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Fate of [^{14}C]poly(DL-lactide-co-glycolide) nanoparticles after intravenous and oral administration to mice

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Abstract

^{14}C-labelled poly(2-hydroxy acid) nanoparticles with a mean diameter of 133 ± 25 nm were prepared according to a solvent evaporation process using polyvinyl alcohol as the surfactant. 51.7% of polyvinyl alcohol remained associated with the colloidal carriers which were intravenously injected to mice. As expected, the fate of the nanoparticles was characterized by high uptake by the RES organs. From these results, it was concluded that the significant rate of residual surfactant on the nanoparticle surface did not influence the overall tissue distribution of this type of passive carrier. Oral administration of an aqueous suspension of these nanoparticles in mice showed that little radioactivity crossed the intestinal barrier (0.5% at 1 h) even if the association of concentrated milk seemed to increase, to some extent, the amount absorbed (2.4% at 1 h). In order to determine whether the measured radioactivity in the organs could be attributed to the passage of intact nanoparticles or degradation products generated in the gastrointestinal lumen, radiolabelled acetic acid was given in parallel to mice. From these in vivo data, it appeared that the percentage of absorption of intact nanoparticles ranged from 1.9 to 2.3%, 1 h after oral administration, when concentrated milk was associated with the nanoparticle suspension.

Key words: Poly(DL-lactide-co-glycolide); Drug carrier; Nanoparticle; Solvent evaporation process; Tissue distribution; Oral administration

1. Introduction

The distribution profiles after intravenous administration of nanoparticles to animals have been widely described in various studies and it is agreed that the reticuloendothelial system (RES) takes up most of the colloidal carriers (Kreuter et al., 1979; Leu et al., 1984; Krause et al., 1985; Couvreur et al., 1986). On the other hand, many discordant results have been reported concerning the intestinal absorption of nanoparticles after oral administration to animals. Thus, 1–2% absorption was described by Couvreur et al. (1986) and Kukan et al. (1989) after oral administration of [^{14}C]poly(hexyl cyanoacrylate) nanoparticles with a mean diameter of 234 nm to mice and [^{14}C]poly(alkyl methacrylate) nanoparticles of 270

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nm to rats. The highest rates of absorption of radioactivity were observed by Nefzger et al. (1984) and Spenlehauer et al. (1991) after oral administration of [^{14}C]poly(methyl methacrylate) nanoparticles of 130 nm and [^{14}C]poly(DL-lactide) of 100 ± 40 nm to rats (10–15 and 20%, respectively).

A number of factors can affect the fate of the colloidal carriers (Lefevre et al., 1985; Eldridge et al., 1990). Among them, it is believed that the methodology used to investigate their tissue distribution is predominant, especially when a radioactive marker is involved. Very few studies focus on the stability of the bond between the radioactive entities and the carrier itself. This can generate situations where the marker or radiolabelled oligomers released by the nanoparticles are followed rather than the carriers themselves. In a previous work, radiolabelling of end-chain groups was achieved on poly(DL-lactide) and the stability of the labelling moieties after processing to nanoparticles was shown to be very high in different aqueous media (Le Ray et al., 1994).

In this paper, we describe the results of further investigations aimed at determining the extent of absorption and the tissue distribution of poly(DL-lactide-co-glycolide) nanoparticles after oral administration to mice. The intravenous route was used as a reference.

2. Materials and methods

2.1. Materials

2.1.1. Polymers (Table 1)

Poly(DL-lactide) PLA 50 (50% of L-lactic units, 50% of D-lactic units) no. 1 and 2 were synthesized by ring-opening polymerization of DL-lactide at 145°C using Zn powder as the initiator (URA CNRS 1465, Montpellier, France). The ^{14}C labelling (Le Ray et al., 1994) allowed three batches of radiolabelled PLA 50 to be obtained. Their activities were, respectively, 145, 569 and 1140 μCi/g of polymer for PLA 50 no. 1, 2a and 2b. The poly(DL-lactide-co-glycolide) PLA 37.5 GA 25 (37.5% of L-lactic units, 37.5% of D-lactic units and 25% of glycolic units) was supplied by BI Chimie (Le Vésinet, France). These aliphatic polyesters were intrinsically amorphous compounds. The emulsifying agent used was an 88% hydrolyzed polyvinyl alcohol (PVA, 4 cp) (Rhodoviol® 4/125) (Rhône-Poulenc, Paris, France).

