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Modification of the body distribution of poly(methyl methacrylate) nanoparticles in rats by coating with surfactants

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Summary

The body distribution of surfactant-coated and non-coated poly(methyl methacrylate) nanoparticles with a size of 131 ± 30 nm was determined in rats. A series of poloxamers/poloxamines, polysorbates as well as polyoxyethylene lauryl ether (Brij 35) were tested. No direct correlation was found between the body distribution pattern obtained and the contact angles of the surfactant solutions on PMMA. Nevertheless, all surfactants reduced the liver accumulation and increased the uptake in other parts of the body. Poloxamer 338 and poloxamine 908 were especially effective in reducing the liver uptake to a value of below 30% of the dose and maintaining significantly higher blood levels up to an increase factor of 103 for up to 6 h. The experiments also showed that spleen uptake is not a result of liver spillover because spleen concentrations reached values up to 50% of the total dose whereas at the same time only 25% was found in the liver. The uptake of nanoparticles in non-RES organs such as heart, GI tract, ovary, kidneys, muscles and brain was significantly increased with all surfactants.

Introduction

Drug targeting with colloidal drug-delivery systems has become a major objective of advanced drug delivery. Colloidal drug delivery systems that have been developed so far include liposomes and nanoparticles. Compared to liposomes, nanoparticles have several advantages such as better stability and ease of preparation. However, a number of problems especially concerning parenteral administration of these colloidal carriers have to be solved. One of these problems is the uptake of the colloidal drug carriers (size < 1000 nm) by the macrophages of the reticuloendothelial system (RES) especially in the liver and in the spleen after i.v. administration (Juhlin, 1960; Kreuter et al., 1979; Lenaerts et al., 1982; Grislain et al., 1983; Leu et al., 1984; Davis and Illum, 1986; Illum et al., 1987; Waser et al., 1987). This uptake represents a major obstacle for their targeting to other parts of the body. The quality and extent of this uptake mainly depend on the size, surface charge and surface properties of the particles (Wilkins and Myers, 1966; Singer et al., 1967; Wilkins, 1967; Gesler and Garvin, 1973; Schroeder et al., 1978; Illum et al., 1982; 1986; Douglas et al., 1986). Microspheres with a size of ≥ 4–7 μm are mechanically filtered and retained in the lungs,

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while smaller spheres pass through the lungs and mainly localize in the liver and the spleen (Wilkins and Myers, 1966; Stuart, 1970; Gesler and Garvin, 1973; Schroeder et al., 1978; Kanke et al., 1980; Illum and Davis; 1983, 1984). To date it has generally been assumed that injected colloidal particles interact with plasma components, and that the nature of this interaction triggers the uptake by certain phagocytotic cells and hence localization in the body (Jenkin and Rowley, 1961; Wilkins, 1971). Leu et al. (1984), Illum et al. (1986, 1987), Douglas et al. (1986) and Davis and Illum (1986) have demonstrated that the liver uptake of nanoparticles can be reduced by the coating of these particles with certain surfactants. Although the distribution of nanoparticles after parenteral administration can be changed by coating the particles with surface active substances, it is not clear whether this change in distribution is caused by an alteration of the nanoparticles surface charge, a change in the hydrophobic interaction with plasma proteins or by steric hindrance preventing opsonins from coating the particles.

In a previous study, surfactants were classified by an in vitro method into four different groups according to their advancing and receding contact angles (Tröster and Kreuter, 1988) in an attempt to predict their ability to prevent or alter the interaction of the nanoparticle surface with the plasma components. The contact angles are an expression of the interaction of the surfactant with the nanoparticle material and consequently its ability to change the surface characteristics of the nanoparticles (Andrade et al., 1979; Coleman et al., 1982; Kreuter, 1983; Zografi and Johnson, 1984; Hogt et al., 1985; Johnson, 1985; Murray, 1986; Johnson et al., 1986).

Poly(methyl methacrylate) (PMMA) was chosen as the model polymer carrier material for the following reasons: (a) It is of intermediate lipophilicity, (b) it can be stably labeled with ^{14}C within the polymer chain, and (c) it is only very slowly biodegradable. The latter two properties enable an accurate determination of the body distribution of virtually undegraded carriers in individual organs which is not possible using a gamma camera.

The purpose of the present investigation is to discover new surfactants with more effective targeting properties and to determine whether contact angles of surfactants on PMMA correlate with their in vivo ability to alter the body distribution (Tröster and Kreuter, 1988). For this reason, the PMMA nanoparticles were coated in the first run with poloxamers/poloxamines with different contact angles on PMMA surfaces (Tröster and Kreuter, 1988). In a second run, a series of polysorbates or polyoxyethylene fatty alcohol ethers yielding similar contact angles were used to challenge the hypothesis that these contact angles are indicative of the body distribution of surfactant coated nanoparticles.

Materials and Methods

Surfactants

The poloxamers and poloxamines were obtained from C.H. Erbslöh (Düsseldorf-Hafen, F.R.G.), the polysorbates from Atlas-Chemie (Essen, F.R.G.), and polyoxyethylene (23) lauryl ether (Brandname: Brij 35) from Fluka (Buchs, Switzerland).

Preparation of ^{14}C-labeled nanoparticles

Methyl [2-^{14}C]methacrylate customer synthesised by Amersham Radiochemical Centre (Bucks, U.K.) was used as the labeled monomer. The nanoparticles were prepared as described previously (Kreuter et al., 1979) and freeze-dried as a suspension in 0.15 M phosphate-buffered saline. The resulting polymer had a specific activity of 4 mCi/g. The freeze-dried powder contained 56.3% of buffer salts (dibasic sodium phosphate dihydrate, monobasic potassium phosphate, sodium chloride (7.6 : 1.45 : 4.8, w/w/w)) and 43.7% of polymethyl [2-^{14}C]methacrylate. Particle size determination by photon correlation spectrometry (Model K 7025 with 64 channels, Malvern Instruments, Malvern, U.K.) (Kreuter, 1983) revealed an average diameter of 131 ± 30 nm.

Preparation of the suspension for injection

Reference group. The above-specified lyophilized nanoparticle-buffer preparation (160 mg) was suspended in 8 ml of distilled water and 8 ml of

phosphate-buffered saline to give a suspension of 4.37 mg nanoparticles/ml and ultrasonicated several times for about 5 min at 50 kHz in a ultrasonicator (Bransonic 12, Branson Europa B.V., Soest, The Netherlands).

Surfactant groups. Lyophilized nanoparticle-buffer preparation (160 mg) was suspended in 8 ml of a 2% (w/v) surfactant solution and 8 ml of phosphate-buffered saline to obtain a 1% (w/v) surfactant solution when both were added. Then the suspension was ultrasonicated in the same way as the reference group.

Specific activity of the suspensions

Five samples of each surfactant suspension as well as the references were counted in a liquid scintillation counter after dissolution and addition of scintillation cocktail as shown below.

An average activity of 0.604 MBq was found. The standard deviation in all cases was less than 2%, and the difference from the theoretical activity of 1 ml less than 1%.

Injection

Groups of four (two male and two female) randomly selected Wistar rats (Savo-Ivanovas, Kisslegg im Allgäu, F.R.G.) with a body weight of 180–220 g were used for each suspension and for each time point (0.5, 2, 6, 24 h, and 7 days). The rats were anesthetized with ether and then an adequate amount of suspension (3.333 ml/kg body weight) was injected at a rate of 1.5 ml/min into the tail vein using a Sterican-Einmalkanüle with Luer Lock no. 18 (B. Braun Melsungen A.G. Medizin-u. Labortechnik, Melsungen, F.R.G.) attached to a plastic syringe (Omnifix Tuberkulin, B. Braun Melsungen).

Immediately before injection, the suspensions were ultrasonicated at 50 kHz for 20 s. The injection dose was 2.013 MBq/kg body weight, adequate to 3.333 ml/kg body weight.

