Lymphatic Targeting of Polymeric Nanoparticles After Intraperitoneal Administration in Rats

Philippe Maincent,[1,6] Pierre Thouvenot,[2] Claude Amicabile,[3] Maurice Hoffman,[1] Jörg Kreuter,[4] Patrick Couvreur,[5] and Jean Philippe Devissaguet[5]

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Following intraperitoneal administration, the lymphatic targeting of polyacrylic nanoparticles has been evaluated in thoracic duct cannulated rats. The dosage forms administered consisted of carbon-14 polyhexylcyanoacrylate nanoparticles (PHCA) and polymethylmethacrylate (PMMA) nanoparticles. The carbon-14 concentrations were much higher in the excreted thoracic lymph than in the blood for both types of particles. The most dramatic results were found in the mediastinal nodes since the carbon-14 concentrations of rats receiving PHCA and PMMA nanoparticles by the ip route were 70- to more than 2000-fold higher than in the corresponding nodes of animals treated by the intravenous route. This potential lymphatic targeting could prove valuable in cancerology to treat tumors that metastasize in the peritoneal cavity or via lymphatic pathways such as colon carcinomas.

KEY WORDS: lymphatic targeting; intraperitoneal administration; polyacrylic nanoparticles; rats.

INTRODUCTION

After intravenous (iv) administration to animals or humans, colloidal drug carriers are taken up by the mononuclear phagocytic system (MPS), mainly by the fixed macrophages of the liver and the spleen. This common fate of most of the colloidal carriers may be attributed to passive targeting to the MPS after opsonization of the carriers. Consequently, it is difficult to modify the fate of drug carriers substantially unless different routes of administration such as oral (1), ocular (2), subcutaneous (3), or possibly other routes are chosen.

An elegant method for reaching the subdiaphragmatic system consists of injecting the carriers via the intraperitoneal (ip) route since liquids or cells, inside the abdominal cavity, are naturally drained to the diaphragm before being absorbed in the lymphatic capillaries. Then, these capillaries go through the mediastinal nodes before reaching the blood circulation.

Parker *et al.* (4) compared the administration of cytosine-β-D-arabinofuranoside in liposomes to an aqueous solution of the drug. Their results yielded, between 4 and 24 hr, an increase in the drug concentration in the renal and thoracic nodes as well as in the diaphragm. Hirano and Hunt (5) administered intraperitoneally ^{14}C-sucrose liposomes of four diameters (from 0.048 to 0.72 μm). These authors showed that the liposome size had almost no effect on the absorption of radioactivity. Indeed for each size there was an average of 50% of radioactivity still present in the abdominal cavity, i.e., not absorbed, after 5 hr. Nevertheless, the size of the liposomes has an important influence on their lymph concentration and on retention by the mediastinal nodes. For 0.048- and 0.17-μm liposome diameters, the percentages of the absorbed radioactivity found in the lymph were 26 and 22%, respectively. For the larger liposomes, i.e., 0.46 and 0.72 μm, the percentages of the absorbed radioactivity were much lower, namely, 6 and 1%, respectively. In contrast, the highest sucrose concentrations were found in the mediastinal nodes after administration of the largest liposomes (0.46 and 0.72 μm). Therefore, if mediastinal node targeting is desired, liposomes larger than 0.5 μm should be prepared.

Other particulate matter has been administered intraperitoneally in animals, including red blood cells (6), cells with a diameter up to 22 μm (7), or even microspheres of 30-μm diameter (8). The common fate of all this particulate matter involves capture by the mediastinal nodes.

In the colloidal drug carrier area, very few systems have been administered *in vivo* by the ip route (except for the liposomes already cited). Grislain (9) administered polyhexylcyanoacrylate nanoparticles in rats in order to study the toxicity of the carrier by this route. Edman and Sjöholm (10) administered intraperitoneally polyacrylamide nanoparticles incorporating L-asparaginase. They showed that the serum concentration of L-asparagine in rats was much lower in comparison to the free form of the drug. Furthermore, the administration of L-asparaginase polyacrylamide microspheres of 18- and 36-μm diameter led to nearly the same serum concentrations. However, hypertrophic nodes have been seen in the abdominal cavity with the microspheres.

