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Evaluation of long-circulating nanoparticles using biodegradable ABA triblock copolymers containing of poly(L-lactic acid) A-blocks attached to central poly(oxyethylene) B-blocks in vivo

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Abstract

The nanoparticles (mean diameter 152–377 nm) consisting of the ABA triblock copolymers (M_w 29000–147000) containing poly(L-lactic acid) (PLA) A-blocks attached to central poly(oxyethylene) (PEG) B-blocks (PEG M_w 6600, 20000) (PLA-PEG-PLA) were prepared, and the effects of the polymer characteristics on the pharmacokinetics of the nanoparticles and the biodistribution of the nanoparticles were studied. Progesterone was used as a model drug. We could make the long-circulating nanoparticles using the triblock copolymer. The degree of burst in early phase, which was estimated by the Co values (Progesterone concentration at time 0) and the circulating-time were affected by the total M_w, the PEG content, the PEG M_w and the M_w/M_n (M_n: where M_n is the number average molecular weight) ratio. The purification of the polymer was an important factor for the control of the burst. These triblock copolymer nanoparticles induced reduction in the liver and spleen uptake of the nonoparticles. These phenomena are probably explained by the avoidence of adsorption of opsonin to the particles as a result of the orientation of PEG on the surface of the particles.

Keywords: In vivo; Long-circulating; Nanoparticle; Poly(L-lactic acid); Poly(oxyethylene); Triblock copolymer

1. Introduction

Nanoparticles are colloidal particles ranging in size from 10 to 1000 nm, and are made of artificial or natural polymers. The rapid removal of

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intravenously administered nanoparticles by the reticuloendothelial system (RES) has been identified as the major barrier to delivery of drugs to the target organ or tissue sites (Guiot and Couvreur, 1986). Illum et al. (1987) showed the model polystyrene nanospheres of 60 nm in diameter, which were coated with polyoxyethylene-polyoxypropylene, could avoid the uptake by the mononuclear phagocyte system. It has been reported that nanoparticles using diblock copolymers containing of poly(oxyethylene) (PEG) and poly(L-lactic acid) (PLA) (Verrecchia et al., 1995), or PEG and poly(L-lactic-co-glycolic acid) (PLGA) (Gref et al., 1994). These phenomena were probably explained by avoiding the adsorption of opsonin to the particles (Davis et al., 1993).

We have reported the synthesis of the ABA triblock copolymers containing of PLA A-blocks attached to central PEG B-blocks (PLA-PEG-PLA), the possibility of the long-circulating nanoparticles using this triblock copolymer (Nakada et al., 1997), and the evaluation of the characteristics of nanoparticles consisted of this ABA triblock copolymer in vitro (Matsumoto et al., 1998).

In this paper, we describe the effects of the polymer characteristics on the pharmacokinetics of the nanoparticles, and the biodistribution of the nanoparticles.

2. Materials and methods

2.1. Materials

^{3}H-progesterone was purchased from Amersham (UK). Progesterone was purchased from Sigma (St. Louis, MO). 4-Hydroxy-2-phenylbenzothiazol which was used as internal standard, was synthesized in our laboratory. Dichloromethane (DCM), dimethylsulfoxide (DMSO), phosphoric acid, Tween80 of reagent grade, methanol of HPLC grade, and PEG (molecular weight (M_w); 6600 and 20000) were purchased from Wako Pure Chemical Industries, (Osaka, Japan). Polyvinyl alcohol (namely, PVA; PVA-203, Kuraray Company, Tokyo, Japan) was used as supplied. L-lactide was purchased from Boehringer Ingelheim (Germany).