The rats were kept at a constant temperature of $24 \pm 2°$ C and a relative air humidity of $55 \pm 10\%$. They had access to water and a standard rat diet (Altromin-Haltungsdiät 1324, Samen Schmidt Jacobi, Frankfurt, F.R.G.) ad libitum.

Organ distribution of the ^{14}C radioactivity

The animals were killed by placing in carbon dioxide and subsequent decapitation. After collection of the blood, the abdomen and chest were opened and liver, lungs, heart, lymph nodes, kidneys, spleen, gonads, GI tract, brain, bone marrow, and a sample of the muscles were removed. The tail was also assayed for residual radioactivity in order to allow the exact quantification of the injected i.v. dose. The organs were weighed immediately after removal, and two aliquots were stored in a glass vial at $-20°$ C for further examination. The whole tail was dissected into 6 parts that were stored and assayed using the same conditions as for the other organ samples.

Determination of the ^{14}C radioactivity

After thawing, 1 ml tissue solubilizer (BTS-450, Beckman Instruments, Munich) (3 ml for the tail samples) was added and the vials stored at 50° C until all tissue was solubilized. Then 30% H_2O_2 was added till the colour was removed. After addition of 70 µl glacial acetic acid and 10 ml scintillation cocktail (Ready Organic Cocktail, Beckman Instruments), the prepared samples were stored for about 1 week in darkness and then counted in the scintillation counter (Beckman LS 1801).

Calculation of radioactivity

For the calculation of the radioactivity of the whole organs (percent dose) and of 1 g of tissue (µg per g), the total radioactivity in the tail was subtracted from the total dose. Since an accurate weight for the bone marrow, muscles and blood could not be determined, average weights were used (Professor Förster and Dr. Forthmeier, JWG University, Frankfurt). These values were: for bone marrow, 3% of body weight; for muscles 15% of body weight; for blood, 6.5% of body weight.

Statistics

The data were analyzed using Student's *t*-test involving heterogenic variances (Sachs, 1984).

Results

The aim of this investigation was to discover new surfactants with more effective targeting

TABLE 1

Classification of the surfactants according to their contact angles on poly(methyl methacrylate)

Study	Group			
	I	II	III	IV
A	Poloxamer 188	Poloxamer 407	Poloxamer 184	Poloxamer 338 Poloxamine 908
B	Polysorbate 60	Polysorbate 80	Polysorbate 20	Brij 35

properties and to investigate a possible correlation between the surface properties and the in vivo ability of the surfactants to alter the body distribution. For this reason, the surfactants were classified into four different groups according to their contact angles as shown in Table 1 (after Tröster and Kreuter, 1988). Nanoparticles coated with these surfactants were injected i.v. into rats. Two studies were carried out: In the first run, five poloxamers/poloxamines were tested; the results are listed in Tables 2 and 4. Tables 3 and 5 show the results of the second run in which a series of polysorbates and a polyoxyethylene fatty alcohol ether were examined. Uncoated nanoparticles were used as a reference.

All values, factors and statistical statements reported below were calculated on a μg nanoparticle per g tissue basis. The expression 'factor' refers to the quotient of surfactant coated particles to the reference.

The organ distribution of the uncoated nanoparticles was very similar in both runs (n.s., 1 random exception out of 65 samples) over the whole time period demonstrating the excellent reproducibility of the experiment.

Blood

After 30 min, all surfactants except poloxamer 188 and 184 showed significantly higher mean values for the blood levels than the reference (increase factor 2–103, $p < 0.05$). The highest values were found for polysorbate 80 (increased by a factor 4), poloxamer 407 (factor 6.5), poloxamer 338 (factor 81) and polxamine 908 (factor 103) (Tables 4 and 5). After 2 h, a marked increase resulted only for poloxamer 338 (factor 81) and poloxamine 908 (factor 80), which were the two most effective surfactants in increasing the blood level, although in both cases the increase was just not significant ($p < 0.1$). After 6 h, a non-significant increase (factor 25, $p < 0.15$) was observed for poloxamine 908 and poloxamer 338 (factor 49, $p < 0.15$) while a significant increase (factor 3, $p < 0.05$) was only found for poloxamer 184. For the other surfactants higher values were still observable at later times, but all increases were very small and non-significant.

Liver

All surfactants decreased the liver uptake of nanoparticles up to 2 h (factor 0.8–0.2). This decrease was found to be significant ($p < 0.05$) for all surfactants except poloxamer 188 ($p < 0.1$) and Brij 35 ($p < 0.3$) at 30 min and poloxamer 188 ($p < 0.15$), polysorbate 60 ($p < 0.1$) and Brij 35 ($p < 0.35$) at 2 h. The most effective decrease was caused by poloxamer 407 (factor 0.4–0.8), poloxamer 338 (factor 0.2–0.4), poloxamine 908 (0.3–0.6) and polysorbate 20 (0.6–0.8). For these four surfactants, a significant decrease in liver uptake was found up to 7 day ($p < 0.05$) except the 7 day value of poloxamer 407 ($p < 0.1$). For poloxamer 188 and Brij 35 a significant decrease was not observed at any time.

Spleen

All surfactants of study A (Tables 2 and 4) increased the spleen uptake up to 7 days (factor 3–27), the most efficient being poloxamer 338 (factor 11–27) and poloxamine 908 (factor 9–22). This increase was significant ($p < 0.05$) for all times and substances except poloxamer 407 at 30 min ($p < 0.1$).

TABLE 2

Body distribution of ^{14}C as percent of the dose after intravenous administration of uncoated and surfactant-coated polymethyl[2-^{14}C]methacrylate nanoparticles to rats (study A)

Time	Sample	Reference		Poloxamer 188		Poloxamer 407		Poloxamer 184		Poloxamer 338		Poloxamine 908	
		Percent dose	S.D.	Percent dose	S.D.	Percent dose	S.D.	Percent dose	S.D.	Percent dose	S.D.	Percent dose	S.D.
30 min	Lymph nodes	0.00	0.00	0.01	0.01	0.01	0.01	0.02	0.01	0.02	0.01	0.02	0.02
	Heart	0.06	0.05	0.18	0.09	0.30	0.09	0.75	0.26	0.84	0.37	0.61	0.29
	Lungs	2.11	1.41	11.74	7.22	42.16	17.01	14.59	8.03	9.26	7.72	8.67	8.24
	Liver	83.20	4.92	61.82	19.64	27.95	14.83	48.82	11.76	15.04	4.67	21.43	16.58
	Spleen	1.83	0.70	5.30	1.37	6.74	3.66	8.19	1.76	20.06	4.67	19.29	7.00
	GI tract	1.21	0.59	2.58	1.09	5.03	2.75	2.93	1.60	3.36	1.24	3.90	1.86
	Ovary	0.02	0.01	0.03	0.01	0.04	0.01	0.08	0.01	0.08	0.01	0.07	0.01
	Testicles	0.01	0.00	0.02	0.01	0.02	0.01	0.04	0.03	0.04	0.01	0.05	0.02
	Kidneys	0.25	0.12	0.86	0.28	1.03	0.45	1.13	0.35	1.43	0.38	1.33	0.66
	Muscles	0.40	0.33	1.17	0.71	1.19	0.32	1.80	0.32	2.25	0.91	1.96	0.79
	Brain	0.05	0.04	0.26	0.16	0.36	0.12	0.37	0.19	0.62	0.33	0.46	0.34
	Blood	0.28	0.14	0.31	0.26	1.81	0.46	0.54	0.38	22.18	9.64	28.21	23.47
	Bone marrow	4.04	2.25	2.10	1.88	1.73	0.05	9.64	2.67	2.52	1.74	2.41	2.00
	Total body	93.03	5.12	86.33	13.77	88.32	7.44	88.81	4.86	77.64	4.76	88.32	12.59
	Total RES	91.18	5.65	80.95	13.81	78.57	6.27	81.22	5.34	46.88	5.94	51.79	13.48
2 h	Lymph nodes	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.01	0.01	0.00
	Heart	0.03	0.02	0.14	0.07	0.15	0.06	0.72	0.48	0.31	0.10	0.28	0.07
	Lungs	1.70	1.62	10.67	7.37	37.39	22.15	15.96	15.09	12.86	9.32	13.88	10.37
	Liver	79.80	9.02	63.66	6.82	33.38	18.08	52.23	11.14	19.93	5.77	23.33	6.28
	Spleen	2.11	0.47	7.03	1.00	9.53	6.55	6.99	3.14	27.24	13.95	30.92	12.64
	GI tract	0.96	0.41	1.33	0.49	1.84	0.55	1.85	0.63	2.00	1.11	2.09	0.99
	Ovary	0.01	0.00	0.02	0.00	0.02	0.01	0.04	0.01	0.03	0.01	0.04	0.01
	Testicles	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.02	0.01	0.03	0.01
	Kidneys	0.14	0.04	0.50	0.15	0.52	0.18	0.62	0.28	0.64	0.24	0.59	0.15
	Muscles	0.20	0.03	1.12	0.71	0.91	0.51	1.41	0.95	1.50	0.73	1.25	0.36
	Brain	0.03	0.02	0.17	0.07	0.16	0.07	0.22	0.18	0.27	0.17	0.25	0.13
	Blood	0.08	0.03	0.07	0.03	0.14	0.06	0.13	0.09	6.49	6.37	6.38	5.61
	Bone marrow	3.90	1.02	2.82	1.49	2.89	0.81	12.64	6.07	2.42	1.11	2.32	0.69
	Total body	88.95	11.64	87.53	8.67	87.02	5.58	92.70	4.99	73.66	9.35	81.33	3.96
	Total RES	87.51	11.46	84.18	8.04	83.19	6.04	87.81	5.00	62.44	4.58	70.45	5.20
6 h	Lymph nodes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
	Heart	0.02	0.01	0.14	0.10	0.12	0.03	0.20	0.10	0.16	0.07	0.10	0.04
	Lungs	0.88	0.65	12.51	8.96	47.16	32.39	21.39	19.30	17.55	13.35	14.17	10.15
	Liver	66.64	11.82	63.05	17.10	34.90	16.89	50.50	16.60	23.50	5.90	34.18	8.27
	Spleen	1.03	0.42	5.07	1.22	10.10	7.79	5.14	3.92	28.35	15.68	26.09	15.51
	GI tract	0.50	0.24	0.50	0.20	0.71	0.09	0.63	0.19	0.61	0.13	0.89	0.25
	Ovary	0.00	0.00	0.02	0.01	0.01	0.00	0.03	0.00	0.01	0.00	0.02	0.00
	Testicles	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00