The present work was undertaken in order to evaluate the targeting of the lymphatic system with colloidal carriers such as nanoparticles after ip administration. Taking into account the results obtained with liposomes (5), we selected nanoparticles with diameters larger than 0.5 μm. The dosage forms administered consisted of biodegradable polyhexylcyanoacrylate nanoparticles and non (or very slowly)-biodegradable polymethylmethacrylate nanoparticles. The nanoparticles were administered in the thoracic duct-cannulated rat model. In order to establish a comparison in the distribution of the particles, some rats received the preparations by the iv route.

MATERIALS AND METHODS

Materials

Polyhexyl-[3-^{14}C]cyanoacrylate (PHCA) nanoparticles

[1] Laboratoire de Pharmacie galénique et Biopharmacie, Faculté des Sciences Pharmaceutiques et Biologiques, BP 403, 5 Rue Lebrun, 54001 Nancy Cédex, France.
[2] Laboratoire de Médecine Nucléaire, CHRU Nancy Brabois, 54511 Vandoeuvre, France.
[3] Polyclinique d'Essey les Nancy, 54270 Essey les Nancy, France.
[4] Institut for Pharmaceutical Technology, J. W. Goethe University, Frankfurt, Germany.
[5] Laboratoire de Pharmacie galénique et Biopharmacie, Centre d'Etudes Pharmaceutiques, UA CNRS 1218, Rue Clément, 92290 Chatenay-Malabry, France.
[6] To whom correspondence should be addressed.

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were prepared according to a previously published method (11,12). The preparation of polymethyl-[2-^{14}C]methacrylate (PMMA) nanoparticles has been described by Kreuter *et al.* (13). The average diameters determined by photon correlation spectrometry (Malvern 4600, Malvern Instruments, Orsay, France) were 543 ± 150 nm and 1.4 ± 0.25 μm for PHCA and PMMA nanoparticles, respectively. Transmission electron microscopy (Philips EM 300) of the PMMA nanoparticles, however, revealed a particle size of 125 ± 30 nm.

Methods

Male Sprague–Dawley rats (300–400 g) were fasted for 24 hr before the experiment. Four hours before surgery, the rats received peanut oil (4 ml/kg) by gastric intubation in order to increase the lymph flow. The rats were then anesthetized by an intraperitoneal injection of ketamine chlorohydrate (100 mg/kg). They were positioned on a heating table (37°C) and systematically tracheotomized. The chest was opened and the left thoracic duct proximal to the jugulosubclavian junction was cannulated, using a heparin-filled Intramedic PE 10 cannula (Clay-Adams, France), according to a previously described method (14). In addition, the left jugulosubclavian junction was clamped in order to suppress the physiological pathway from lymph to blood. Then the right femoral vein was cannulated using a Vasculon cannula (Viggo Laboratories, Paris, France). The cannula allowed both for the intravenous administration of the nanoparticle preparations and for blood sampling. Further, before starting the experiment, the rats received an intravenous injection of heparin (100 IU/kg). After the intraperitoneal or intravenous administration of the nanoparticles, lymph was sampled continuously for intervals of 15 min, and 0.2 ml of blood was collected at the following times: 30, 90, 150, 210, and 240 min. After the withdrawal of each blood sample, the rats received intravenously 1 ml of 0.9% NaCl in order to compensate for fluid loss. At the end of the experimental time (4 hr), the rats were sacrificed and the abdominal cavity was rinsed with 20 ml of 0.9% NaCl. After a gentle massage of the peritoneal cavity, the sodium chloride solution was removed and the radioactivity found in the washing solution was measured. The radioactivity recovered from the peritoneal cavity was then substracted from the activity of the administered dose to allow determination of the dose actually absorbed (ΣD). The main organs (liver, spleen, kidneys, heart, lungs) and the main nodes (iliac, mesenteric, renal, and mediastinal) were removed, washed in 0.9% NaCl, blotted dry, and kept at −20°C until radioactivity measurement.