2.2. Synthesis of polymer

Bulk polymerization of L-lactide initiated by PEG's was carried out in 50 wt% Tin(II) bis(2-ethylhexanonate) chloroform according to Leenslag and Pennigs' method (Leenslag and Pennigs, 1987) with some minor modifications. All polymerized products were fractionated and purified by the fractional precipitation using chloroform and methanol (Nakada et al., 1997). The weight-average molecular weight (M_w) and the molecular weight distribution (M_w/M_n, where M_n is the number-average molecular weight) of PLA-PEG-PLA measured by Gel permeation chromatograms (GPC). The GPC was performed using a model L-7200 GPC system (Hitachi, Tokyo) and a refractive index meter (detector, RI-8010, Tosoh, Tokyo, Japan) at a flow rate of 1.0 ml/min at 35°C. The PLA or PLA-PEG-PLA dissolved in 100 μl of chloroform were separated with GMHXL column (TSK-GEL Tosoh, Tokyo). The M_w was calibrated by the use of standard polystylene. The PEG content of polymers was measured by NMR. The composition and the properties of PLA-PEG-PLA used in this study are summarized in Table 1. All other chemicals were of reagent grade.

2.3. Preparation of nanoparticles containing drugs

A solvent evaporation method (Vauthier-Holtzscherer et al., 1991) was used to prepare the

Table 1
Profiles of triblock copolymers for the preparation of nanoparticles

Lot	PEG M_w	M_W	M_w/M_n	PEG contents (%)
A	0	129 000	1.9	0
B	6600	147 000	2.0	5.2
C	6600	119 000	1.6	10.3
C-1	6600	83 000	11.7	10.5
F	6600	29 000	2.0	23.8
E	20 000	121 000	1.3	15.8

nanoparticles. Drug (4 mg) and polymer (40 mg) were dissolved in 4 ml of dichloromethane. The solution was loaded into a 10 ml syringe to which a tubelcrine needle was attached and then added dropwise to a polyvinyl alcohol solution (0.5% w/v, 16 ml). After homogenization (2 min), the emulsion was sonicated for 5 min. And after the dichloromethane in the emulsion was evaporated under reduced pressure and nanoparticles were obtained as dispersions. Free drug was removed by GPC with Bio Rad Econo-Pac® 10DG (Bio Rad, CA, USA) using 0.5%w/v polyvinylalcohol solution. The particles diameters of nanoparticles were measured by the dynamic light scattering method (ELS-8000 Ohtsuka Electronics, Japan).

The drug content in the PLA-PEG-PLA nanoparticles was determined by the method as below. The 50 μl drug-loaded nanoparticles dispersion was added into the definite volume DMSO. The drug concentration in DMSO was determined by HPLC. For HPLC analysis, a reverse-phase Unisil Pack 250A (250 × 4.6 mm i.d., pore size 5 μm, GL science, Tokyo, Japan) was used and the column temperature was controlled at 35°C. The mobile phase was a mixture of methanol and water (75:25). The flow-rate was 1.0 ml/min and the absorbance of the eluate at 254 nm was monitored. The HPLC system consisted of a Model 600E flow pump, a model U6K injector, a model 481UV detector (Millipore, Waters Chromatography Div.).

To determine the trapping efficiency of drug to the nanoparticles, the nanoparticle suspension before GPC as described previously was subjected to the same procedure. The trapping efficiency of drug into the PLA-PEG-PLA nanoparticles was calculated as below.

Trapping efficiency (%) = amount of drug in the nanoparticle suspension after gel permeation chromatography/amount of drug in the nanoparticle suspension before gel permeation chromatography × 100

2.4. Analytical procedure of progesterone in plasma

To 0.1 ml of plasma were added 0.2 ml of water, 0.05 ml of acetonitrile, 0.2 ml DMSO, 0.05 ml of the internal solution (1 μg/ml, in acetonitile), and 5 ml of n-hexane. The mixture was shaken for 15 min. After the centrifugation (15 min at 800 × g), 4 ml of the organic supernatant was transferred to a clean tube and dried under a stream of nitrogen gas at 40°C. The residue was redissolved in 100 μl of a mixture of acetonitle and methanol (1:3), and a 20 μl aliquot was injected onto the HPLC column. The HPLC condition is similar to the above mentioned in Section 2.3.

2.5. Animal experiments

2.5.1. Plasma concentration

Male Wistar rats (8–10 weeks) fasted overnight received injections of drug loaded nanoparticles or drug in 0.5% PVA solution at a dose of 1 mg/5 ml/kg to the tail vein. The blood samples were withdrawn periodically from the jugular vein. The blood samples were then centrifuged, and 0.1 ml of each plasma sample was used for the HPLC assay.