(continued)

TABLE 2 (continued)

Time	Sample	Reference		Poloxamer 188		Poloxamer 407		Poloxamer 184		Poloxamer 338		Poloxamine 908	
		Percent dose	S.D.	Percent dose	S.D.	Percent dose	S.D.	Percent dose	S.D.	Percent dose	S.D.	Percent dose	S.D.
6 h	Kidneys	0.10	0.02	0.26	0.08	0.40	0.10	0.31	0.04	0.31	0.09	0.32	0.09
	Muscles	0.24	0.04	0.76	0.26	0.94	0.32	0.89	0.42	0.81	0.30	0.81	0.33
	Brain	0.01	0.01	0.07	0.04	0.11	0.04	0.08	0.04	0.13	0.09	0.06	0.04
	Blood	0.03	0.01	0.05	0.02	0.06	0.04	0.10	0.05	1.54	1.76	0.78	0.97
	Bone marrow	2.53	1.08	3.56	3.15	4.21	1.37	11.22	6.17	2.61	1.08	3.44	2.97
	Total body	71.98	13.56	85.98	6.01	98.71	10.51	90.48	6.92	75.57	9.68	80.83	4.98
	Total RES	71.07	13.58	84.19	6.28	96.37	10.78	88.25	7.02	72.01	9.21	77.87	5.05
24 h	Lymph nodes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Heart	0.01	0.01	0.02	0.00	0.03	0.00	0.06	0.02	0.03	0.00	0.04	0.01
	Lungs	0.50	0.10	2.38	1.57	6.08	5.48	1.82	1.14	2.29	2.61	1.95	2.05
	Liver	70.07	6.72	66.86	9.18	48.96	5.58	67.28	3.46	26.54	6.97	28.38	9.89
	Spleen	2.06	0.40	10.44	3.43	18.02	8.08	9.66	1.38	44.07	8.07	43.69	13.41
	GI tract	0.30	0.10	0.37	0.07	0.44	0.13	0.72	0.18	0.68	0.25	0.90	0.42
	Ovary	0.00	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.02	0.00	0.02	0.01
	Testicles	0.01	0.00	0.02	0.01	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00
	Kidneys	0.09	0.04	0.14	0.04	0.22	0.08	0.24	0.02	0.15	0.02	0.15	0.05
	Muscles	0.14	0.05	0.28	0.13	0.35	0.12	0.28	0.07	0.27	0.12	0.32	0.21
	Brain	0.01	0.00	0.01	0.00	0.02	0.00	0.02	0.01	0.01	0.00	0.02	0.02
	Blood	0.03	0.03	0.01	0.01	0.02	0.00	0.12	0.09	0.08	0.10	0.07	0.05
	Bone marrow	3.22	1.33	3.43	1.34	10.26	7.92	16.17	8.31	7.87	6.75	3.74	1.12
	Total body	76.45	6.80	83.95	8.33	84.42	11.67	96.37	11.29	82.01	5.89	79.27	3.13
	Total RES	75.86	6.79	83.11	8.36	83.32	11.82	94.93	11.35	80.77	5.75	77.76	2.93
7 days	Lymph nodes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01
	Heart	0.00	0.00	0.01	0.00	0.01	0.01	0.02	0.01	0.01	0.00	0.02	0.01
	Lungs	0.26	0.06	0.59	0.29	2.33	1.81	0.26	0.10	0.20	0.10	0.20	0.10
	Liver	73.14	10.01	73.40	11.93	55.98	9.61	70.67	7.40	19.87	3.70	28.96	8.45
	Spleen	2.90	0.17	9.10	3.55	19.67	9.78	10.53	3.61	51.51	2.59	46.34	7.89
	GI tract	0.16	0.09	0.20	0.13	0.43	0.18	0.39	0.23	0.77	0.32	0.67	0.48
	Ovary	0.00	0.00	0.01	0.01	0.01	0.01	0.00	0.00	0.02	0.00	0.01	0.00
	Testicles	0.01	0.00	0.01	0.00	0.03	0.02	0.01	0.00	0.03	0.02	0.05	0.05
	Kidneys	0.03	0.01	0.03	0.00	0.05	0.01	0.04	0.02	0.04	0.01	0.05	0.02
	Muscles	0.12	0.04	0.46	0.36	0.41	0.36	0.58	0.57	0.55	0.23	0.70	0.76
	Brain	0.01	0.01	0.00	0.00	0.01	0.01	0.01	0.00	0.01	0.00	0.01	0.01
	Blood	0.01	0.01	0.01	0.00	0.01	0.01	0.03	0.03	0.01	0.00	0.01	0.00
	Bone marrow	6.09	3.16	4.01	1.25	5.56	0.60	13.78	5.23	7.26	2.94	8.13	2.23
	Total body	82.71	12.04	87.81	12.99	84.48	5.33	96.27	11.45	80.19	4.67	85.11	5.03
	Total RES	82.38	12.05	87.10	12.89	83.54	5.28	95.24	11.29	78.85	4.65	83.63	4.88

TABLE 3

Body distribution of ^{14}C as percent of the dose after intravenous administration of uncoated and surfactant-coated polymethyl[2-^{14}C]methacrylate nanoparticles to rats (study B)