Dose Administered

In the case of PHCA nanoparticles, the radioactivity administered was 0.0185 MBq/100 g (300 μl/100 g, i.e., 0.227 mg of polymer/100 g of rat) and 0.006 MBq/100 g (100 μl/100 g, i.e., 0.075 mg of polymer/100 g of rat) for the intraperitoneal and the intravenous routes, respectively. For the PMMA nanoparticles, due to a higher specific activity, the doses were 0.037 MBq/100 g (100 μ1/100 g, i.e., 0.25 mg of polymer/100 g of rat) for the intraperitoneal route and 0.018 MBq/100 g (50 μl/100 g, i.e., 0.125 mg of polymer/100 g of rat) for the intravenous route.

Analytical Procedures

Carbon-14 radioactivity in lymph, blood, washings of the peritoneal cavity, organ samples after homogenization ($m \approx 50$ mg, $n = 3$), and nodes were quantified using a scintillation counter equipped with automatic quench correction (Beckman LS 1801, Paris, France). Blood (100 μl) was mixed with 1 ml of Protosol (Dupont, Paris, France), decolored with 200 μl of t-butyl hydroperoxide and mixed with 10 ml of scintillator (4 g 2,5-diphenyloxazol, 100 mg 2-2′-phenylenebis-4-methyl-5-phenyloxazol, 1 l toluene). The same procedure was applied to the lymph samples except for the decoloring process. Lymph nodes and organ tissues were mixed with 1.5 ml of Protosol, kept at 37°C for 6 hr, and then mixed with 10 ml of scintillator. All the samples were kept at +4°C for 12 hr before starting radioactivity quantification.

Statistical Analysis

Results are given as mean ± standard error of the mean (SE). Means were compared by the unpaired t test. Differences were considered significant at a level of $P < 0.05$.

RESULTS

Particle Size

The particle sizes were determined by transmission electron microscopy and photon correlation spectroscopy. In contrast to the PHCA nanoparticles, where the results obtained with the two methods correspond very well, a significant difference was observed between photon correlation spectroscopy (1.4 ± 0.25 μm) and electron microscopy (125 ± 30 nm) in the case of the PMMA nanoparticles. The reason for this discrepancy is probably the hydrophobic nature of the PMMA nanoparticles. As a consequence, the particles tend to aggregate in water or aqueous media without the use of detergents. The size of these nanoparticle aggregates was measured by photon correlation spectrometry, whereas electron microscopy enabled the size determination of single particles.

Disappearance from the Peritoneal Cavity (Global Recovery of Radioactivity)

The percentages of the administered dose recovered from the peritoneal washings were 22 ± 7% ($n = 7$) and 32 ± 5% ($n = 11$), respectively, for the rats receiving PHCA and PMMA nanoparticles. Therefore the doses actually absorbed (see Materials and Methods) were 78% (for PHCA nanoparticles) and 68% (for PMMA nanoparticles) of the administered radioactivity. Those values were used for both fluid concentration and tissue distribution calculations.

Lymph and Blood Concentrations

Figure 1 shows the lymph and blood concentrations of carbon-14 following the intraperitoneal administration of PHCA and PMMA nanoparticles. For both types of nanoparticles a lag time of about 30 min was observed before the radioactivity appeared in the lymph. After 2 hr of experiment, the concentrations reached a plateau. At this time lymph concentrations obtained with the PMMA nanoparti-

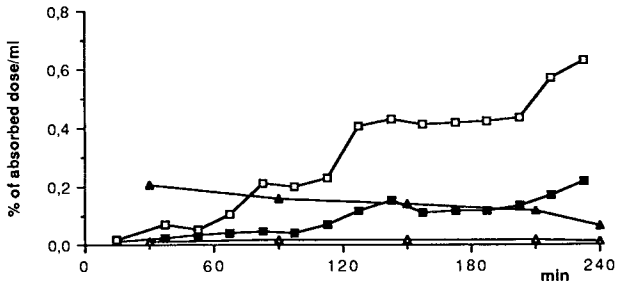

Fig. 1. Blood (triangles) and lymph (squares) carbon-14 concentrations in thoracic duct-cannulated rats following ip administration of PHCA (filled symbols) and PMMA (open symbols).

