) of ^{14}C-Labelled PLA Microspheres and Lectin-PLA Microspheres in the Presence or Absence of Their Specific Inhibiting Sugar (mean ± sd, n = 4)

	% of recovered radioactivity				
Organ	PLA	LT-PLA	LT-PLA/fucose	LE-PLA	LE-PLA/chitin
Stomach	1.8 ± 1.5	10.1 ± 2.5	15.5 ± 2.1	11.1 ± 7.9	17.8 ± 4.9
Small intestine	23.0 ± 9.0	20.1 ± 11.3	27.5 ± 6.4	22.9 ± 11.8	8.5 ± 2.4
Caecum	0.1 ± 0.0	0.1 ± 0.0	0.4 ± 0.3	0.1 ± 0.1	0.2 ± 0.1
Colon	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.0 ± 0.0
Lumen content	72.3 ± 10.0	67.0 ± 11.3	52.7 ± 5.7	62.6 ± 14.3	71.2 ± 6.4
Faeces	—	—	—	—	—
Blood	0.6 ± 0.3	0.4 ± 0.1	1.1 ± 0.7	0.9 ± 0.5	1.0 ± 0.4
Liver	1.3 ± 0.4	0.6 ± 0.1	0.7 ± 0.0	0.9 ± 0.4	0.8 ± 0.2
Spleen	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Lung	0.1 ± 0.0	0.9 ± 1.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Urine	0.0 ± 0.0	0.0 ± 0.0	—	0.2 ± 0.3	0.0 ± 0.0
Peyer's patches	1.3 ± 1.1	1.0 ± 0.4	2.2 ± 0.9	1.9 ± 0.9	0.6 ± 0.3

and 48% of the recovered radioactivity, whereas the rest of the particles remained in the fluids of the lumen. No feces were produced during the experiments.

Whatever the type of particles, only 2% of the radioactivity was recovered into other organs, including liver, spleen, lung, blood, and urine. Although Hussain *et al.* (13) have reported that *Lycopersicon esculentum* lectin-conjugation to polystyrene nanospheres (500 nm in diameter) resulted in a considerable enhancement in the overall uptake of particles after repeated administrations for 5 days, it was probably not surprising in the present case to observe no uptake enhancements only 1 h after the administration of lectin-conjugates or controls. The amounts of conjugates associated to the Peyer's patches, which have been suggested to be an important site for particle uptake processes (20), ranged from 0.59 to 2.2%, which was not significantly different from the control particles (1.26%). These values are comparable to the results described by Hodges *et al.* (21) for polystyrene microspheres (2 μm). These authors showed that 1.78% and 0.38% of particles were associated to the Peyer's patches 0.5 and 2 h after administration, respectively.

Gastrointestinal Distribution of PLA Microspheres and Lectin-PLA Microsphere Conjugates

From the radioactivity in the segments, overall distributions of the particles (including mucosae-bound and luminally unbound particles) along the stomach and the small intestine were obtained and further characterized by their geometric centers. This value indicates the location of the particles along the intestine. As shown in Figure 1, the geometric centers of the gastrointestinal distributions of the lectin conjugates (including stomach and intestine) were decreased by half when compared to control PLA microspheres, indicating that the particles remained in the upper part of the tract and that the transit of the conjugates could be strongly delayed.

Figure 2 presents in more detail an histogram representing the overall gastrointestinal distribution of the lectin-PLA microsphere conjugates and PLA control microspheres along the gastrointestinal tract. It was confirmed that the gastrointestinal distribution of the particles was completely modified by the conjugation of the particles to lectins, which delayed the overall transit. Gastrointestinal distributions of the conjugates were bimodal, 1 h after administration, 30–60% of the conjugates were still in the stomach and the rest in the lower part of the small intestine, whereas 91% of the control particles were present in the lower intestine. The high amount of conjugates in the stomach could be partly explained by an increase in the adhering fraction (10–18% and 1.8 % for the conjugates and the control, respectively). The amount of conjugates in the stomach was composed roughly of one-third of adhering particles and two-thirds of particles in the lumen. This latter fraction was surprisingly high, considering that unbound particles should have undergone rapid gastric emptying, as did control PLA microspheres. However, it should be remembered that the rinsing process may have increased artificially the unbound fractions and reduced the fraction adhering. As a consequence of the delayed transit, only 35–54% of the conjugates, compared to 91% for the unconjugated PLA microspheres, were recovered in the jejunum and ileum.