Table 1
Mass parameters of poly(2-hydroxy acid) determined by size-exclusion chromatography (SEC) on unlabelled polymers

Polymer	Mass parameters			
	Before chemical modification		After chemical modification	
	$\bar{M}_n$	I	$\bar{M}_n$	I
PLA 50 no. 1	48000	1.5	7500	2.4
PLA 50 no. 2	27500	1.5	28000	1.4
PLA 37.5 GA 25	38500	1.9	–	–

2.1.2. Radiolabelled acetic acid

[1-^{14}C]Acetic acid, sodium salt (57 mCi/mmol) was purchased from Amersham (Paris, France).

2.1.3. Solvents

Acetone, hydrogen chloride and methylene chloride (Normapur® grade) were supplied by Prolabo (Paris, France). H_2O_2 (35%) was obtained from Riedel-de-Haen (Seelze, Germany). Isopropanol (Codex®) was provided by Farmitalia Carlo Erba (Romilly-sur-Andelle, France). All these products were used without further purification.

2.1.4. Scintillation reactants

Instagel®, Picofluor® 30 and Soluene® 350 were purchased from Packard Instrument S.A. (Rungis, France).

2.1.5. Powder

Potassium hydroxide was obtained from Prolabo.

2.1.6. Animals

8-week-old female Swiss mice weighing 25 g were selected. They were fasted for 24 h prior to experimentation.

Table 2
Composition and mean diameter of the studied nanoparticles

Nanoparticle batch	Starting polymers	Mean diameter (nm)
1	50 mg PLA 50-14C no. 1 270 mg PLA 37.5 GA 25	134 ± 23
5	105 mg PLA 50-14C no. 2a 215 mg PLA 50 no. 2	141 ± 26
6	88 mg PLA 50-14C no. 2a 232 mg PLA 50 no. 2	145 ± 28
7	40 mg PLA 50-14C no. 2b 280 mg PLA 50 no. 2	128 ± 27

2.2. Methods

2.2.1. Nanoparticle preparation procedure

Typically, 320 mg of poly(2-hydroxy acid) were dissolved in 32 ml of 50:50 acetone/methylene chloride. The organic phase was emulsified in 100 ml of a polyvinyl alcohol aqueous solution (0.5%, w/v) using a high-pressure homogenizer (ALM2®, Guérin, Mauzé, France) for 30 s under 160 bar, at 4°C. The solvent was evaporated for 2 h at 210 rpm under reduced pressure in a rotary evaporator (Heidolph®, Bioblock, Illkirch, France). The final nanoparticulate suspension was concentrated by ultrafiltration under a nitrogen pressure of 2 bar, for 6 h (Amicon®, Grace S.A., Epernon, France) through a disc having a 100 kDa mass cut-off (ultrafiltration disc membrane, PTHK, Millipore, Saint-Quentin-en-Yvelines, France). The exact composition of nanoparticles in poly(2-hydroxy acid) is shown in Table 2.

2.2.2. Nanoparticle characterization

The mean diameter of nanoparticles ($n = 5$) was measured by photon correlation spectroscopy (PCS) (Nanosizer N4MD®, Coulter Electronics Inc., FL, U.S.A.). 10 μl of the concentrated nanoparticle suspension were added to 10 ml of Instagel® and the radioactivity was counted by β spectrometry (RackBeta®, 1211, LKB, Wallac Oy, Turku, Finland). The residual PVA percentage was determined by gravimetry after extraction of PLA and PVA with the appropriate solvents (methylene chloride for PLA, warm water for PVA). This was expressed according to: [PVA (mg)/freeze-dried nanoparticles (mg)] × 100.

2.2.3. Intravenous distribution of nanoparticles

Nanoparticles releasing 1.5–1.7% of their initial radioactivity after 3 h under model blood conditions (batch 1) were used (Le Ray et al., 1994). 250 μl (10^{12} nanoparticles, 0.5 mg of polymer with an activity of 0.5 μCi) of the aqueous nanoparticle suspension was administered to 35 mice ($n = 5$) in one of the four caudal veins. The animals were placed in metabolic cages and killed after 0.5, 1, 2, 4, 24, 48 h and 1 week.

2.2.4. Oral administration

Nanoparticles releasing 0.8–1.5% of their initial radioactivity under model intestinal conditions after 48 h (batches 5–7) were used (Le Ray et al., 1994). 500 μl (10^{13} nanoparticles, 5 mg of polymer with an activity of 5 μCi) of the aqueous nanoparticle suspension were orally administered to mice by gastric intubation. 48 mice ($n = 6$) were administered with the aqueous nanoparticle suspension. Among them, 24 mice then received in addition 500 μl of concentrated milk without sugar (Gloria®, Sopad Nestlé, Courbevoie, France). In parallel, 24 mice ($n = 6$) were administered with 0.04 μCi of an aqueous acetic acid solution. The mice were placed in metabolic cages and killed at 1, 4, 24 and 48 h.