Time	Sample	Reference		Polysorbate 60		Polysorbate 80		Polysorbate 20		Brii 35	
		Percent dose	S.D.	Percent dose	S.D.	Percent dose	S.D.	Percent dose	S.D.	Percent dose	S.D.
30 min	Lymph nodes	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00
	Heart	0.06	0.05	0.32	0.09	0.61	0.17	0.38	0.10	0.21	0.08
	Lungs	4.03	3.49	23.70	16.28	43.20	18.55	16.85	3.02	19.32	11.43
	Liver	76.66	12.70	51.71	11.59	26.94	8.11	54.10	3.24	68.68	17.58
	Spleen	1.27	0.23	2.79	1.72	4.55	2.42	2.54	0.92	1.52	0.27
	GI tract	2.34	1.93	2.05	1.69	5.35	2.43	3.26	1.37	3.71	3.10
	Ovary	0.01	0.01	0.03	0.01	0.07	0.02	0.05	0.01	0.02	0.00
	Testicles	0.01	0.00	0.02	0.00	0.03	0.01	0.01	0.00	0.01	0.01
	Kidneys	0.26	0.15	0.93	0.20	2.51	1.11	0.84	0.19	0.66	0.18
	Muscles	0.40	0.17	2.45	1.07	2.21	1.04	2.17	1.07	0.96	0.55
	Brain	0.08	0.08	0.44	0.13	0.72	0.20	0.30	0.04	0.26	0.10
	Blood	0.13	0.02	0.24	0.04	0.55	0.31	0.23	0.08	0.33	0.09
	Bone marrow	2.27	1.38	4.13	2.75	2.51	1.02	7.36	2.92	1.51	0.75
	Total body	87.49	10.58	88.78	5.37	89.19	5.61	88.06	3.96	97.17	9.59
	Total RES	84.23	11.55	82.33	5.39	77.20	7.98	80.84	4.12	91.03	11.80
2 h	Lymph nodes	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00
	Heart	0.04	0.03	0.26	0.09	0.44	0.19	0.18	0.07	0.10	0.02
	Lungs	3.83	4.55	33.25	21.14	7.65	6.45	27.68	19.49	16.14	9.28
	Liver	73.50	11.37	52.36	14.49	49.96	7.10	49.82	18.39	62.06	22.48
	Spleen	2.03	0.73	1.92	0.55	9.19	2.97	1.83	1.58	1.77	0.87
	GI tract	1.75	2.17	2.20	0.55	3.03	0.86	1.60	0.92	1.47	0.50
	Ovary	0.00	0.00	0.03	0.01	0.10	0.02	0.02	0.01	0.02	0.00
	Testicles	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.01
	Kidneys	0.22	0.18	0.62	0.05	2.21	1.04	0.53	0.08	0.37	0.09
	Muscles	0.33	0.22	1.42	0.75	1.86	0.82	0.99	0.46	0.95	0.45
	Brain	0.03	0.02	0.29	0.11	0.33	0.21	0.22	0.17	0.11	0.04
	Blood	0.07	0.04	0.10	0.05	0.10	0.01	0.07	0.01	0.09	0.02
	Bone marrow	2.67	0.72	5.60	3.72	3.33	0.49	4.46	2.79	1.78	1.31
	Total body	84.46	8.77	98.02	10.05	78.03	8.40	87.37	9.78	84.85	20.13
	Total RES	82.02	9.04	93.12	10.80	70.13	6.19	83.78	9.99	81.75	20.44
6 h	Lymph nodes	0.00	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00
	Heart	0.03	0.02	0.14	0.06	0.25	0.10	0.10	0.03	0.09	0.04
	Lungs	4.77	7.03	27.41	15.42	2.51	1.37	30.95	21.69	26.56	19.73
	Liver	70.97	11.81	50.68	11.38	58.64	10.61	48.59	14.03	56.01	17.73
	Spleen	1.69	0.47	2.04	0.75	12.63	4.67	1.70	0.63	2.00	0.96
	GI tract	0.44	0.17	0.67	0.47	1.88	0.63	0.60	0.20	0.66	0.35
	Ovary	0.00	0.00	0.02	0.01	0.06	0.00	0.01	0.00	0.02	0.00
	Testicles	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00
	Kidneys	0.17	0.14	0.36	0.06	1.49	0.29	0.28	0.06	0.38	0.13
	Muscles	0.34	0.23	0.82	0.35	1.13	0.58	0.75	0.33	0.59	0.22
	Brain	0.02	0.02	0.12	0.03	0.20	0.14	0.08	0.05	0.09	0.03
	Blood	0.04	0.01	0.05	0.03	0.04	0.01	0.04	0.02	0.05	0.01
	Bone marrow	2.85	0.90	5.46	3.67	5.19	1.49	4.64	2.12	2.66	1.32
	Total body	81.34	11.25	87.76	4.35	84.00	11.44	87.74	7.07	89.11	4.62
	Total RES	80.29	11.13	85.58	4.58	78.97	11.78	85.88	6.90	87.23	4.70

(continued)

TABLE 3 (continued)

Time	Sample	Reference		Polysorbate 60		Polysorbate 80		Polysorbate 20		Brii 35	
		Percent dose	S.D.	Percent dose	S.D.	Percent dose	S.D.	Percent dose	S.D.	Percent dose	S.D.
24 h	Lymph nodes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Heart	0.02	0.01	0.06	0.02	0.12	0.03	0.06	0.02	0.03	0.01
	Lungs	2.26	1.80	13.64	3.64	9.25	3.20	18.33	7.01	4.10	2.92
	Liver	74.39	7.62	76.00	11.58	65.44	7.24	59.01	4.43	81.81	13.26
	Spleen	2.04	0.28	2.41	0.43	6.31	1.85	2.43	0.56	2.83	0.56
	GI tract	0.49	0.66	0.39	0.11	0.94	0.91	0.36	0.09	0.32	0.17
	Ovary	0.00	0.00	0.01	0.00	0.03	0.02	0.01	0.00	0.01	0.01
	Testicles	0.01	0.00	0.02	0.01	0.01	0.00	0.01	0.00	0.01	0.00
	Kidneys	0.12	0.05	0.31	0.08	0.43	0.11	0.26	0.06	0.18	0.01
	Muscles	0.31	0.24	0.60	0.17	0.89	0.51	0.43	0.18	0.38	0.21
	Brain	0.01	0.01	0.03	0.01	0.15	0.03	0.03	0.01	0.02	0.01
	Blood	0.02	0.01	0.02	0.00	0.03	0.01	0.02	0.01	0.02	0.01
	Bone marrow	4.15	1.62	5.18	2.13	6.81	0.90	5.70	1.29	3.48	0.90
	Total body	83.81	7.02	98.64	13.21	90.39	8.06	86.64	4.83	93.13	11.82
	Total RES	82.84	7.04	97.22	13.12	87.82	7.98	85.46	4.86	92.22	11.81
7 days	Lymph nodes	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	Heart	0.01	0.01	0.03	0.02	0.04	0.01	0.03	0.01	0.02	0.01
	Lungs	7.53	8.40	26.28	7.93	30.87	12.85	28.29	8.01	21.09	6.88
	Liver	71.14	16.69	56.01	10.94	53.28	7.97	51.58	9.30	58.57	7.75
	Spleen	2.73	0.77	3.44	0.61	4.22	1.34	3.16	0.66	2.93	0.17
	GI tract	0.12	0.06	0.24	0.08	0.27	0.16	0.17	0.06	0.20	0.05
	Ovary	0.00	0.00	0.01	0.00	0.02	0.01	0.01	0.00	0.01	0.01
	Testicles	0.01	0.01	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.01
	Kidneys	0.03	0.01	0.12	0.02	0.16	0.02	0.15	0.06	0.16	0.11
	Muscles	0.17	0.12	0.28	0.27	0.35	0.26	0.31	0.20	0.32	0.17
	Brain	0.00	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00
	Blood	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00	0.01	0.00
	Bone marrow	5.11	1.72	6.55	1.54	5.86	1.34	6.15	0.83	4.94	0.42
	Total body	86.86	13.95	92.98	6.53	95.09	11.30	89.86	5.29	88.27	6.89
	Total RES	86.50	13.89	92.28	6.51	94.23	11.33	89.18	5.18	87.54	6.78

In study B (Tables 3 and 5) only a few significant increases were found: polysorbate 20 at 30 min (factor 2, $p < 0.05$) and polysorbate 80 (factor 4–6, $p < 0.05$). Polysorbate 80 was the only study B surfactant which was able to increase the spleen uptake significantly up to 24 h and non-significantly at 7 days ($p < 0.1$).