Finally, the distributions of the lectin conjugates were not modified by preliminary incubation of the LE-PLA and LT-PLA microsphere conjugates with chitin and L-fucose, respectively.

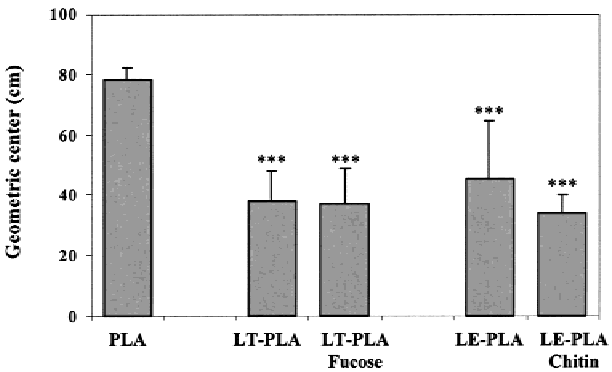

Fig. 1. Geometric centers of the overall distribution profiles (including mucosae bound and luminally unbound particles) of lectin-PLA microsphere conjugates or PLA microspheres in the stomach and the small intestine. (mean ± SD, n = 4). ***Significant difference from PLA (P = 0.1).

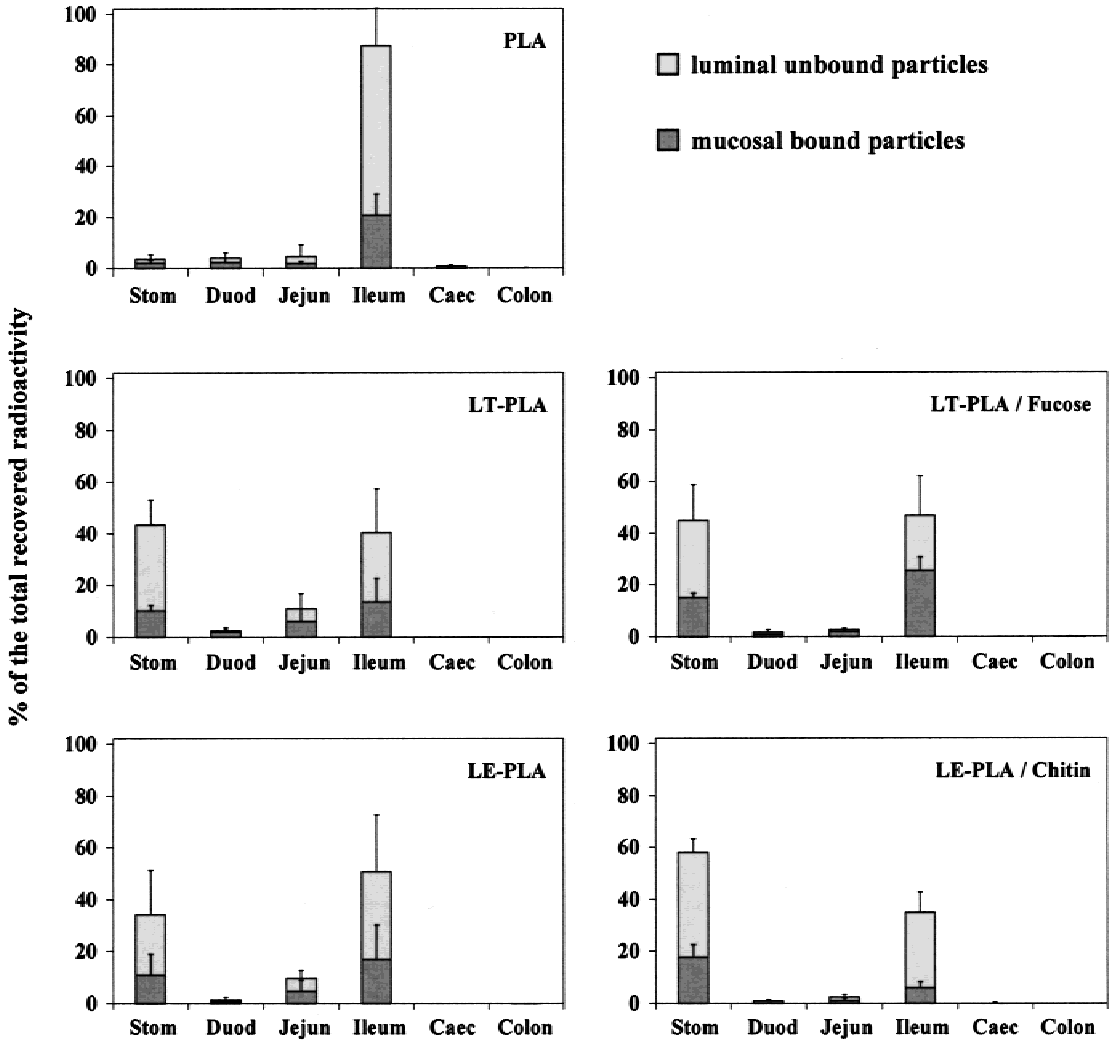

Fig. 2. Distribution profiles of lectin-PLA microspheres and control PLA microspheres along the gastrointestinal tract in rat 1 h after intragastric gavage (n = 4). No feces were produced during the experimental period. (Stom = stomach, Duod = duodenum, Jejun = jejunum, and Caec = caecum).

Gastric Emptying of PLA Microspheres and Lectin-PLA Microsphere Conjugates

The stomach emptying was described by a gastric emptying ratio for each type of particle 1 h after administration (Fig. 3). On one hand, the gastric emptying of the PLA microspheres was close to 100%, meaning that the emptying was almost complete for the non-conjugated particles. On the other hand, the ratio dropped to 40–65 % for the conjugates, corresponding to the presence of 35–60% of the conjugates in the stomach 1 h after administration. For each lectin-conjugate, the incubation of the conjugate in a solution of the inhibiting sugar had no influence on the emptying ratio.