2.2.5. Radioactivity counting

Each mouse was anaesthetized, then decapitated to collect the blood. A blood sample of 100 μl was taken. The total blood volume was evaluated according to Gipps et al. (1986) and Bannerman (1983): 100 g of mouse weight corresponds to a blood volume of 5.85 ml. 11 organs were then removed: brain, colon, heart, kidney, liver, lung, lymph nodes, mesentery, small intestine, spleen and stomach. They were then weighed and a sample of 50 mg was taken from each. The urines, faeces and the content of the gastrointestinal tract were kept. The carcass and the head were preserved to determine the total radioactivity administered in the mice. The tail was removed to avoid the radioactivity counts that could remain at the injection point.

50 mg of each organ (Kante et al., 1980) were supplemented with 1 ml of Soluene®350. After one night at 50°C, 0.2 ml of H_2O_2 (35%) were added. They were then placed for 30 min at 50°C. After cooling, a mixture of 9 ml of Instagel® and 1 ml of 0.5 M HCl was added. The stomach, small intestine and colon were digested in Soluene®350, and then 1 ml was taken and treated as described above. 100 μl of blood (Illum et al., 1984) were added to a mixture of Soluene®350 and isopropanol (750 μl each). The sample was then placed for 10 min at 50°C. After cooling, 500 μl of H_2O_2 (35%) were added via a drip feed. A mixture of Instagel® and 0.5 M HCl (9–1 ml) was finally added. The carcass as well as the head (Combes, personal communication; INSTN, SACLAY, CEA) were digested in 100 and 50 ml of alcoholic 3 N potassium hydroxide, respectively. After 24 h at room temperature, 700 μl were taken up and mixed with 10 ml of Picofluor®30. 1 ml of urine (Packard Instrument S.A. protocol) was directly added with 10 ml of Instagel®. 20 mg of dried faeces (Packard Instrument S.A. protocol) were rehydrated for 30 min with 0.1 ml of water. 1 ml of Soluene®350 was added to the sample which was then placed for 1–2 h at 50°C. 0.5 ml of isopropanol followed by 0.2 ml of H_2O_2 (35%) were added. The sample was placed for 10 min at room temperature, then for 2 h at 50°C before adding 10 ml of Instagel®.

Table 3
Radioactivity of the nanoparticle batches

Nanoparticle batch	Activity (μCi/ml)
1	1.50
5	12.00
6	9.40
7	8.80

All the preparations were allowed to stand for 24 h at 4°C in the dark before β spectrometry. The results were expressed as a percentage of radioactivity per organ or biological medium as a function of the total radioactivity recovered per mouse.

3. Results

3.1. Nanoparticles

The nanoparticle batches were prepared according to a solvent evaporation process (Julienne et al., 1992) with an organic phase comprising equal quantities of acetone and methylene chloride. The use of acetone and methylene chloride, in equal amounts, as the organic phase in the solvent evaporation process permitted the reproducible formation of nanoparticles with a diameter of 134 ± 23 nm (Table 2).

Table 4
Percentages of radioactivity recovered per organ as a function of the total recovered radioactivity after intravenous administration of [^{14}C]PLAGA nanoparticles (n = 5) (mean ± SD)

Organ	Time						
	0.5 h	1 h	2 h	4 h	24 h	48 h	1 week
Blood	38.33 ± 4.04	3.24 ± 1.58	1.25 ± 1.00	0.14 ± 0.03	0.24 ± 0.26	0.13 ± 0.03	0.14 ± 0.08
Brain	0.16 ± 0.10	0.13 ± 0.04	0.01 ± 0.01	0.01 ± 0.01	0.03 ± 0.01	0.04 ± 0.03	0.02 ± 0.01
Carcass	21.72 ± 3.70	19.74 ± 1.48	16.3 ± 3.18	8.41 ± 0.98	13.00 ± 4.64	13.60 ± 7.15	5.77 ± 1.09
Colon	0.31 ± 0.07	0.37 ± 0.10	0.22 ± 0.11	0.06 ± 0.06	0.45 ± 0.21	0.23 ± 0.17	0.09 ± 0.01
Faeces	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.08 ± 0.00	0.76 ± 0.52	1.54 ± 1.51	11.16 ± 1.87
Heart	0.22 ± 0.15	0.22 ± 0.03	0.06 ± 0.03	0.06 ± 0.06	0.07 ± 0.03	0.05 ± 0.03	0.03 ± 0.01
Kidney	1.21 ± 0.68	1.02 ± 0.06	0.50 ± 0.17	0.36 ± 0.11	1.12 ± 0.60	0.55 ± 0.19	0.60 ± 0.07
Liver	28.79 ± 6.11	37.01 ± 2.16	72.50 ± 3.64	83.00 ± 1.70	69.20 ± 6.76	63.60 ± 5.70	56.96 ± 8.06
Lung	5.15 ± 1.31	4.67 ± 1.51	3.66 ± 1.36	3.37 ± 1.57	0.86 ± 0.33	0.08 ± 0.26	0.42 ± 0.17
Small intestine	1.35 ± 0.93	1.17 ± 0.55	0.88 ± 0.30	0.98 ± 0.22	2.29 ± 1.43	1.53 ± 1.51	0.72 ± 0.31
Spleen	2.48 ± 1.01	3.01 ± 0.75	4.46 ± 0.84	3.07 ± 0.98	5.46 ± 2.20	5.37 ± 2.86	6.25 ± 1.57
Stomach	0.27 ± 0.13	0.24 ± 0.09	0.07 ± 0.02	0.02 ± 0.02	0.12 ± 0.06	0.06 ± 0.04	0.05 ± 0.03
Urine	0.00 ± 0.00	0.00 ± 0.00	0.31 ± 0.43	2.34 ± 0.00	6.42 ± 3.19	12.40 ± 1.16	17.78 ± 6.83