2.5.2. Distribution

Male Wistar rats (8–10 weeks) fasted overnight received injections of ^{3}H-progesterone nanoparticles or ^{3}H-progesterone in 0.5% PVA solution at a dose of 1 mg/5 ml/kg to the tail vein. At regular intervals, test animals were killed. The concentration of ^{3}H-progesterone in blood, plasma, liver, spleen, lung, bone marrow, kidney, brain, heart, thymus, duodenum and inframandibular lymph was measured by liquid scintillation counting after digestion of the tissues with SOLUEN® 350 (Packard, USA).

3. Results

3.1. Long-circulating nanoparticles using triblock copolymer

Fig. 1 shows the plasma concentration of progesterone solution, progesterone loaded polymer C nanoparticles and polymer A nanoparticles at a dose of 1 mg/kg in rats. The plasma concentration of progesterone at 24 h after injection of

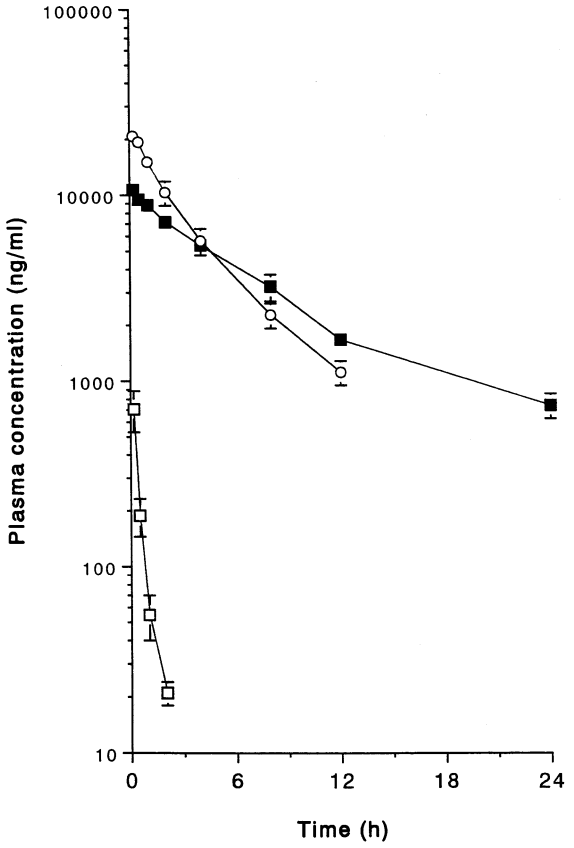

Fig. 1. Plasma concentration of progesterone after intravenous administration of progesterone solution or progesterone-loaded nanoparticles to rats at a dose of 1 mg/kg (mean ± S.D.; $n = 3–5$). □, progesterone solution; ■, progesterone-loaded polymer C nanoparticles; ○, progesterone-loaded polymer A nanoparticles.

polymer A nanoparticles was below the detection limit. Both the plasma half-life after injection of progesterone loaded nanoparticle was strongly enhanced compared with that of progesterone solution, and the plasma half-life of progesterone of polymer C nanoparticles was about twice as long as that of polymer A nanoparticles as shown in Table 2.

3.2. Effects of various polymers

3.2.1. M_w

The effect of the M_w on the plasma concentration of progesterone after administration of progesterone loaded nanoparticles is shown in Fig. 2. The M_w of PEG of both polymer is 6600. M_w of polymer F is 29000 and that of polymer C is 119000. The plasma half-life of polymer F nanoparticles was lower than that of polymer C nanoparticles, as shown in Table 2.

3.2.2. Content of PEG

Fig. 3 shows the plasma concentration of progesterone after administration of nanoparticles consisted of various PEG contents whose M_w was almost the same. The plasma concentration of progesterone at 24 h after injection of polymer A nanoparticles was below the detection limit. The order of the plasma half-life was polymer C (PEG content:10.3%), polymer B (PEG content:5.2%) and polymer A (PEG content:0%) nanoparticles, but the order of the Co which would be the parameter indicated the burst, is polymer A, polymer B and polymer C nanoparticles. Pharmacokinetics parameters are shown in Table 2.