Lungs

The lung levels are very variable, generally leading to higher standard deviations than are observable in other organs. Up to 24 h, the lung uptake was increased by all surfactants for various amounts (factor 2–38) with the exception of polysorbate 80 at 6 h. Because of the very high standard deviation, these increases were often not significant (n.s., 30 min: poloxamer 338, poloxamine 908, polysorbate 60; 2 h: poloxamer 184, polysorbate 80; 6 h: poloxamer 184, 338, Brij 35; 24 h: all study A surfactants and Brij 35). At 7 days a significant increase was found for all study B surfactants. The most dramatic increase over all times was exhibited by poloxamer 407 (factor 8–38) and some polysorbates. After 7 days, a reduction of the lung values for all poloxamers/poloxamines down to the reference value was observed except for poloxamer 407, while the polysorbates still showed very high values for the

TABLE 4

Body distribution (in μg nanoporticles per g tissue weight) after intravenous administration of uncoated and surfactant-coated polymethyl[2-^{14}C]methacrylate nanoparticles to rats (study A)

Time	Sample	Reference		Poloxamer 188		Poloxamer 407		Poloxamer 184		Poloxamer 338		Poloxamine 908	
		μg/g	S.D.	μg/g	S.D.	μg/g	S.D.	μg/g	S.D.	μg/g	S.D.	μg/g	S.D.
30 min	Lymph nodes	0.46	0.24	1.79	1.13	2.15	1.13	4.63	2.21	11.20	11.31	3.77	2.67
	Heart	2.33	2.09	7.33	3.83	11.57	4.39	27.05	10.69	28.31	9.34	22.69	10.58
	Lungs	51.86	38.46	316.20	220.66	844.51	196.04	335.15	179.13	201.85	158.63	208.00	199.48
	Liver	258.19	51.55	189.47	49.68	96.82	47.14	161.48	24.93	47.01	11.01	65.56	39.51
	Spleen	111.79	50.30	315.34	158.39	404.96	193.36	447.16	131.15	1 216.96	466.05	993.06	419.03
	GI tract	1.31	0.42	3.06	1.37	6.01	3.39	3.62	2.12	3.99	1.17	4.93	1.93
	Ovary	2.45	0.69	6.91	3.07	8.82	0.89	14.53	1.88	19.08	2.23	13.81	4.22
	Testicles	0.11	0.01	0.15	0.02	0.18	0.02	0.39	0.38	0.50	0.05	0.51	0.25
	Kidneys	4.58	2.55	15.64	4.80	19.51	8.25	21.07	6.39	26.00	5.13	24.53	14.41
	Muscles	0.39	0.32	1.13	0.68	1.15	0.31	1.74	0.31	2.20	0.89	1.91	0.78
	Brain	0.70	0.67	3.89	2.07	6.03	2.77	5.91	2.95	10.17	5.40	6.69	5.07
	Blood	0.62	0.31	0.69	0.57	4.03	1.02	1.20	0.87	50.02	21.84	63.60	52.69
	Bone marrow	19.59	10.97	10.18	9.04	8.39	0.23	46.62	12.80	12.31	8.50	11.76	9.71
2 h	Lymph nodes	0.30	0.15	1.35	1.11	1.65	0.73	2.12	1.13	2.10	0.61	2.27	1.39
	Heart	1.03	0.89	5.47	2.34	5.24	2.14	22.13	13.59	11.04	3.44	10.57	2.97
	Lungs	33.44	23.91	241.45	137.38	745.54	391.68	312.09	291.49	262.90	169.61	262.50	186.70
	Liver	258.15	59.05	207.08	20.10	103.03	60.20	164.77	30.04	62.69	17.87	74.97	21.75
	Spleen	132.25	46.88	452.99	137.63	593.07	396.00	397.82	198.75	1 546.32	742.87	1 671.03	429.72
	GI tract	1.09	0.43	1.63	0.59	2.02	0.43	2.05	0.74	2.24	1.05	2.57	1.53
	Ovary	2.32	0.28	5.24	1.88	3.51	2.16	8.47	1.78	6.26	2.35	8.11	2.28
	Testicles	0.08	0.01	0.15	0.06	0.12	0.01	0.12	0.01	0.24	0.05	0.29	0.07
	Kidneys	2.61	1.16	9.83	2.75	9.12	3.19	11.44	4.43	11.61	3.37	10.95	3.44
	Muscles	0.19	0.03	1.09	0.68	0.89	0.49	1.37	0.93	1.46	0.71	1.22	0.35
	Brain	0.42	0.42	2.77	1.11	2.42	1.19	3.31	2.33	4.09	2.63	4.12	2.33
	Blood	0.18	0.07	0.15	0.07	0.31	0.13	0.29	0.18	14.60	14.38	14.37	12.65
	Bone marrow	18.92	5.04	13.72	7.21	14.06	3.81	61.57	29.95	11.77	5.44	11.32	3.42
6 h	Lymph nodes	0.36	0.23	1.30	1.10	0.98	0.37	1.49	0.61	0.89	0.17	0.95	0.22
	Heart	0.78	0.28	5.17	3.65	4.20	0.71	7.19	2.67	5.51	2.14	3.71	1.47
	Lungs	21.20	12.65	286.81	202.91	799.96	485.68	385.69	339.69	341.18	258.72	281.31	191.94
	Liver	214.55	46.04	225.68	61.04	110.76	45.41	173.98	50.08	79.21	26.07	120.42	36.63
	Spleen	58.25	24.93	304.15	113.37	549.59	379.73	308.47	224.78	1 594.88	744.21	1 297.78	674.52
	GI tract	0.56	0.24	0.63	0.29	0.97	0.20	0.76	0.25	0.79	0.09	1.22	0.33
	Ovary	0.93	0.08	3.05	1.18	2.31	0.32	4.54	1.12	3.57	0.84	3.77	0.65
	Testicles	0.09	0.01	0.10	0.02	0.10	0.02	0.10	0.02	0.14	0.00	0.11	0.01
	Kidneys	1.84	0.26	4.84	1.53	7.48	1.76	5.75	1.41	5.56	1.81	6.14	1.93
	Muscles	0.23	0.04	0.73	0.26	0.91	0.30	0.87	0.41	0.79	0.29	0.79	0.32
	Brain	0.20	0.10	1.11	0.72	1.58	0.62	1.21	0.64	2.10	1.39	0.90	0.53
	Blood	0.07	0.02	0.12	0.05	0.14	0.08	0.23	0.13	3.43	3.92	1.73	2.17
	Bone marrow	12.28	5.31	17.23	15.19	20.42	6.62	54.55	29.85	12.63	5.20	16.66	14.26
24 h	Lymph nodes	0.54	0.20	0.98	0.53	0.79	0.06	1.16	0.19	0.70	0.31	0.90	0.37
	Heart	0.39	0.19	0.70	0.10	1.16	0.14	2.39	0.95	1.22	0.16	1.33	0.53

(continued)

TABLE 4 (continued)