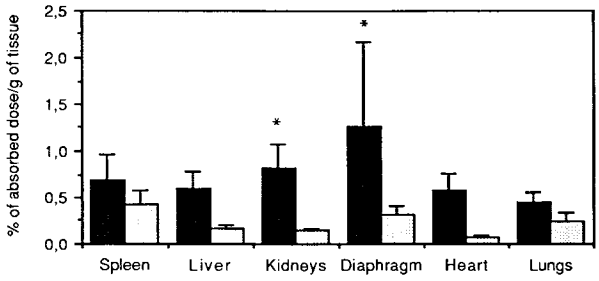

Fig. 3. Carbon-14 concentrations in the main organs of rats receiving PHCA (black) or PMMA (stippled) nanoparticles after ip administration. *Significantly different ($P < 0.05$).

cles were at least threefold higher than the corresponding PHCA concentrations. There was a major difference between the blood concentrations obtained with the two types of particles since the maximum carbon-14 concentration with PHCA nanoparticles was observed at the first blood sampling time (30 min); afterward the radioactivity slowly decreased. In contrast with PMMA nanoparticles, very little radioactivity appeared in the blood and there was no detectable peak during the experimental time.

Lymph carbon-14 activity increased with time after dosing, attaining the highest concentrations at 4 hr. At this time the experiment was terminated. The lymph concentrations were 0.5- to 2- or 5- to 20-fold higher than the corresponding blood levels at all times after dosing with PHCA or PMMA nanoparticles, respectively. The cumulated percentages of the absorbed dose which appeared in the lymph are presented in Fig. 2. It is obvious that the highest radioactivity amount is found in rats treated with PMMA nanoparticles, although the overall percentages are low (always less than 1%). The mean lymph flow ($n = 22$) was 0.87 ± 0.25 ml/hr, which is in good agreement with previous studies (4,5).

In the case of iv administration, the radioactivity in lymph was lower than after ip administration. The maximum amount recovered in lymph was 0.21 and 0.013% of the dose actually absorbed for the PHCA and PMMA nanoparticles, respectively.

Tissue Distribution Studies

The carbon-14 tissue distribution after intraperitoneal injection of the two types of nanoparticles is presented in Fig. 3. Except for the diaphragm and the kidneys, the differences in organ concentrations between PHCA and PMMA nanoparticles are not statistically different ($P < 0.05$). The general trend of this study is that the radioactivity concentrates in the organs to a higher degree in the case of PHCA nanoparticles. The tissue distribution results obtained after iv administration are not presented since only two animals were used with each type of particle. As generally observed with colloidal carriers, there was a major uptake in the MPS. After 4 hr, the mean carbon-14 accumulation in the liver was 56 and 89% and that in the spleen was 10 and 4% of the administered dose for PHCA and PMMA nanoparticles, respectively. The lymph node carbon-14 concentrations after ip and iv injection are summarized in Table I. After ip administration, for both PHCA and PMMA nanoparticles, the radioactivity concentrates mainly in the mediastinal nodes. There was almost no difference in the carbon-14 concentrations between the different types of nodes for rats receiving nanoparticles by the iv route. Concentration differences between each type of node were not dependent on the type of particle ($P < 0.05$). It is important to notice that carbon-14 concentrations in mediastinal nodes of rats receiving PHCA and PMMA nanoparticles by the ip route are 70- to more than 2000-fold higher than in the corresponding nodes of animals treated by the iv route: The corresponding figures for the three other types of lymph nodes range only between 0.2 and 15.

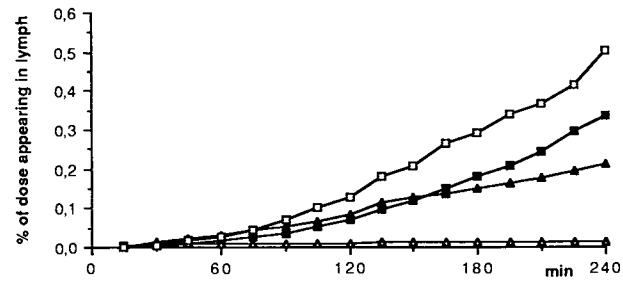

Fig. 2. Cumulated percentages of radioactivity in the thoracic lymph after ip (squares) or iv (triangles) administration of PHCA (filled symbols) or PMMA (open symbols) nanoparticles.