The use of PVA as emulsifier led to rather high residual adsorption, since after a single concentration of the nanoparticle suspension, the content of PVA was evaluated as 51.7%.

The suspension activity varied from 1.5 to 12 μCi/ml depending on the activity of the polymer batch used (Table 3).

3.2. Intravenous distribution of nanoparticles

After i.v. administration of suspensions (Table 4), the nanoparticles did not remain for a long time in the blood compartment (1.25% after 2 h). From this compartment, the majority of the nanoparticles were targeted to the liver, the main organ of the RES (83% at 4 h). The rate of hepatic elimination was very slow. Thus, the nanoparticles were still present in this organ after 1 week (57%). The other organs which exhibited some degree of uptake were the lungs and the spleen. The percentage of nanoparticles in the lungs reached a maximum at 0.5 h (5.15%) and gradually decreased. The radioactivity profile in the spleen was different, since the maximum was only reached after 1 week (6.25%). Radioactivity was also found in the elimination organs (colon, small intestine and kidneys) at low levels (below 2.5%). The brain, heart and stomach did not concentrate radioactivity (below 0.3%). The elimination of radioactivity in the faeces and urine progressively increased over 1 week.

3.3. Oral administration

After oral administration of nanoparticles in aqueous suspension (Table 5), it was observed that their gastrointestinal transit was very rapid. In fact, after 1 h, 90.5% of the administered dose had already appeared in the small intestine chyme. After 24 or 48 h, the majority had been evacuated in the faeces (65.6 and 69.6%, respectively). After absorption through the intestinal barrier, the radioactivity was essentially found in the carcass (1% after 4 h decreasing to 0.64% after 48 h). Also, a significant amount of radioactivity was observed in the liver (0.24% after 4 h followed by a decrease to 0.06% after 48 h). The radioactivity found in the other RES organs (lungs and spleen) was negligible (less than 0.02%). However, we found that the distribution profiles were similar for those two organs with a maximum after 4 h followed by a slow decrease until

Table 5
Percentages of radioactivity recovered per organ as a function of the total recovered radioactivity after oral administration of [^{14}C]PLA nanoparticles in aqueous suspension ($n = 6$) (mean ± SD)

Organ	Time (h)			
	1	4	24	48
Blood	0.0209 ± 0.0082	0.0241 ± 0.0085	0.0159 ± 0.0121	0.0434 ± 0.0329
Brain	0.0027 ± 0.0017	0.0037 ± 0.0025	0.0029 ± 0.0021	0.0046 ± 0.0015
Carcass	0.4118 ± 0.1407	0.9860 ± 0.3929	0.7098 ± 0.3219	0.6441 ± 0.1632
Colon	0.0562 ± 0.0554	1.4236 ± 0.4262	0.1525 ± 0.2708	0.0296 ± 0.0271
Faeces	0.0037 ± 0.0003	22.8991 ± 16.8881	65.5653 ± 32.4693	69.5953 ± 27.1799
Heart	0.0014 ± 0.0007	0.0019 ± 0.0010	0.0018 ± 0.0012	0.0015 ± 0.0004
Kidney	0.0145 ± 0.0071	0.0108 ± 0.0058	0.0100 ± 0.0077	0.0091 ± 0.0025
Liver	0.0267 ± 0.0151	0.2426 ± 0.0986	0.0827 ± 0.0532	0.0642 ± 0.0274
Lung	0.0053 ± 0.0027	0.0090 ± 0.0072	0.0070 ± 0.0054	0.0066 ± 0.0025
Lymph nodes	0.0010 ± 0.0008	0.0025 ± 0.0019	0.0022 ± 0.0021	0.0018 ± 0.0016
Mesentery	0.0094 ± 0.0094	0.0123 ± 0.0047	0.0134 ± 0.0110	0.0123 ± 0.0043
Small intestine	1.7849 ± 0.8210	0.1575 ± 0.1706	0.0328 ± 0.0181	0.0212 ± 0.0217
Spleen	0.0043 ± 0.0056	0.0044 ± 0.0022	0.0034 ± 0.0025	0.0031 ± 0.0021
Stomach	0.2572 ± 0.4130	0.0212 ± 0.0155	0.0071 ± 0.0044	0.0077 ± 0.0039
Urine	0.0107 ± 0.0056	0.1912 ± 0.1473	0.2855 ± 0.2280	0.5943 ± 0.2429
Absorption	0.4980 ± 0.1487	1.2972 ± 0.3441	0.8487 ± 0.3513	0.7906 ± 0.1737