Time	Sample	Reference		Poloxamer 188		Poloxamer 407		Poloxamer 184		Polaxamer 338		Poloxamine 908	
		μg/g	S.D.	μg/g	S.D.	μg/g	S.D.	μg/g	S.D.	μg/g	S.D.	μg/g	S.D.
24 h	Lungs	12.69	3.53	57.58	44.03	149.28	128.72	45.43	33.49	52.63	56.92	49.19	55.40
	Liver	192.75	19.89	187.66	25.51	149.83	14.66	191.70	16.52	69.19	12.17	75.53	26.42
	Spleen	115.28	25.61	577.20	274.90	1 070.83	452.34	457.27	81.60	2 236.95	750.72	2 060.26	178.59
	GI tract	0.31	0.11	0.41	0.10	0.54	0.14	0.77	0.20	0.79	0.35	0.95	0.34
	Ovary	0.71	0.09	0.98	0.58	1.67	0.73	1.71	0.45	5.32	0.39	3.74	1.74
	Testicles	0.08	0.01	0.16	0.12	0.13	0.02	0.13	0.02	0.09	0.01	0.11	0.01
	Kidneys	1.61	0.61	2.48	1.03	4.16	1.73	4.11	0.48	2.67	0.37	2.67	1.02
	Muscles	0.14	0.05	0.28	0.12	0.35	0.12	0.27	0.07	0.26	0.11	0.31	0.20
	Brain	0.10	0.01	0.18	0.04	0.31	0.07	0.29	0.14	0.20	0.08	0.29	0.27
	Blood	0.07	0.07	0.04	0.01	0.04	0.01	0.26	0.18	0.19	0.23	0.15	0.12
	Bone marrow	15.74	6.50	16.68	6.44	50.08	38.38	78.46	39.74	38.43	32.78	18.10	5.38
7 days	Lymph nodes	0.78	0.29	0.59	0.25	0.86	0.38	1.10	0.30	0.74	0.28	1.09	0.26
	Heart	0.15	0.04	0.20	0.05	0.48	0.19	0.73	0.38	0.46	0.09	0.55	0.23
	Lungs	5.82	2.71	12.86	6.51	48.19	37.71	5.37	2.57	4.52	2.33	4.15	2.23
	Liver	195.25	51.53	194.04	46.94	136.06	40.07	178.33	31.38	49.72	12.95	75.15	25.34
	Spleen	155.16	21.23	463.74	210.05	943.85	278.52	508.05	151.10	2 216.52	189.00	2 079.49	348.21
	GI tract	0.14	0.08	0.19	0.18	0.38	0.16	0.35	0.21	0.69	0.21	0.61	0.37
	Ovary	0.32	0.11	1.05	0.52	0.87	0.45	0.46	0.09	3.05	0.16	2.35	0.37
	Testicles	0.08	0.02	0.10	0.07	0.23	0.17	0.07	0.01	0.29	0.27	0.39	0.34
	Kidneys	0.39	0.05	0.45	0.13	0.78	0.15	0.64	0.35	0.58	0.10	0.69	0.20
	Muscles	0.10	0.04	0.40	0.33	0.32	0.27	0.48	0.48	0.45	0.22	0.58	0.63
	Brain	0.08	0.02	0.07	0.02	0.09	0.04	0.08	0.01	0.09	0.02	0.08	0.01
	Blood	0.03	0.01	0.02	0.00	0.03	0.02	0.06	0.05	0.03	0.01	0.03	0.01
	Bone marrow	26.21	13.84	16.74	5.75	23.01	4.15	57.42	23.72	30.73	15.25	33.73	10.43

lungs.

Bone marrow

In the bone marrow, the uptake of nanoparticles was increased or decreased by coating with surfactants. The most significant and striking increase was caused by poloxamer 184 (factor 2–5) which is the only surfactant that increased the uptake persistently and significantly up to 7 days ($p < 0.05$). In study B only polysorbate 80 and polysorbate 20 showed an effect (significant increase: polysorbate 80 at 6 h and 24 h, polysorbate 20 at 30 min). The only surfactant that decreased the bone marrow values for every time period examined was Brij 35, however none of these decreases or of those induced by other surfactants were significant.

Lymph nodes

The uptake by the lymph nodes was increased by all surfactants up to 2 h (factor 1.3–24) except Brij 35 which showed no increase after 2 h. This increase was not significant for many surfactant (n.s.: poloxamer 407, 338 at 30 min; at 2 h poloxamer 188, polysorbate 20). The highest values were observed at 30 min for poloxamer 338 (factor 24, $p < 0.1$), poloxamer 184 (factor 10, $p < 0.025$), poloxamine 908 (factor 8, $p < 0.05$) and polysorbate 80 (factor 7, $p < 0.05$). At 6 h, a significant increase was found for poloxamer 407, 184, 338, poloxamine 908 and polysorbate 80. At 24 h, only poloxamer 407, 184 and polysorbate 80 showed a significant increase. In all cases, this increase became smaller in extent with increasing time. No significant increase was observed after 7 days.

Kidneys

All surfactants increased the uptake by the kidneys for various amounts (factor 1.2–10) up to

TABLE 5

Body distribution (in μg / g tissue weight) after intravenous administration of uncoated and surfactant-coated polymethyl[2-^{14}C]methacrylate nanoparticles to rats (study B)

Time	Sample	Reference		Polysorbate 60		Polysorbate 80		Polysorbate 20		Brij 35	
		μg/g	S.D.	μg/g	S.D.	μg/g	S.D.	μg/g	S.D.	μg/g	S.D.
30 min	Lymph nodes	0.58	0.17	1.57	0.45	3.95	2.68	1.41	0.25	0.88	0.10
	Heart	2.35	2.07	12.98	3.96	24.54	7.17	13.55	3.94	7.84	2.95
	Lungs	110.21	110.32	484.47	348.48	936.98	320.75	426.61	93.60	469.27	305.07
	Liver	246.27	44.23	155.33	23.20	89.59	28.54	160.34	17.21	222.18	65.42
	Spleen	81.16	16.43	183.95	134.16	289.55	182.07	167.64	61.95	96.22	20.49
	GI tract	2.73	2.22	2.84	2.21	7.31	3.65	3.83	1.58	4.86	4.41
	Ovary	0.96	0.52	6.10	0.69	13.77	7.63	10.77	2.30	5.03	0.96
	Testicles	0.11	0.00	0.19	0.02	0.23	0.05	0.14	0.01	0.15	0.01
	Kidneys	4.52	2.69	15.39	2.88	44.60	22.53	14.74	2.49	11.57	3.39
	Muscles	0.39	0.16	2.38	1.05	2.14	1.01	2.10	1.04	0.94	0.54
	Brain	1.23	1.34	6.86	1.96	11.71	3.44	4.80	0.48	4.45	1.83
	Blood	0.29	0.05	0.54	0.08	1.23	0.70	0.51	0.19	0.74	0.19
	Bone marrow	11.03	6.78	20.02	13.38	12.20	4.93	35.61	14.15	7.33	3.65
2 h	Lymph nodes	0.63	0.09	1.01	0.10	2.42	0.59	0.81	0.16	0.55	0.07
	Heart	1.40	1.26	9.50	2.35	15.89	6.56	6.73	2.43	4.02	0.82
	Lungs	96.56	118.66	756.14	459.17	165.48	136.41	679.24	472.05	352.86	183.68
	Liver	236.65	41.49	173.21	54.04	160.88	30.99	159.34	59.40	209.65	101.78
	Spleen	141.50	64.05	115.11	39.75	494.10	145.15	104.21	67.92	114.69	86.88
	GI tract	2.37	3.12	2.70	0.76	4.28	1.58	2.10	1.34	1.82	0.70
	Ovary	0.73	0.04	5.74	1.15	18.33	3.13	3.32	1.20	2.95	0.29
	Testicles	0.10	0.02	0.11	0.01	0.12	0.03	0.11	0.02	0.12	0.05
	Kidneys	3.61	3.03	10.51	0.74	36.47	17.43	9.30	1.90	6.24	1.13
	Muscles	0.33	0.22	1.37	0.73	1.79	0.79	0.96	0.45	0.92	0.44
	Brain	0.48	0.35	4.24	1.38	5.27	3.43	3.39	2.38	1.70	0.54
	Blood	0.15	0.09	0.22	0.11	0.23	0.02	0.15	0.02	0.21	0.04
	Bone marrow	12.95	3.52	27.06	17.94	16.09	2.36	21.58	13.56	8.63	6.37
6 h	Lymph nodes	0.62	0.17	0.68	0.17	1.82	0.33	0.61	0.18	0.66	0.16
	Heart	1.06	0.89	4.95	2.30	9.76	4.22	3.99	0.90	3.71	1.42
	Lungs	112.67	172.04	564.04	346.54	58.70	36.49	578.78	393.21	556.46	402.78
	Liver	234.05	44.67	172.87	41.77	195.36	22.68	150.57	38.51	190.24	67.62
	Spleen	103.25	29.10	130.03	48.42	649.78	278.74	103.00	34.88	119.20	65.38
	GI tract	0.61	0.20	0.98	0.67	2.77	0.98	0.76	0.22	1.00	0.58
	Ovary	0.73	0.42	2.80	0.25	11.01	0.32	1.89	0.84	4.25	0.86
	Testicles	0.10	0.01	0.09	0.01	0.10	0.01	0.09	0.02	0.10	0.03
	Kidneys	3.27	2.93	6.17	1.22	26.78	6.26	4.50	0.85	6.18	1.61
	Muscles	0.33	0.22	0.79	0.34	1.09	0.57	0.73	0.32	0.58	0.21
	Brain	0.35	0.30	1.91	0.53	3.46	2.53	1.17	0.69	1.30	0.50
	Blood	0.08	0.04	0.12	0.07	0.09	0.01	0.10	0.04	0.11	0.03
	Bone marrow	13.81	4.36	26.42	17.64	25.07	7.20	22.47	10.27	12.98	6.46
24 h	Lymph nodes	0.78	0.09	0.76	0.17	1.18	0.32	0.63	0.09	0.63	0.25
	Heart	0.60	0.25	2.05	0.52	4.38	0.88	2.40	0.79	1.20	0.32
	Lungs	44.67	34.99	259.69	62.96	212.80	79.16	334.76	114.19	91.21	61.20