Distribution of PLA Microspheres and Lectin-PLA Microsphere Conjugates in the Small Intestine

The distribution of PLA microspheres and lectin-PLA microsphere conjugates in the small intestine were determined respectively, for adhering particles, the non-adhering luminal particles and the total particles.

An example of histograms representing the distribution of small intestine-adhering LE-PLA microsphere conjugates (Fig. 4A) and the corresponding luminal content (Fig. 4B) is given. Figure 4 gives the intestinal distribution of the particles that have been emptied from the stomach during 1 h. As can be seen, the particles were spread irregularly along the intestine, although a set of pikes corresponding to the majority of the particles could be seen in the jejunum and ileum. Similar results were obtained by Lehr and Pusztai (22) for LE-conjugates. The existence of multiple pikes could probably be related to segmentation of the intestine occurring during the transit. The distribution of the LE-conjugates in the luminal content (Fig. 4B), which was less resolved (due to experimental difficulty), showed few pikes located in front of the zone of maximum adhesion observed for the intestinal tissue.

Due to the fact that intestinal lengths varied from rat to rat, an exact comparison of these distributions was performed by calculating their geometric centers. The geometric centers corresponding to the different distribution profiles, are summarized in Figure 5, not only for the adhering particles but also for the luminally contained particles and the total distri-