Table 6
Percentages of radioactivity recovered per organ as a function of the total recovered radioactivity after oral administration of [^{14}C]PLA nanoparticles in aqueous suspension followed by the administration of concentrated milk ($n = 6$) (mean ± SD)

Organ	Time (h)			
	1	4	24	48
Blood	0.0620 ± 0.0410	0.0419 ± 0.0257	0.0438 ± 0.0168	0.0291 ± 0.0137
Brain	0.0069 ± 0.0042	0.0094 ± 0.0073	0.0129 ± 0.0065	0.0087 ± 0.0059
Carcass	2.0829 ± 1.5387	1.2923 ± 0.4101	0.9947 ± 0.1251	0.7074 ± 0.1830
Colon	1.4041 ± 1.1803	3.6117 ± 3.0100	0.1007 ± 0.0277	0.0430 ± 0.0297
Faeces	0.2335 ± 0.3268	15.4426 ± 13.8228	45.5480 ± 29.6353	65.5643 ± 27.3486
Heart	0.0066 ± 0.0099	0.0029 ± 0.0022	0.0034 ± 0.0016	0.0038 ± 0.0031
Kidney	0.0348 ± 0.0261	0.0262 ± 0.0221	0.0277 ± 0.0119	0.0133 ± 0.0051
Liver	0.1380 ± 0.1040	0.4239 ± 0.1283	0.2847 ± 0.1148	0.0910 ± 0.0402
Lung	0.0143 ± 0.0138	0.0077 ± 0.0044	0.0121 ± 0.0049	0.0084 ± 0.0055
Lymph nodes	0.0179 ± 0.0221	0.0083 ± 0.0075	0.0094 ± 0.0106	0.0037 ± 0.0027
Mesentery	0.0548 ± 0.0272	0.0362 ± 0.0209	0.0255 ± 0.0029	0.0218 ± 0.0024
Small intestine	7.0125 ± 6.1172	0.6612 ± 0.7639	0.1020 ± 0.0302	0.0563 ± 0.0439
Spleen	0.0321 ± 0.0396	0.0093 ± 0.0076	0.0081 ± 0.0057	0.0065 ± 0.0067
Stomach	0.4882 ± 0.5963	0.1773 ± 0.2506	0.0167 ± 0.0067	0.0298 ± 0.0470
Urine	0.0136 ± 0.0191	0.5712 ± 0.6334	2.1352 ± 0.6955	1.2963 ± 1.5341
Absorption	2.4353 ± 1.6305	1.6643 ± 0.5149	1.4693 ± 0.1835	0.8973 ± 0.2264

48 h. The radioactivity in the other organs (brain, heart, kidneys and lymph nodes) was too low to be interpreted.

In conclusion, the general profile of absorption of radioactivity reached a maximum of 1.3% after 4 h and slowly decreased during the course of the study (0.85% after 24 h and 0.79% after 48 h).

Administration of the aqueous suspension of nanoparticles was then followed by the administration of concentrated milk in order to stimulate

Table 7
Percentages of radioactivity recovered per organ as a function of the total recovered radioactivity after oral administration of [^{14}C]acetic acid in aqueous solution ($n = 6$) (mean ± SD)