(continued)

TABLE 5 (continued)

Time	Sample	Reference		Polysorbate 60		Polysorbate 80		Polysorbate 20		Brij 35	
		μg/g	S.D.	μg/g	S.D.	μg/g	S.D.	μg/g	S.D.	μg/g	S.D.
24 h	Liver	220.89	28.60	209.95	41.75	238.46	35.69	176.60	12.35	236.17	30.32
	Spleen	136.28	17.01	127.34	22.82	429.21	83.84	145.25	30.79	185.37	68.54
	GI tract	0.59	0.78	0.42	0.12	1.44	1.25	0.50	0.14	0.42	0.20
	Ovary	0.44	0.16	2.26	0.25	4.87	4.25	2.27	0.44	0.76	0.28
	Testicles	0.10	0.03	0.16	0.06	0.10	0.02	0.09	0.01	0.10	0.01
	Kidneys	2.08	0.74	4.75	0.84	7.88	1.69	4.14	0.93	3.04	0.13
	Muscles	0.30	0.23	0.58	0.17	0.86	0.49	0.42	0.17	0.37	0.20
	Brain	0.18	0.10	0.48	0.12	2.38	0.56	0.46	0.10	0.24	0.07
	Blood	0.04	0.01	0.04	0.01	0.05	0.02	0.04	0.01	0.04	0.01
	Bone marrow	20.07	7.80	25.05	10.49	32.97	4.28	27.49	6.24	16.71	4.36
7 days	Lymph nodes	0.67	0.07	0.88	0.22	0.89	0.34	0.58	0.19	0.67	0.19
	Heart	0.33	0.21	0.82	0.45	1.16	0.22	0.68	0.08	0.69	0.24
	Lungs	167.15	195.25	520.16	204.74	573.14	199.34	524.93	190.68	417.28	120.89
	Liver	219.60	63.85	171.72	44.32	154.22	30.77	132.63	30.06	162.33	39.06
	Spleen	164.25	42.56	210.50	42.75	221.88	49.26	164.26	17.77	172.87	20.05
	GI tract	0.13	0.06	0.26	0.09	0.29	0.16	0.18	0.04	0.20	0.04
	Ovary	0.43	0.15	1.70	0.69	3.78	2.22	1.34	0.56	0.95	0.02
	Testicles	0.07	0.03	0.07	0.02	0.05	0.01	0.05	0.01	0.06	0.02
	Kidneys	0.55	0.21	1.90	0.41	2.64	0.54	2.18	0.86	2.43	1.53
	Muscles	0.14	0.11	0.25	0.23	0.31	0.25	0.27	0.17	0.26	0.14
	Brain	0.07	0.02	0.11	0.02	0.15	0.03	0.09	0.03	0.12	0.04
	Blood	0.02	0.00	0.03	0.01	0.02	0.01	0.02	0.00	0.02	0.00
	Bone marrow	22.31	7.37	28.92	7.20	25.75	7.20	26.50	4.48	20.30	2.36

7 d. Even though a few increases were very small, nearly all were significant. Non-significant increases were observed for Brij 35 at 2 h, polysorbate 60, 20 and Brij 35 at 6 h, poloxamer 188, poloxamine 908 at 24 h and after 7 days for poloxamer 188 and 184. The highest values were attained after 30 min (factor 3–10) and normally decreased quickly with increasing time. Polysorbate 80 yielded the highest increase in kidney uptake.

Heart

All surfactants increased the heart uptake up to 7 days (factor 1.3–22), whereby this increase after short times was much higher than that after 7 days. All 65 values except 3 (poloxamer 188 at 6 h and 7 days, polysorbate 60 after 7 days) were significant ($p < 0.05$). Again the values decreased with increasing time. The best surfactants were poloxamer 184, poloxamer 338, poloxamine 908 and polysorbate 80 (factor 3–22).

Brain

In the brain, the uptake of nanoparticles was increased by all surfactants up to 24 h (factor 1.3–15). Nearly all differences were significant (non-significant: poloxamer 188, 338 at 6 h; poloxamine 908 and Brij 35 at 24 h). The most effective increase was caused by polysorbate 80, 60 and all study A surfactants except poloxamer 188. At later times the values decreased and approached more and more those of the reference.

Muscles

In the muscles an increase in uptake also was found (factor 1.2–8) up to 7 days. Up to 6 h, all increases were significant ($p < 0.05$) except poloxamer 188/30 min, and Brij 35/30 min and 6 h.

TABLE 6

Variation of the organ weights after intravenous administration of coated particles to rats after 30 min, 24 h and 7 days

Sample	Organ	30 min	S.D.	24 h	S.D.	7 days	S.D.
Reference A	Lungs	1.320	0.147	1.212	0.239	1.298	0.323
	Liver	9.324	2.030	10.779	0.988	10.402	1.936
	Spleen	0.525	0.121	0.533	0.031	0.509	0.067
Poloxamer 188	Lungs	1.148	0.194	1.331	0.136	1.210	0.122
	Liver	9.757	1.710	10.707	1.156	9.954	1.051
	Spleen	0.568	0.175	0.574	0.153	0.523	0.045
Poloxamer 407	Lungs	1.385	0.320	1.210	0.068	1.282	0.106
	Liver	8.882	0.798	9.906	1.104	10.911	1.062
	Spleen	0.491	0.094	0.500	0.032	0.506	0.069
Poloxamer 184	Lungs	1.299	0.076	1.305	0.220	1.316	0.078
	Liver	9.114	1.569	10.539	0.840	10.418	1.018
	Spleen	0.569	0.122	0.636	0.039	0.531	0.036
Poloxamer 338	Lungs	1.299	0.240	1.243	0.139	1.143	0.099
	Liver	9.594	1.649	11.245	1.193	10.415	0.588
	Spleen	0.525	0.098	0.623	0.150	0.601	0.078
Poloxamine 908	Lungs	1.281	0.179	1.286	0.167	1.299	0.223
	Liver	9.221	1.536	11.033	0.471	10.155	1.160
	Spleen	0.634	0.182	0.619	0.180	0.577	0.068
Reference B	Lungs	1.383	0.352	1.464	0.069	1.284	0.093
	Liver	9.693	0.682	9.960	0.336	8.992	0.592
	Spleen	0.486	0.031	0.448	0.097	0.454	0.032
Polysorbate 60	Lungs	1.460	0.311	1.532	0.196	1.436	0.201
	Liver	9.962	1.616	10.633	0.533	9.092	0.537
	Spleen	0.485	0.057	0.553	0.057	0.455	0.060
Polysorbate 80	Lungs	1.351	0.176	1.349	0.061	1.453	0.240
	Liver	9.123	0.722	8.444	0.810	9.542	0.964
	Spleen	0.504	0.076	0.440	0.054	0.514	0.075
Polysorbate 20	Lungs	1.187	0.092	1.639	0.216	1.489	0.190
	Liver	10.138	1.043	10.115	1.330	10.517	0.536
	Spleen	0.454	0.030	0.502	0.051	0.512	0.075
Brij 35	Lungs	1.309	0.136	1.283	0.122	1.287	0.083
	Liver	9.484	0.919	10.200	0.774	9.456	0.772
	Spleen	0.485	0.022	0.477	0.095	0.437	0.013

The values are standardized to rats with a body weight of 200 g.