DISCUSSION

Targeting drugs linked to specific carriers has been the aim of most studies on colloidal carriers for the last 20 years. Yet *in vivo*, after iv administration of such carriers, including liposomes, it was almost impossible to target to organs and tissues other than those belonging to the MPS. By choosing different routes of administration, the goal of targeting to other organs may possibly be reached more easily. The present study demonstrates that, like liposomes, it is possible to target to the lymphatic system after ip administration of nanoparticles: indeed there was a dramatic difference between blood and lymph concentrations as well as in the organ distribution with regard to the administration route.

A common behavior with previous studies, i.e., a large MPS uptake, has been confirmed for PMMA and PHCA nanoparticles after iv administration (13,15). Previous distribution reports after iv administration described the poor lymphatic uptake of such particles (13).

After ip administration and in comparison to the iv re-

Table I. Carbon-14 Concentrations (% of Absorbed Dose/g) in Various Lymph Nodes of Rats Receiving PHCA and PMMA Nanoparticles by iv or ip Routes[a]

Treatment	Number of rats	Route	Mediastinal	Renal	Iliac	Mesenteric
PHCA	7	ip	118 (92)	33 (31)	0.3 (0.07)	0.5 (0.16)
PHCA	2	iv	1.7	2.2	0.5	2.6
PMMA	11	ip	226 (176)	9 (7.3)	0.3 (0.1)	1.8 (1.4)
PMMA	2	iv	0.08	0.1	0.06	0.1

[a] Values are means (SE).

sults, the carbon-14 concentrations in the lymph appeared much higher for the two types of particles, the major differences being found with the PMMA nanoparticles. However, the differences in the percentages of carbon-14 accumulated in the excreted thoracic lymph obtained with the two types of nanoparticles were not statistically significant ($P < 0.05$).

An interesting parameter to display the thoracic lymph targeting was the lymph/blood concentration ratio (L/B ratio). Therefore the time course of L/B ratios is shown in Fig. 4. With the two types of particles, after iv administration, the L/B ratios were lower than 1, which confirmed the lack of lymphatic targeting. After ip administration of PHCA nanoparticles, the L/B ratio was first lower than 1, was then equal to 1 at between 150 and 200 min, and was, finally, more than 1 after 200 min. This behavior reflects less intense targeting of the thoracic lymph with PHCA than with PMMA nanoparticles. Indeed, for PMMA nanoparticles, the L/B ratios were always greater than 1 (between 3.4 at 30 min and 35.3 at 240 min).

The lag time of about 30 min before radioactivity appears in the lymph should represent the time necessary for nanoparticles to arrive on the diaphragmatic surface and hence the beginning of lymphatic absorption. PHCA nanoparticles are rapidly biodegradable (15) and are sensitive to hydrolysis in an aqueous medium. Therefore, the peak of blood radioactivity as early as 30 min, observed with PHCA nanoparticles, could be due to the very fast absorption of hydrolysis products by the blood system. In the case of PMMA nanoparticles, the polymer is virtually undegradable over the short time periods covered in this study (3) and not sensitive to hydrolysis. The blood carbon-14 levels after ip administration of those particles were very low and the only significant absorption route in the abdominal cavity seems to be the lymphatic route. PMMA is a much more hydrophobic

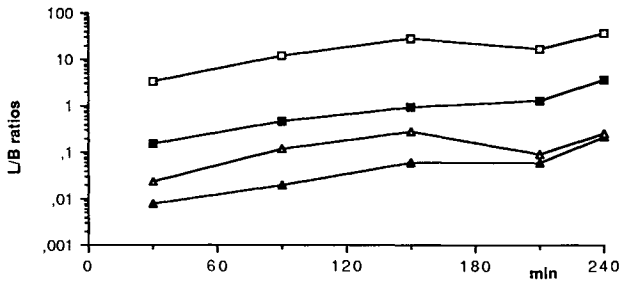

Fig. 4. Chronological evolution of the lymph/blood (L/B) ratios after ip (squares) or iv (triangles) administration of PHCA (filled symbols) or PMMA (open symbols) nanoparticles.