3.2.3. M_w of PEG

The effects of M_w of PEG on the plasma concentration of progesterone were investigated using almost the same M_w polymer (A,C,E) whose M_w of PEG was different. As shown in Fig. 4, the plasma concentration at 24 h after injection of polymer A nanoparticles was under the detection limit. The Co of polymer A (PLA) was highest when compared with polymer C (PEG M_w:6600) and polymer E (PEG M_w:20000) nanoparticles. The order of the Co was polymer A, polymer C and polymer E nanoparticles, but the half-life of polymer A, polymer E and polymer C nanoparticles were 3.9, 7.6 and 7.9 h, respectively, as shown in Table 2.

3.2.4. M_w/M_n

Fig. 5 shows the effect of M_w/M_n on the pharmacokinetics of progesterone loaded nanoparticles. The Co of polymer C (M_w/M_n:1.62), which is after purification, nanoparticles are higher than that of polymer C-1 (M_w/M_n:11.7), which is before purification. The half-lives of polymer C and C-1 nanoparticles were 7.9, 6.0 h, respectively.

Table 2
Pharmacokinetic parameters of various progesterone loaded nanoparticles

	Co (μg/ml)	$t_{1/2}$ (h)	$AUC_{0\rightarrow\infty}$ (μg · h/ml)	Mean diameter (nm)	Drug trapping efficiency (%)	n
Polymer A nanoparticles	21.7 ± 1.4	3.9 ± 0.1	76.8 ± 7.8	205	65	3
Polymer B nanoparticles	16.8	6.0	79.7	305	73	2
Polymer C nanoparticles	13.8 ± 1.1	7.1 ± 0.7	55.2 ± 15.2	197	49	5
Polymer C-1 nanoparticles	5.4 ± 0.3	6.0 ± 0.8	19.9 ± 1.7	152	64	4
Polymer F nanoparticles	3.6 ± 0.1	2.2 ± 0.3	6.7 ± 0.3	222	32	3
Polymer E nanoparticles	6.9 ± 0.4	7.6 ± 2.6	28.6 ± 2.2	377	67	3
Solution	1.4 ± 0.4	0.4 ± 0.0	0.3 ± 0.1	—	—	3

3.3. Biodistribution

As shown in Table 3, the organ distribution of ^{3}H-progesterone was different among the solution, polymer C (PLA-PEG-PLA) nanoparticles and polymer A (PLA) nanoparticles. The elimination of ^{3}H-progesterone from RES (liver, spleen, lung, bone marrow) after administration of ^{3}H-progesterone solution was fast and directly proportional to that from plasma, but that of ^{3}H-progesterone loaded nanoparticles was not directly proportional to that from plasma. The dis-

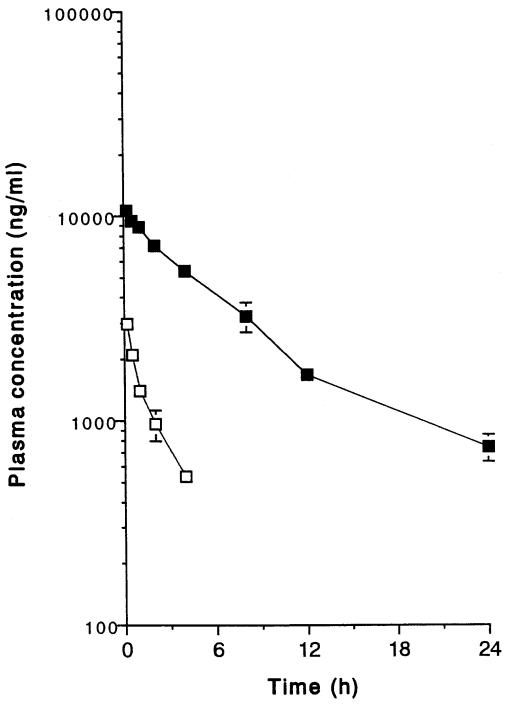

Fig. 2. Effect of M_w of polymer on plasma concentration of progesterone after intravenous administration of progesterone-loaded nanoparticles to rats at a dose of 1 mg/kg (mean ± S.D.; $n = 3$ or 5). ■, progesterone-loaded polymer C nanoparticles; □, progesterone-loaded polymer F nanoparticles.