At 24 h, significant increases were found for poloxamer 188, 407, 184 and polysorbate 60 ($p < 0.05$). After 7 days only poloxamer 338 showed a significant increase ($p < 0.05$). The values again decreased rapidly at later time points.

Ovary

All surfactants increased uptake in the case of the ovary up to 7 days (factor 1.4–25) but only a few of those increases were significant due to the reduced number of animals ($n = 2$ females), although the factors were sometimes very high. The highest increase was exhibited by polysorbate 80 (factor 9–25). On a basis of μg nanoparticles per g organ, even the reference yielded quite high ovary values. In contrast to the ovaries, the uptake of nanoparticles by the testicles was much lower.

Testicles

Only in a few cases was a small increase in uptake detected. Poloxamer 338/30 min (factor 5) represents the sole example of an important increase being observed.

GI tract

A few significant increases were found (factor

2–5). The most effective surfactants were poloxamer 407, 338, poloxamine 908 and polysorbate 80.

Organ weights

Changes in organ weights are an indication of toxicity. The organ weights of lungs, liver and spleen were determined after 30 min, 24 h and 7 days (Table 6). Only very small increases were found. After comparison of the 24 h and 7 day values with those for 30 min that served as reference, only four random, statistically significant differences out of 66 were found. Therefore, it can be concluded that the particles do not influence organ weight.

Discussion

Body distribution experiments are very arduous. Therefore, an in vitro method that is capable of predicting the fate of colloidal drug carriers after injection is a much desired requirement. Tröster and Kreuter (1988) investigated the possibility of using contact angle measurements for such a purpose. In addition, such in vitro investigations regarding interactions of coating materials with polymers and also with blood components or cells could provide insight into the mechanisms that may influence the body distribution.

Surfactants were classified in four different groups (Table 1) according to their contact angles on the polymeric material used for the manufacture of the nanoparticles. Comparison of the results with the data from animal studies taken from the literature suggested that these surfactant groups have a characteristic potential for changing the body distribution of intravenously injected nanoparticles. Group IV seemed to possess the greatest potential to reduce the RES uptake of intravenously administered nanoparticles. The results of the present study, however, demonstrate that the above-described observations cannot be generalized: Although the poloxamers and poloxamines used in the first part of the present study (part A) seemed to indicate a correlation of the contact angles with their ability to reduce RES uptake, especially liver uptake (Tables 2 and 4), the surfactants used in the second part of the study (part B; involving polysorbates and polyoxyethylene (23) lauryl ether) did not show such a correlation: The group IV surfactant polyoxyethylene (23) lauryl ether, which should have been most powerful in reducing liver uptake according to the above-outlined hypothesis, did not reduce liver uptake significantly (Tables 3 and 5). Polysorbate 80, on the other hand, which should have been much less effective, yielded a considerable degree of reduction in liver uptake (Tables 3 and 5).

Nevertheless, a number of important general observations can be made: All surfactants decrease liver uptake and increase uptake in all other organs and tissues except the testicles and bone marrow. The decrease in liver uptake and increase in other parts of the body were not significant in every case for surfactants especially at later time points.

Poloxamer 338 and poloxamine 908 were the most powerful surfactants in decreasing the liver uptake and increasing the blood levels of nanoparticles. They increased blood concentrations up to 6 h by a factor of about 100 or more, although large variations in concentrations decreased the level of statistical significance after 2 h. These results confirm data reported previously by Davis and co-workers (Illum and Davis, 1983; 1984; Illum et al., 1986; 1987). Both surfactants also yielded very high spleen concentrations. The uptake of nanoparticles determined on the basis of the weight of nanoparticles per g of tissue base revealed 10–45-fold higher nanoparticle concentrations in the spleen than in the liver. This observation demonstrates that spleen uptake is not necessarily, probably even infrequently, the result of liver spillover, confirming earlier observations by Wilkins and Myers (1966). Possibly the alteration in the nature of the surface of these nanoparticles leads to the interaction with different opsonins which in turn direct the particles to different phagocytosing cells. The lung uptake of nanoparticles was also much higher after coating, this time with all of the surfactants, in some cases up to a factor of over 35. The lung uptake, however, was very variable. Although these findings could indicate that the high uptake would be the

result of secondary agglomeration of the surfactant coated nanoparticles after injection and subsequent lung capillary filtration of large aggregates, in vitro investigations did not reveal the formation of large aggregates at any significant level. The aggregation tendency of surfactant-coated nanoparticles in plasma determined by light microscopy or laser light scattering also did not correlate with the observed lung uptake data (unpublished observations). Therefore, the rather high and variable lung uptake of nanoparticles after coating with surfactants remains unexplained.

Poloxamer 184, but not poloxamer 407, yielded a very significant increase in bone marrow uptake up to a level of 16% of the dose. The relatively low uptake with poloxamer 407 somewhat contradicts earlier results by Davis and Illum (1986) who reported a bone marrow uptake of 70% with polystyrene nanoparticles. It should be borne in mind, however, that their particle sizes (about 60 nm) may have allowed the particles to traverse readily through the fenestration of the bone marrow blood capillaries (about 80 nm), while the larger particles used in this study (130 nm) may do so only with difficulty. For this reason, the high uptake of 16% after 24 h and 14% after 7 days with poloxamer 184 is very surprising and noteworthy.

Another important observation is that the surfactants also increase uptake in the organs and tissues that do not belong to the reticuloendothelial system. This enhanced uptake was significant for most of the surfactants and persisted for up to 7 days. It was especially pronounced in the heart (increase by a factor up to 22), brain (up to 13-fold), kidneys (up to 10-fold) and muscles (up to 8-fold). With the latter organs and tissues, polysorbate 80 and poloxamer 184 were the most powerful surfactants in increasing organ uptake. It is possible that the particles are not engulfed by cells of these organs, but rather adhere to the endothelial cells lining the vasculature as suggested by Illum et al. (1987).

Labeling of the nanoparticles with ^{14}C proved to be a more reliable and accurate method for the determination of the fate of nanoparticles or other colloidal drug carriers after injection as compared to gamma-scintigraphy after labeling with a gamma-emitter. The latter method does not allow an accurate quantitative and even qualitative determination in the cases of smaller organs and of tissues with lower levels of uptake of labeled particles. Gamma-scintigraphy, for instance, would not allow accurate distinction between the uptake for liver and that for spleen due to the vicinity of these organs. ^{14}C labeling also ensures high stability of the label when the labeled atom is incorporated into the polymer backbone.

Conclusions

All surfactants used in this study decrease the liver uptake and increase the uptake in other organs and tissues. Poloxamer 338 and poloxamine 908 were the most powerful surfactants in enhancing blood levels of the nanoparticles and in increasing spleen uptake while reducing liver uptake. Polysorbate 80 and poloxamer 184 yielded the highest concentrations in the heart, brain, kidneys and muscles and therefore may be most useful in directing nanoparticles away from the reticuloendothelial system to other parts of the body. However, contact angles of surfactants on polymer surfaces do not allow prediction of the fate of the nanoparticles in the body after coating with these surfactants.

None of the surfactants altered the weights of the organs which may be an indication of their relatively low toxicity in vivo at low concentrations.

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