Organ	Time (h)			
	1	4	24	48
Blood	3.53 ± 0.45	2.86 ± 0.61	2.66 ± 0.28	2.08 ± 0.91
Brain	0.76 ± 0.61	0.74 ± 0.49	0.49 ± 0.25	0.45 ± 0.34
Carcass	28.79 ± 9.19	28.85 ± 10.45	32.56 ± 9.01	30.82 ± 8.03
Colon	4.11 ± 1.22	4.80 ± 1.64	2.69 ± 0.70	2.91 ± 0.49
Faeces	0.08 ± 0.06	2.63 ± 0.95	16.92 ± 6.37	25.00 ± 6.96
Head	17.04 ± 9.54	17.22 ± 6.54	18.37 ± 9.46	17.99 ± 9.65
Heart	0.22 ± 0.21	0.14 ± 0.06	0.10 ± 0.07	0.10 ± 0.04
Kidney	0.97 ± 0.42	0.80 ± 0.42	0.85 ± 0.33	0.62 ± 0.24
Liver	10.87 ± 4.46	6.63 ± 3.69	7.78 ± 4.15	5.91 ± 5.10
Lung	0.95 ± 0.53	0.67 ± 0.24	0.65 ± 0.29	0.38 ± 0.11
Lymph nodes	0.17 ± 0.09	0.18 ± 0.09	0.16 ± 0.11	0.09 ± 0.04
Mesentery	1.32 ± 0.40	0.59 ± 0.21	0.49 ± 0.23	0.56 ± 0.44
Small intestine	3.91 ± 1.13	4.06 ± 1.10	1.91 ± 1.02	1.01 ± 0.58
Spleen	0.42 ± 0.14	0.31 ± 0.17	0.25 ± 0.15	0.16 ± 0.05
Stomach	4.97 ± 2.78	4.49 ± 2.13	1.25 ± 0.54	0.92 ± 0.65
Urine	0.52 ± 0.00	4.92 ± 4.06	3.40 ± 1.33	7.19 ± 4.90
Absorption	63.95 ± 9.25	59.40 ± 12.56	66.90 ± 11.53	64.48 ± 7.40

the lymphatic absorption of the carriers (Charman and Stella, 1986) (Table 6). After administration, nearly the same profile as described above was observed in the gastrointestinal tract. Likewise, after absorption, the majority of the radioactivity was detected in the carcass. The only difference was found to be in the kinetics of the uptake. In fact, a maximum of 2.1% of radioactivity was reached after 1 h followed by a slow decrease during the study (0.7% after 48 h). Similarly, the liver was the only organ to concentrate significantly the radioactivity. The highest rate was reached after 4 h (0.42%). Subsequently, it decreased to 0.09% after 48 h. In the other RES organs (lungs and spleen), the radioactivity was very low and followed the same pattern as that in the carcass with a maximum 1 h after administration. The radioactivity in the other organs (brain, heart, kidneys and lymph nodes), as previously mentioned, was too low to be considered.

In conclusion, the general profile of absorption of radioactivity when concentrated milk is simultaneously given reached a maximum of 2.4% after 1 h and slowly decreased to a value of 0.9% after 48 h.

Finally, a solution of [^{14}C]acetic acid was orally administered in an attempt to attribute the absorbed radioactivity to degradation products or to intact nanoparticles. After administration, this molecule followed the same path as the nanoparticles (Table 7). Thus, during the first hour, it was recovered in the small intestine chyme (15.5%) and had already appeared in the colon (2.6%). The radioactivity was recovered in the faeces 24 and 48 h after administration, however, the amounts were lower than those following the administration of nanoparticles (16.9 and 25%, respectively). After absorption, the radioactivity accumulated in the carcass with a quasi constant value of 50% throughout the study. The liver was the other major organ of uptake with a mean percentage of radioactivity of 6–7% during the 48 h of study. However, a higher percentage of 11% was observed within the first hour. In the lungs, the radioactivity was maximum during the first hour (0.9%), after which it decreased to 0.4% at 48 h. The spleen exhibited the same profile but lower percentages were observed (0.4% after 1 h and 0.16% after 48 h). The level of radioactivity in the kidneys was maximal after 1 h (0.97%). It then decreased to reach 0.62% after 48 h. The brain uptake was nearly the same with percentages of 0.76% after 1 h and 0.45% after 48 h. The radioactivity found in the heart and the lymph nodes was the lowest with maxima of 0.22% after 1 h and 0.10% after 4 h.

In conclusion, the general profile of acetic acid absorption was constant throughout the study with a mean value of 65%.