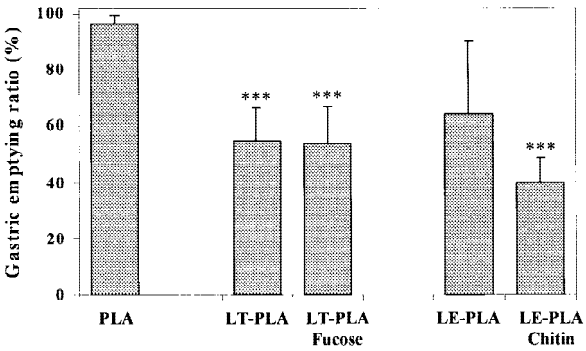

Fig. 3. Gastric emptying ratio of the different PLA microsphere conjugates and PLA microspheres calculated as the ratio between the total amount of radioactivity (adhering and luminal) in the intestine and the sum of the total radioactivity (adhering and luminal) in the stomach and the intestine, expressed as a percentage. (mean ± SD, n = 4). ***Significant difference from PLA ($P = 0.1$).

bution profiles. Depending on the lectin investigated, no or only moderate differences between the distributions were observed.

In the case of LT (Fig. 5A), the geometric centers of the total and luminal conjugated particles were slightly shifted back when compared to the non-conjugated PLA microspheres (shift: 20 cm), resulting in a similar shift in the distribution of adhering conjugated particles. Moreover, the incubation of the conjugates in α-L-fucose, which should have inhibited the LT lectin, had no effect on the distributions of the conjugates.

Similarly, in the case of LE (Fig. 5B), the intestinal distribution of the total conjugates was shifted back (shift: 15 cm), resulting in a similar shift in the distribution of adhering conjugated particles (shift: 20 cm). This shift was reversed by incubating the conjugates in a saturated chitin solution (a polymer of *N*-acetyl-D-glucosamine, specific for LE lectin), which may be attributed to a blocking of the lectin.