polymer than PHCA, which may explain the higher thoracic lymph and lymph nodes concentrations. As shown by Kreuter (16), PMMA material is more hydrophobic than polyalkylcyanoacrylates. In addition, poloxamer 188 and dextran 70 were added during the preparation of PHCA nanoparticles (12), whereas PMMA nanoparticles were produced without any additives (13). This further increases the hydrophobicity/hydrophilicity ratio in favor of the PMMA. Eldrige *et al.* (17) also reported increased absorption of nano- and microparticles by Peyer's patches and interaction with draining lymph nodes with increasing hydrophobicity after peroral administration. It can be concluded, therefore, that hydrophobicity seems to have paramount influence in governing lymphatic uptake.

The influence of particle size is very difficult to determine in this study because of the occurrence of aggregation in the case of PMMA nanoparticles. Although in water these aggregates may be the relevant species, the fluids in the peritoneum may act as detergents leading to an occurrence of single primary particles.

Depending on the route of administration, the carbon-14 distribution in the organs was also totally different. After iv administration the major part of the radioactivity administered was found in the liver and spleen. Accordingly, due to the inability of the carriers to escape the vascular space (18), the carbon-14 concentrations in the lymphatic system (lymph and the considered nodes) were very low. After ip administration, carbon-14 concentrations in the organs were also very low, especially compared to the nodal concentrations. The general trend, although the differences were not statistically different, is in favor of a greater accumulation of radioactivity in the organs with PHCA nanoparticles. The diaphragm and, to a lesser extent, the kidneys were the organs concentrating the most radioactivity in the case of PHCA nanoparticles. For PMMA nanoparticles the main organs of uptake were, first, the spleen and, second, the diaphragm. The most striking result of the present work, however, was the increased radioactivity concentration in the mediastinal nodes after ip administration.

After IP administration, lymphatic targeting is restricted to the thoracic lymph fluid, renal nodes, and mediastinal nodes (by increasing order of carbon-14 concentrations): This dramatic lymphatic targeting may be explained by the physiology of the peritoneal lymphatic drainage. Indeed, drugs, macromolecules, or colloidal particles present in the abdominal cavity may be absorbed by two major pathways (5,19). The first absorption route is through the portal vein via the splenic blood capillaries: This is the route for the absorption of small hydrophilic molecules of low molecular

weights. The kinetics of absorption of drugs by this route are very fast. The second route has to be considered for drugs with higher molecular weights (20) or for colloidal particles (21). In this case, in order to be absorbed, drugs or colloidal particles must be drained to the abdominal diaphragmatic surface, onto which lymphatic stomates open alternatively in synchrony with the breathing rhythm; drugs or particulate systems are thereby aspirated into the lymphatic channels and will pass through the mediastinal nodes where particles can be trapped. It is also well established that the peritoneal lymph drains, to a lesser extent, caudally via retroperitoneal lymphatics to renal lymph nodes (22), which nevertheless explains the greater uptake of radioactivity in the latter nodes in the case of ip administration. Finally, in the case of lymph nodes (mesenteric and iliac) which do not participate in peritoneal lymphatic drainage, the carbon-14 concentrations were very low and equivalent for the two routes of administration.