4. Discussion

4.1. Intravenous distribution of nanoparticles

After intravenous administration of the suspension, the tissue distribution study showed that the nanoparticles had a high tropism for the liver. The highest rate of radioactivity was achieved 4 h after injection (83%), subsequently decreasing to reach 57% after 1 week. This result was in accordance with those found in the literature for poly(methyl methacrylate) (50, 100 and 800 nm) (Juhlin, 1960) and polystyrene (230 nm) (Adlersberg et al., 1969; Singer et al., 1969) nanoparticles injected intravenously in mice (65–85% after 3–4 h). Interestingly, nanoparticles made from biodegradable polymers such as poly(isobutyl cyanoacrylate) (254 nm) (Grislain et al., 1983) and gelatin (400 nm) nanoparticles (Oppenheim et al., 1978) reached these organs after i.v. injection to mice at lower percentages (42 and 27% after 4 and 3 h, respectively). One could argue that these low percentages might be the result of faster degradation of the polymer backbone and/or poor stability of their radiolabelling. Our data demonstrated the good stability of the polymeric matrices and the end-chain group radiolabelling of the poly(2-hydroxy acid) used over 4 h. Likewise, it has been reported that only 42.9% of ^{99m}Tc-PLA nanoparticles ($< 1\ \mu$m) reached the liver 2 h after i.v. administration to rats (Krause et al., 1985). In addition to the rate of biodegradation of the polymer and the stability of the marker, this difference in behaviour could also be related to physicochemical parameters such as

the nature of the surface or the diameter of the carriers. It was not mentioned, in this case, whether some traces of the surfactant, gelatin, remained on the particle surface. It has been reported that the presence of adsorbed surfactant on nanoparticles might influence their biodistribution (Illum et al., 1984; Illum and Davis, 1987; Tröster et al., 1990). In our case, we believed that the presence of residual PVA in our preparations conferred a hydrophilic character to the nanoparticles. Consequently, they should have remained longer in the blood compartment and their liver and spleen uptake should have been minimised according to the results of the above studies. Our conflicting results were consistent with those of Müller and Wallis (1993) who found that poly(β-hydroxybutyric acid) nanoparticles with a mean diameter of 180 nm, coated with PVA, behaved like uncoated polystyrene nanoparticles with a mean diameter of 60 nm, with regard to their rapid uptake by the liver and spleen. Compared to some results in the literature, our findings showed that the hydrophilic or lipophilic nature alone of the carrier surface could not explain the body distribution of these systems.

In terms of kinetics we also observed that the liver uptake of the studied nanoparticles was relatively slow when compared to other investigations (maximum after 4 h). Our results were comparable to those obtained with poly(diethylmethylidene malonate) (140–250 nm) (De Keyser et al., 1991), poly(methylmethacrylate) (Juhlin, 1960) and polystyrene (Adlersberg et al., 1969; Singer et al., 1969) nanoparticles. For poly(isobutyl cyanoacrylate) (Grislain et al., 1983; Couvreur et al., 1986), poly(hexyl cyanoacrylate) (276 nm) (Couvreur et al., 1986), human serum albumin (300–1000 nm) (Scheffel et al., 1972) and gelatin (Oppenheim et al., 1978) nanoparticles, the liver uptake kinetics were extremely fast (maximum after 15 min). Polyacrylamide (253 ± 20 nm) (Sjöholm and Edman, 1979) or polyacryldextran (500–1500 nm) (Edman and Sjöholm, 1983) nanoparticles, on the other hand, displayed very slow liver uptake (maximum after 1–2 weeks). One criterion retained in the literature (Scheffel et al., 1972) to explain the differences in kinetics was the nanoparticle diameter. Thus, larger particles would be eliminated more rapidly from the blood compartment and taken up more rapidly by the liver and the spleen than smaller particles which would remain longer in the blood circulation. The reported results showed that this was not always the case. A clear relationship between the diameter of our carriers and their uptake kinetics cannot be established, on the basis of literature data. In other words, the carrier diameter alone does not govern the rate of uptake by the liver. Apart from the surface nature and the mean diameter of the carriers, other factors should be considered to predict precisely liver uptake, for instance, the presence of chemical structures which would create an 'inactive' surface, i.e., a surface not recognized by RES cells (Müller and Wallis, 1993).

The percentage of nanoparticles found in the spleen, the second major organ of the RES system, was lower (6.25% after 1 week). This difference as compared to uptake by the liver (57.0%) could be explained by the 10-fold factor of weight difference between these two organs. The distribution profile was different as compared to that observed in the liver. The concentration of nanoparticles in the spleen increased throughout the week. No explanation for this observation was found, however, the same profile was described for [^{125}I]polystyrene nanoparticles (230 nm) after i.v. administration to mice (Adlersberg et al., 1969).

The presence of nanoparticles in the lungs after 0.5 h (4.7%) could be accounted for by two phenomena. Firstly, the nanoparticles could be taken up by the alveolar macrophages and secondly, the agglomerated nanoparticles could be retained by mechanical filtration. The lower amount of nanoparticles (0.86%) observed 24 h after injection was probably due to the resuspension of nanoparticle agglomerates by blood components acting as surfactants (Kreuter et al., 1979; Edman and Sjöholm, 1983).

The radioactivity found in the other organs after 0.5 and 1 h was probably the result of the presence of nanoparticles in the intravascular compartment of these organs.

The presence of radioactivity in the excretory organs and in the elimination products reflects

the biodegradation process occurring on the nanoparticles.