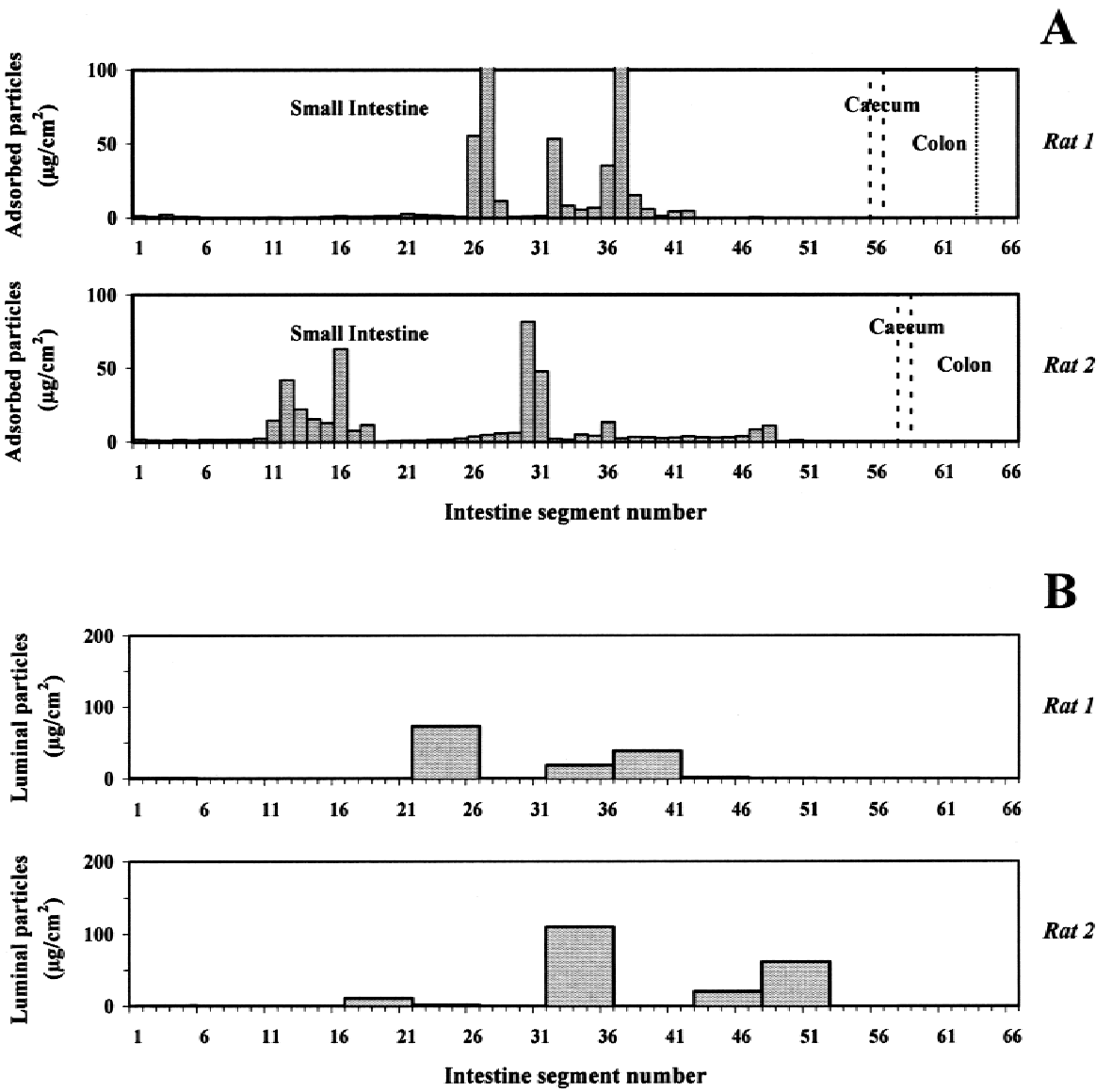

Fig. 4. (A) Bioadhesion profiles of *Lycopersicon esculentum* lectin-PLA microsphere conjugates along the small intestine 1 h after intragastric gavage in two rats. (B) Distribution profiles of *Lycopersicon esculentum* lectin-PLA microsphere conjugates in the lumen 1 h after intragastric gavage in two rats.