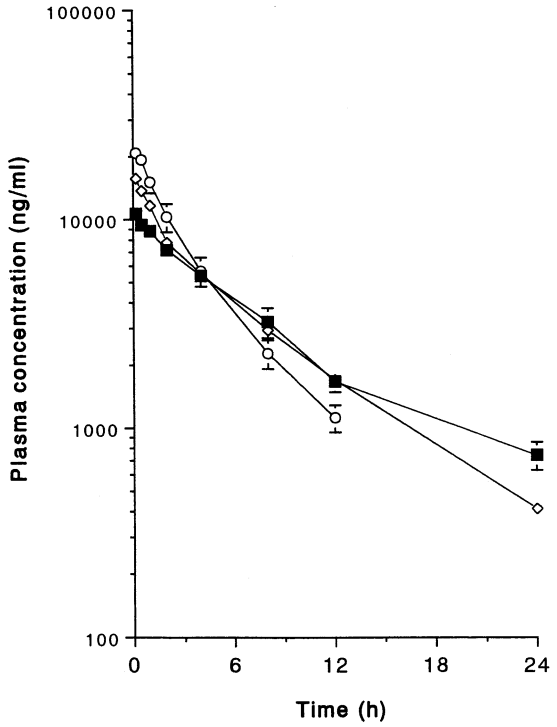

Fig. 3. Effect of PEG content on plasma concentration of progesterone after intravenous administration of progesterone-loaded nanoparticles to rats at a dose of 1 mg/kg (mean ± S.D.; $n = 2$–5). ○, progesterone-loaded polymer A nanoparticles; ◇, progesterone-loaded polymer B nanoparticles; ■, progesterone-loaded polymer C nanoparticles.

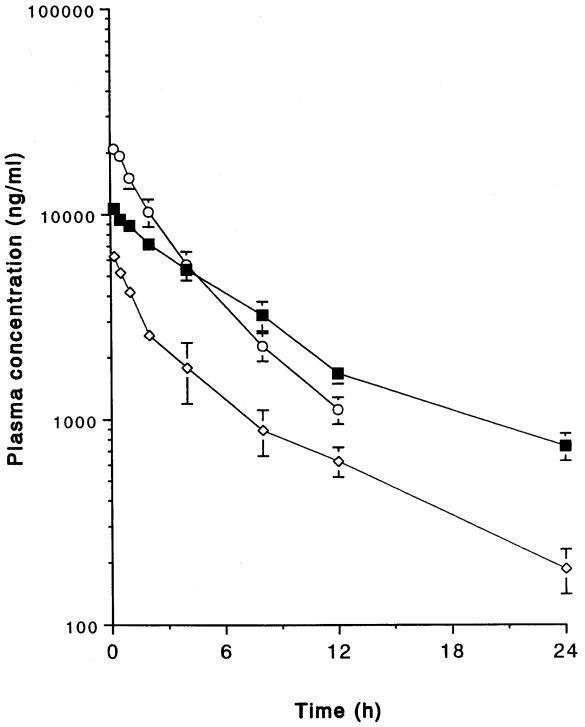

Fig. 4. Effect of M_w of PEG on plasma concentration of progesterone after intravenous administration of progesterone-loaded nanoparticles to rats at a dose of 1 mg/kg (mean ± S.D.; $n = 3–5$). ○, progesterone-loaded polymer A nanoparticles; ■, progesterone-loaded polymer C nanoparticles; ◇, progesterone-loaded polymer E nanoparticles.

tribution to liver and spleen at all sampling points after administration of ^{3}H-progesterone polymer C nanoparticles was about half of that of ^{3}H-progesterone polymer A nanoparticles, and the distribution to bone marrow after injection of polymer C nanoparticles was lower than that of polymer A nanoparticles in early time. The distribution to other organs was almost the same in both nanoparticles.