4.2. Oral administration

The gastrointestinal transit of the nanoparticles was very fast. Most of the radioactivity was observed very rapidly in the colon 4 h after administration and in the faeces 24 h after administration. It appeared that there was no interaction between the nanoparticles and the mucus. This could be explained by the lack of ionized groups at the surface of the studied carriers to allow their bioadhesion (Ranga Rao and Buri, 1989). Thus, as the phenomenon of absorption of any substance appeared essentially in the small intestine, if nanoparticles are absorbed, they should cross the gastrointestinal barrier during the first hour. As a matter of fact, the general profile of absorption showed a maximum of radioactivity at 4 h (1.3%) for the nanoparticles administered in an aqueous suspension. When the aqueous suspension was followed by the administration of concentrated milk, the maximum of radioactivity absorption was observed sooner, at 1 h (2.4%), and the rate of absorption was significantly greater at 1 and 24 h ($\alpha = 1\%$, Mann-Whitney test). It was thought that the concentrated milk according to its lipid composition stimulated the lymphatic absorption of the carriers and, consequently, increased its absorption rate.

Irrespective of the suspension administered, most of the radioactivity was observed in the carcass and the liver. The presence of radioactivity in the liver was believed to be the result of uptake of nanoparticles by the RES macrophages as previously reported in the case of intravenous administration. Two hypotheses could be proposed to explain the presence of radioactivity in the carcass. The first might correspond to the accumulation of acetate residues resulting from the initiation of degradation of the polymeric matrices in the lipids remaining in the carcass (Harper, 1989). The high percentage of radioactivity found in the carcass after [^{14}C]acetic acid administration agreed with this assumption. The second might be attributed to nanoparticle uptake by the lymph nodes (mediastin, poplite, axillar, aortic, etc.) (Hebel and Stromberg, 1986).

A question remained to be elucidated: was the absorbed radioactivity (0.5 and 2.4% at 1 h and 1.3 and 1.7% at 4 h for the administration of the aqueous suspension alone and followed by the administration of concentrated milk, respectively) due to the absorption of nanoparticles through the intestinal barrier or to the absorption of radiolabelled acetic acid generated by hydrolysis of the radioactive poly(2-hydroxy acid)?

The in vitro stability results reported in a previous paper (Le Ray et al., 1994) and the data on [^{14}C]acetic acid absorption allowed this question to be resolved. The in vitro stability data showed release of 0.12 and 0.21% of radioactivity at 1 and 4 h, respectively, in an artificial intestinal medium (USP XXII). The administration of [^{14}C]acetic acid was followed by absorption of 64 and 59.4% of radioactivity at 1 and 4 h, respectively. One could consider that the radioactivity release under artificial conditions could be lower than that really occurring in the gastrointestinal tract. We have calculated that the in vivo release data could not be more than 6.5-fold those of in vitro release, otherwise our results on absorption would have been negative at the first hour, without using concentrated milk. Taking into account the factor of 6.5 for the in vitro data, the lowest amount of absorbed nanoparticles would have been 0 and 1.9% at 1 h and 0.5 and 0.9% at 4 h for the two modes of nanoparticle administration.

One could conclude that the percentage of absorption of nanoparticles ranged from 0 to 0.4% and from 1.9 to 2.3%, 1 h after the respective administration of an aqueous suspension alone and the same suspension followed by a given amount of concentrated milk.

However, when the radioactivity liver clearances were taken into account between 4 and 48 h after i.v. and oral administration of the nanoparticles, it was observed that they were quite different (Tables 4–6).23.4% of the radioactivity was eliminated within the considered time range, following i.v. infusion. In contrast, 73.5 and 78.5% of the radioactivity were cleared, in the case of oral administration. If one considered that the elimination of radioactivity reflected the

biodegradation processes occurring on nanoparticles, the observed differences concerning the liver clearances remained unexplained and might again minimize the weak percentages of absorption previously found.

5. Conclusion

After intravenous injection, PLAGA nanoparticles were targeted principally to the RES organs, e.g., the liver and spleen.

The oral administration of nanoparticles in aqueous suspension followed by the administration of concentrated milk showed roughly 2% absorption of the radioactivity with redistribution of the radioactivity essentially in the carcass and liver. We believe that this absorption reflects the uptake of the nanoparticles. However, the carcass uptake and elimination profiles of radioactivity from the liver should be elucidated.

In all cases, the administration of an encapsulated drug in the aim of obtaining a pharmacological effect appears to have inconclusive effects.

Attempts to increase the absorption of nanoparticles are now underway focusing on their mean diameter, their surface state and on the nature of the dispersion medium which could influence their lymphotropism.

6. References

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