A note of caution should be added since the figures in this report are probably an underestimation of the extent of lymphatic absorption because the right-side lymph duct, which also collects lymph from the peritoneal cavity, was not cannulated (22). In addition, the distribution results obtained after ip administration are probably an underestimation of the actual total lymphatic targeting. Indeed, if the radioactivity levels found in the sampled organs, the thoracic lymph, and the blood are cumulated, the dose actually absorbed (ΣD) (see Materials and Methods) appears to be overestimated. This may be expressed by the following equation, where T is the dose administered, W is the amount washed out, P is the amount not available for distribution but not washed out using the procedure described, and ΣD is the sum of total radioactivity found in the various organs, lymph, and blood.

$$T - W - P = \Sigma D \quad (1)$$

The quantity P may thus be calculated from the experimental data. The distribution is then more accurately based not on $T - W$ (Fig. 3) but on $T - W - P$ to give more realistic and higher values for the distribution percentages as shown in Table II. Neglecting the amount P results in a total radioactivity recovery of only 18 ± 13 and 8 ± 5% of the dose actually absorbed, respectively, for PHCA and PMMA nanoparticles. In the case of iv administration the mass balance consideration led to a radioactivity recovery of ca. 95% of the administered dose for the two types of particles. These results show that the peritoneal washings (W) do not permit the complete recovery of all radioactivity still present in the abdominal cavity at the end of the experimental time. Due to the liquid administration of these dosage forms, nanoparticles (P) may be found disseminated in the mass of the intestines, from where it would be difficult to recover them in the peritoneal washings (W). Further, the potential bioadhesive properties of nanoparticles (23) could make them adherent to the intestinal tissues and the gentle massage of the peritoneal cavity may not suffice to release nanoparticles from their locations.

Table II. Corrected Distribution Percentages of Carbon-14 After ip Administration of PHCA and PMMA Nanoparticles[a]

Organs	PHCA nanoparticules	PMMA nanoparticles
Spleen	2.9	3.0
Liver	40.0	23.6
Kidneys	13.6	5.0
Diaphragm	7.8	3.4
Heart	3.9	1.5
Lungs	3.9	5.3
Renal nodes	1.3	3.1
Mediastinal nodes	15.0	38.8

[a] See text for explanations.

Table II presents the new distribution pattern of PHCA and PMMA nanoparticles according to the percentages of the actual cumulated figures of radioactivity (18 and 8% of the dose actually absorbed for PHCA and PMMA nanoparticles, respectively). Although theoretical, Table II confirms our previous results. First, it should be noted that the targeting to the MPS (mainly the liver) appears more important and can be related to lymphatic absorption by the right-side lymph duct (22) since the passage of particles from the thoracic duct to the blood was made impossible in our study. When present in the bloodstream, the behavior of particles would therefore be the same as after iv administration and would explain this greater uptake by the liver. Furthermore, the effect of the hydrophilicity/hydrophobicity ratio (allowing a lower uptake of PHCA nanoparticles by the mediastinal nodes) is still evident since, in the case of PHCA nanoparticles, the liver is the organ concentrating the most radioactivity. The importance of the diaphragm in lymphatic absorption (especially for PHCA nanoparticles) is also evident from the figures in Table II. As already known (15), PHCA polymer is much more rapidly biodegradable than PMMA, which is confirmed by the higher radioactivity figures found in both kidneys and urine with PHCA nanoparticles. It is with PMMA nanoparticles that lymphatic targeting still appears the most important; the carbon-14 level in mediastinal nodes is more than 1.5 times the hepatic level, whereas it should be kept in mind that the average weight of the liver is about 10 g and that of the mediastinal nodes is only 60 to 100 mg.

In conclusion, it has been demonstrated that polymeric nanoparticles can target the lymphatic system after ip administration. Furthermore, the lymphatic absorption rate is very slow (a theoretical maximum of 18% of the dose actually absorbed is absorbed in 4 hr) and nanoparticles could act as slow-release formulations after ip administration.

The interest in targeting the lymphatic system with colloidal carriers such as liposomes has been well documented in the case of cytostatic (4,5) and antibiotic (24) drugs. The advantages of nanoparticles are that they generally incorporate greater amounts of drug and that they are more stable than liposomes. Cancers such as colon, rectum, or ovarian, which associate dissemination of metastases in the abdominal cavity and metastases targeting to local and regional nodes, could benefit from the slow release of drugs by diffusion from nanoparticles in the abdominal cavity associated with the targeting of drug loaded to nanoparticles to distant lymph nodes as long as lymphatic circulation is efficient (25). In addition, it is necessary to study the fate of drugs bound to the nanoparticles by this administration pathway.

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