4. Discussion

For the evaluation of the circulation time of nanoparticles itself, we used progesterone which was quickly eliminated from blood, as a model drug. The elimination half-life of progesterone in plasma after intravenous administration is 0.4 h, as shown in Fig. 1 and Table 2. Therefore, a large portion of the plasma concentration of progesterone after administration of polymer A or C nanoparticles in Fig. 1 would express the concentration of progesterone loaded in nanoparticles. Fig. 1 shows the triblock copolymer polymer C nanoparticles has the availability of long-circulation compare with the polymer A (PLA) nanoparticles.

The Co volumes calculated each plasma concentration of polymer A and C nanoparticles were 21.7 and 13.8 μg/ml, respectively. These results suggest the burst of polymer C nanoparticles in early phase is greater than that of polymer A nanoparticles. In fact, the progesterone release

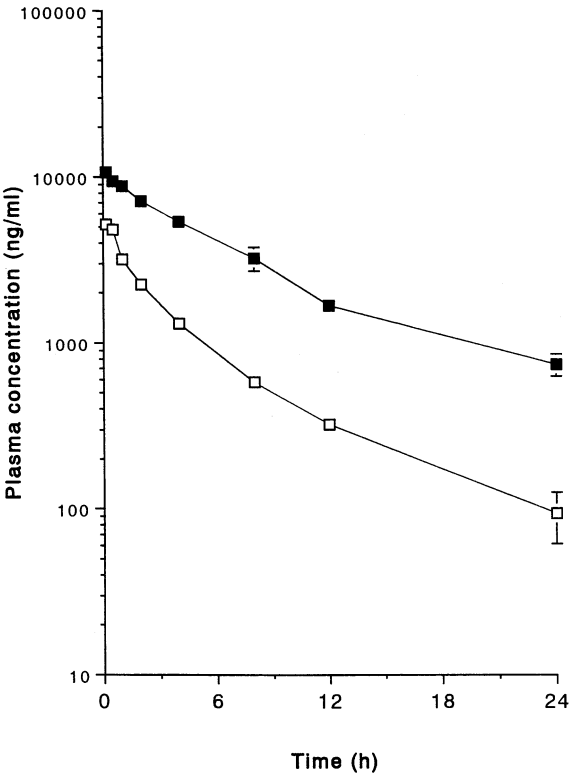

Fig. 5. Effect of M_w/M_n of polymer on plasma concentration of progesterone after intravenous administration of progesterone-loaded nanoparticles to rats at a dose of 1 mg/kg (mean ± S.D.; $n = 4$ or 5). □, progesterone-loaded polymer C-1 nanoparticles; ■, progesterone-loaded polymer C nanoparticles.

Table 3
Tissue distribution of ^{3}H-progesterone after intravenous administration of ^{3}H-progesterone loaded nanoparticles or ^{3}H-progesterone solution to rats at a dose of 1 mg/kg