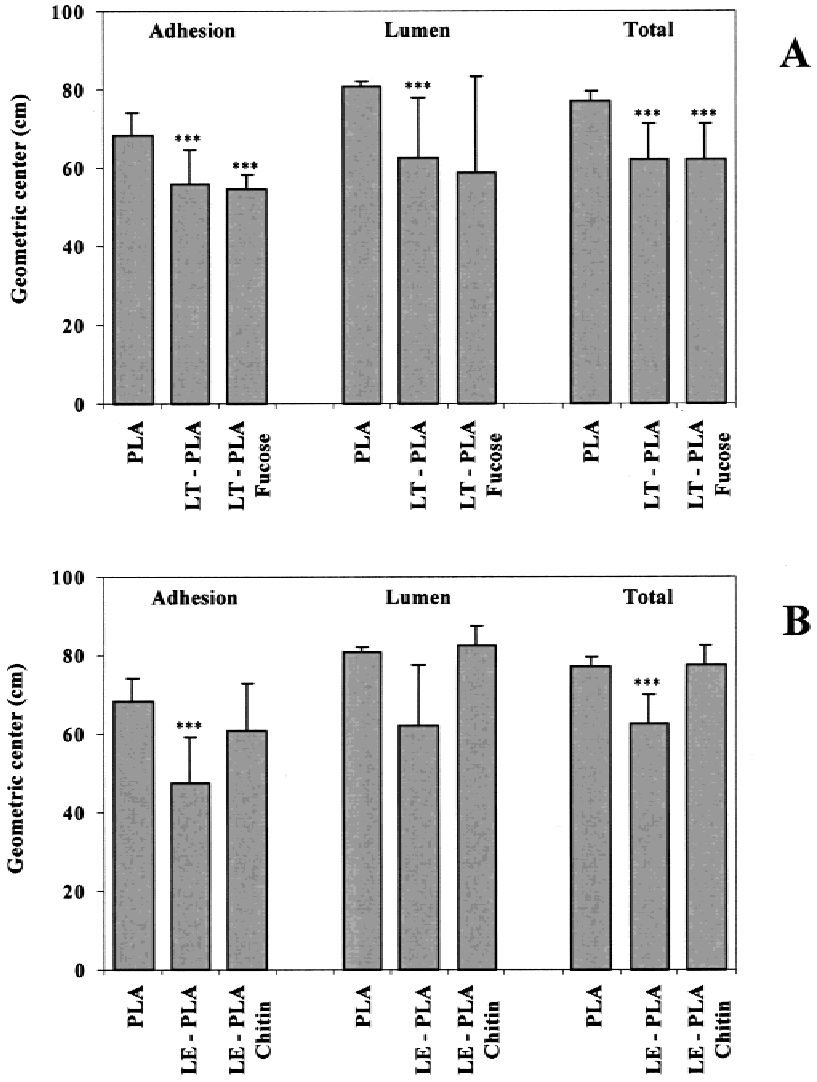

Fig. 5. Geometric centers of the distribution profiles for (i) adhering, (ii) luminal, and (iii) total amount of the following: (A) *Lotus tetragonolobus* lectin-PLA microsphere conjugates or PLA microspheres in the small intestine and (B) *Lycopersicon esculentum* lectin-PLA microsphere conjugates or PLA microspheres in the small intestine. ***Significant difference from PLA ($P = 0.1$).

DISCUSSION

It has been shown *in vitro* that the conjugation of lectins to bioadhesive drug delivery systems can (i) enhance the intensity of the interactions of such systems with the mucosal surfaces and (ii) increase their site specificity (23,24). Obviously, many variables in the alimentary canal can affect greatly the behavior of such systems after their oral administration. Therefore, it was of interest to examine in detail the fate of model particle-lectin conjugates *in vivo*. Small PLA microspheres were chosen as model particles for the preparation of conjugates. PLA was chosen, owing to its biodegradability, which would probably be a prerequisite for such systems, as it is now well documented that such particles are likely, at least to some extent, to be absorbed by the intestinal mucosa and are likely to accumulate in the lymphatics (25).

Bioadhesion and Gastrointestinal Transit of Lectin-PLA Microsphere Conjugates

The transit of solid matter in the gastrointestinal tract is the result of a series of complex events, depending simultaneously on physiological parameters and solid-matter characteristics. In the case of multiparticulate systems, the size and the density of the particles were shown to influence the transit (26). However, it is generally assumed that no interactions occur between the dosage form itself and the mucosal surfaces, and such interactions are generally neglected in transit descriptions. In the present case, the investigation of potentially bioadhesive lectin-microsphere conjugates was examined in detail, not only the overall distribution but also the pattern of their distribution in the gastrointestinal tract. This was achieved by determining simultaneously the spatial distribution of the radioactivity associated to the mucosal sur-

faces and that present in the luminal content after sacrifice of the animals.

The present work clearly shows that the overall transit of the microspheres was delayed by their conjugation to the LE and LT lectins. This delay was due to the adhesion and the retention in the lumen of a significant fraction of the particles in the stomach and the intestine. The emptying of the stomach depends not only on physiological characteristics but also on the dosage form itself. The gastric emptying of a single-unit nondisintegrating dosage form is an all-or-none process, whereas the emptying of multiunit formulations is more complex and variable. In the fasted state, the stomach generally shows minimal motor activity. Yuen *et al.* demonstrated that for small pellets, the emptying can occur after a lag time and that the emptying period was highly variable (27). In the present case, interactions with the stomach wall retarded considerably the gastric emptying.

The delay observed in the intestinal transit was more likely because of the progressive emptying of the stomach than to an increased intestinal adhesion of the conjugate compared to the control. The general behavior of the particles in the intestine could be compared to a chromatographic behavior for which a species in constant interaction equilibrium with the chromatographic substrate is progressively moving along the column. In the present case, the interactions of the conjugates with the intestine were clearly not sufficiently different from the controls to obtain significant transit delays at this level.