	Organ	Distribution (% of dose)				
		Time (h)				
		0.17	1	4	8	24
^{3}H-progesterone solution	Plasma	1.90 ± 1.09	0.77 ± 0.25	0.46 ± 0.21	0.45 ± 0.26	0.26 ± 0.15
	Blood	2.98 ± 1.49	1.53 ± 0.49	1.27 ± 0.25	0.71 ± 0.17	0.83 ± 0.61
	Liver[a]	9.45 ± 6.09	4.67 ± 1.68	1.70 ± 0.66	1.35 ± 0.53	0.72 ± 0.34
	Spleen[b]	0.15 ± 0.09	0.05 ± 0.02	0.04 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
	Bone marrow[c]	2.47 ± 1.25	0.69 ± 0.28	0.37 ± 0.14	0.36 ± 0.17	0.15 ± 0.13
	Lung[d]	2.70 ± 2.81	0.12 ± 0.05	0.07 ± 0.04	0.04 ± 0.02	0.03 ± 0.01
	Kidney	1.44 ± 0.89	0.38 ± 0.09	0.14 ± 0.06	0.08 ± 0.07	0.05 ± 0.02
	Brain	0.59 ± 0.45	0.09 ± 0.03	0.06 ± 0.03	0.07 ± 0.05	0.02 ± 0.02
	Heart	0.29 ± 0.27	0.06 ± 0.02	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
	Thymus	0.06 ± 0.04	0.02 ± 0.01	0.01 ± 0.00	0.00 ± 0.00	0.01 ± 0.00
	Duodenum	0.20 ± 0.17	0.06 ± 0.01	0.03 ± 0.01	0.01 ± 0.00	0.00 ± 0.00
	Inframandibular lymph	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Muscle	3.37 ± 1.15	1.49 ± 0.50	0.94 ± 0.39	0.68 ± 0.51	0.27 ± 0.23
	Total[e]	22.6 ± 14.2	8.39 ± 2.84	3.85 ± 1.52	3.10 ± 1.60	1.54 ± 0.28
	Total of RES[f]	14.8 ± 10.2	5.54 ± 2.00	2.18 ± 0.82	1.79 ± 0.72	0.92 ± 0.25
^{3}H-progesterone loaded polymer A nanoparticles	Plasma	44.5 ± 5.4	23.0 ± 4.2	7.83 ± 2.30	3.03 ± 0.73	0.34 ± 0.02
	Blood	43.7 ± 7.8	21.3 ± 3.6	6.78 ± 3.00	2.91 ± 0.62	0.64 ± 0.23
	Liver[a]	14.2 ± 1.8	20.2 ± 3.3	20.1 ± 3.9	20.3 ± 4.4	20.2 ± 3.7
	Spleen[b]	3.45 ± 0.32	13.1 ± 1.5	17.2 ± 1.0	19.9 ± 1.3	16.3 ± 0.2
	Bone marrow[c]	5.35 ± 0.96	6.63 ± 1.57	6.33 ± 0.17	5.82 ± 0.77	6.73 ± 1.67
	Lung[d]	0.99 ± 0.40	0.68 ± 0.08	0.38 ± 0.09	0.37 ± 0.04	0.38 ± 0.05
	Kidney	0.99 ± 0.11	0.58 ± 0.16	0.28 ± 0.04	0.26 ± 0.01	0.16 ± 0.01
	Brain	0.27 ± 0.03	0.12 ± 0.04	0.08 ± 0.01	0.08 ± 0.02	0.08 ± 0.01
	Heart	0.23 ± 0.04	0.12 ± 0.03	0.05 ± 0.01	0.04 ± 0.01	0.03 ± 0.00
	Thymus	0.04 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.00	0.01 ± 0.00
	Duodenum	0.11 ± 0.02	0.12 ± 0.08	0.06 ± 0.02	0.06 ± 0.02	0.04 ± 0.03
	Inframandibular lymph	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.02	0.01 ± 0.00	0.02 ± 0.01
	Muscle	1.96 ± 0.48	1.49 ± 0.38	1.66 ± 1.42	0.96 ± 0.73	0.94 ± 0.37
	Total[e]	72.2 ± 5.7	66.0 ± 2.3	54.0 ± 2.9	50.8 ± 2.6	45.3 ± 5.8
	Total of RES[f]	24.0 ± 2.1	40.5 ± 2.5	44.0 ± 3.7	46.4 ± 3.0	43.6 ± 5.5
^{3}H-progesterone loaded polymer C nanoparticles	Plasma	52.1 ± 0.5	39.2 ± 1.5	20.7 ± 3.7	12.4 ± 0.8	2.69 ± 0.39
	Blood	37.4 ± 2.8	30.4 ± 4.6	14.3 ± 0.9	10.3 ± 0.7	2.15 ± 0.38
	Liver[a]	6.58 ± 0.56	8.85 ± 0.28	11.9 ± 1.9	11.9 ± 1.8	9.84 ± 1.57
	Spleen[b]	1.69 ± 0.17	5.34 ± 0.31	9.41 ± 1.06	12.0 ± 1.2	13.2 ± 0.9
	Bone marrow[c]	3.65 ± 0.44	4.23 ± 0.82	5.48 ± 0.44	8.18 ± 1.25	6.34 ± 1.14
	Lung[d]	1.10 ± 1.03	0.57 ± 0.36	0.39 ± 0.26	0.32 ± 0.08	0.18 ± 0.03
	Kidney	0.82 ± 0.14	0.60 ± 0.06	0.35 ± 0.06	0.29 ± 0.02	0.19 ± 0.03
	Brain	0.28 ± 0.05	0.13 ± 0.02	0.09 ± 0.01	0.09 ± 0.02	0.10 ± 0.02
	Heart	0.22 ± 0.01	0.13 ± 0.02	0.09 ± 0.02	0.06 ± 0.01	0.05 ± 0.01
	Thymus	0.05 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.00	0.02 ± 0.01
	Duodenum	0.11 ± 0.01	0.08 ± 0.02	0.07 ± 0.03	0.06 ± 0.03	0.07 ± 0.03
	Inframandibular lymph	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00

Table 3 (Continued)

	Organ	Distribution (% of dose)				
		Time (h)				
		0.17	1	4	8	24
	Muscle	1.90 ± 0.15	1.48 ± 0.09	1.18 ± 0.05	0.79 ± 0.12	1.00 ± 0.14
	Total[e]	68.5 ± 1.4	60.7 ± 2.2	49.6 ± 3.9	46.1 ± 0.7	33.7 ± 2.9
	Total of RES[f]	13.0 ± 1.7	19.0 ± 0.6	27.2 ± 2.4	32.3 ± 0.8	29.5 ± 2.5

Mean ± S.D. ($n = 3$).
[e] Total of the distribution to each organ except blood.
[f] Total of RES (a + b + c + d).

rate from polymer C nanoparticles was faster than that of polymer A nanoparticles in vitro release test (Matsumoto et al., 1998).

The circulating-time and the Co values indicated the burst in early phase were affected by the total M_w, the PEG content, the PEG M_w and the M_w/M_n ratio, as shown in Figs. 2–5 and Table 2. From the evaluation of the circulating-time among the nanoparticles consisted of polymer A, B, C and E in which the total M_w and the M_w/M_n ratios were almost the same, the circulating time was proportional to the PEG content, but the Co and AUC was inversely proportional to the PEG content. This means the increase of the PEG content avoids trapping by RES, but promotes the burst in early phase. Furthermore, as compare with the $t_{1/2}$,Co and AUC of the polymer E (PEG M_w 20000) and that of the polymer C (PEG M_w 6600), the $t_{1/2}$ was almost same, but the Co of the polymer E nanoparticles was lower than that of the polymer C nanoparticles, and this result indicates the PEG M_w is a critical factor for the burst.

Fig. 5 shows the purification of the polymer (M_w/M_n ratio) is the important factor for the burst and the circulating-time. The depressing burst could be ascribed to the monodispersity for these two dates. Though this may not be always valid in ultra-thin layers such as the surface of nanoparticles, the presence of low molecular weight polymers in polymer-bulk makes the mechanical strength lower than in the case of an absence of them. All these results suggest it is possible to control the circulating-time and the release rate of drugs for the modification of the triblock copolymer.

From the results of the biodistribution of ^{3}H-progesterone loaded nanoparticles in Table 3, the reason why the increase of the circulating availability of nanoparticles used the triblock copolymer, in other words, the increase of the distribution of nanoparticles to plasma would be the decrease of the distribution to liver and spleen. And these results are consisted with the report which described the biodistribution of the nanoparticles using PLA-PEG, diblock copolymer (Verrecchia et al., 1995). We believe the decrease of the distribution of our triblock copolymer nanoparticles to liver and spleen is explained by the avoiding the adsorption of opsonin to the particles as a result of the orientation of PEG in the surface of the particles like the diblock, PLA-PEG nanoparticles (Peracchia et al., 1997).

In conclusion, we could make the long-circulating nanoparticles avoid distribution to the liver and spleen, and control the circulating-time and the burst in plasma using biodegradable ABA triblock copolymers containing PLA A-blocks attached to central PEG B-blocks.

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