Interactions of Lectin-PLA Microsphere Conjugates with Mucosal Surfaces

Obviously, the conjugation of LE or LT lectins to PLA microspheres resulted in a slight increase in the fraction of adhering particles and, as a result, in a modification of the overall distribution of the particles along the gastrointestinal tract. From *in vitro* considerations, it was expected that adhesion of the conjugates should arise predominantly from specific interactions between the lectins and mucus or membrane-bound glycoproteins (23,28). Although some qualitative indications could be drawn from the differences between the distributions of the lectin-conjugates and the previously inhibited conjugates, the exact physicochemical nature of the interactions arising *in vivo* can not be deduced with certainty from this type of study because numerous factors (including kinetics factors, see above) are continuously modified *in vivo*.

Although lectin-conjugates were designed to develop specific binding to glycoproteins, it could not be excluded that the observed interactions with the mucosal surfaces and subsequent transit delays were due to nonspecific interactions. Clearly, the adhesion depended on the balance between nonspecific and specific interactions, which was created under *in vivo* conditions. On one hand, significant interactions of the conjugates with the stomach wall were seen after intragastric administration, contrary to the control particles, suggesting their stronger reactivity. However, the previously inhibited conjugates (prepared by incubation in their specific sugars) behaved very similarly to the noninhibited conjugates in the stomach. The only difference in transit, which was likely to be due to an inhibitory effect, was observed with the LE lectin conjugates in the intestine.

These trends may suggest that nonspecific interactions were predominant compared with specific interactions. Although the lectin-conjugates were shown *in vitro* to interact specifically with pig gastric mucin (Table I), the modification of the surface properties during the grafting procedure could favor non-specific adhesion under *in vivo* conditions. In this respect, it should be remembered that the hydrophilic surface of the PVA coated PLA microspheres, which was probably not favorable to adhesion, could be hydrophobized to some extent, (i) by the introduction of hydrophobic groups originating from the reaction of glutaraldehyde with hydroxyl groups at the surface of the microspheres and (ii) by the presence of the lectins, which are large proteins [MW = 75,000 and 54,000 for the LE and LT, respectively (11)] and as such would favor aggregative behaviors and finally adhesion. However, the development of specific interactions could not be excluded, as kinetic parameters could mask this effect under *in vivo* conditions. In this case, it could be hypothesized that a fraction of the lectin-conjugates interacted very rapidly with the gastric mucosa through specific interactions.

The interacting capacity and the nature of the interactions of the luminal pool of particles remained unknown. It was very likely that the luminal fraction of the particles that had not yet interacted with the mucosal surface could interact with soluble mucus glycoproteins, preventing any further specific adhesion of the particles in the intestine. A similar situation was probably encountered with the conjugates detached from the mucosa, due to mucus turnover, which were probably associated to detached mucus aggregates. The dissociation of these lectin-mucin complexes and the subsequent unmasking of the lectins, which would then be free for developing new interactions was unlikely, and may constitute a serious limitation for these systems.

In this situation, the previously inhibited-conjugates behaved similarly to the noninhibited conjugates, which could be attributed either to the dissociation of the lectin-sugar complex in the presence of mucus glycoproteins, leading to a subsequent unmasking of the lectin and interaction with mucus glycoproteins (adherent mucus or soluble mucus), or to a change in surface characteristics after sugar-lectin interactions, leading to predominant nonspecific interactions. A relatively low stability of the lectin-sugar complexes in digestive media, as well as a lower affinity of these lectins for free sugars compared with sugars incorporated in the glycan domains of mucin glycoproteins, cannot be excluded.

CONCLUSION

Obviously, the conjugation of lectins to PLA microspheres resulted in the adhesion of a significant fraction of the particles at the mucosal surface of the gastrointestinal tract. Such a capture by the mucous layer or the epithelial surface is potentially of interest not only for delaying the gastrointestinal transit but also for increasing particle absorption by the intestinal mucosa, and further improving drug delivery. However, this study demonstrated also the necessity of a better understanding of the interaction phenomena *in vivo,* as well as a need for the development of systems capable of adhering more efficiently to the intestinal mucosa, which obviously will require a better regulation of the specific interactions of the conjugates in the gastrointestinal tract.

ACKNOWLEDGMENTS

M.-J. Montisci had received a grant from the Ministère de la Recherche et de l'Enseignement Supérieur de la République Française, which enabled her to conduct this study.

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