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Comparison of Body Distribution of Poly(vinyl alcohol) with Other Water-soluble Polymers after Intravenous Administration

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Abstract

The body distribution of poly(vinyl alcohol) (PVA) with molecular weights (MW) from 14800 to 434000 Da was investigated after intravenous administration and compared with that of other water-soluble polymers such as poly(ethylene glycol) (PEG), gelatin, dextran, and pullulan.

The half-life of PVA in the circulation was prolonged from 90 min (MW 14800 Da) to 23 h (MW 434000 Da), similar to that of PEG which had a half-life of 30 min (MW 6000) and 20 h (MW 170000). However, the half-life of PVA was much longer than that of other polymers when compared at a similar molecular weight. PVA was located in most organs but with very small accumulation. An insignificant interaction of PVA with cell components, such as macrophages and blood cells, was observed. Similar to PEG, the excretion rate of PVA at the glomeruli was rapidly reduced around 30000 Da, as the molecular weight increased.

These results indicate that the half-life of intravenously injected PVA in the blood was mainly determined by the permeation characteristics of the kidney.

Drug conjugation with water-soluble polymers enables the half-life of the drug in the blood to be prolonged and the distribution profile to be changed, but the body distribution of the drug chemically modified by polymers has been found to be greatly affected by the molecular weight (Garlick & Renkin 1970; Kagawa & Tomizawa 1980; Takakura et al 1987b; Nagy et al 1989; Nakagomi et al 1989), the electric charge (Brenner et al 1978; Takakura et al 1987a; Barrowcliffe et al 1990), the biological properties (Varga et al 1977; Shen & Ryser 1979; Davis & Preston 1981; Kulkarni et al 1981; Kobayashi et al 1988), and the type of the carrier polymer used (Przybylski et al 1978a, b). For the drug modification, poly(ethylene glycol) (PEG) has been most widely used because of its long half-life in the blood and its low interaction with organs (Abuchowski et al 1977; Savoca et al 1979; Cecchi et al 1981; Hershfield et al 1987; Katre et al 1987; Veronese et al 1989). However, a PEG molecule cannot couple with more than one or two drug molecules since PEG has only one or two functional groups per molecule. In contrast, other polymeric carriers, such as dextran, pullulan, gelatin, and poly(vinyl alcohol) (PVA), have numerous functional groups which are capable of covalently coupling drug molecules.

The present paper makes a comparison of the half-life of these water-soluble polymers in the blood after intravenous injection in mice.

Materials and Methods

Reagents

PVA was kindly supplied from Unitika Kasei Ltd, Osaka, Japan. The weight-average molecular weight (MW) was determined on gel filtration chromatography using PEG as standard samples. Chromatographic experiments were carried out at 40°C and a flow rate of 0·9 mL min^{-1} using TSKgel3000PW$_{XL}$ and TSKgel6000PW$_{XL}$ columns purchased from Tosoh Corporation, Tokyo, Japan. PVA samples with MW of 14800, 68000, 125000, 196000, and 434000 Da were used in the present study and abbreviated as PVA-15, PVA-70, PVA-120, PVA-200, and PVA-430, respectively. All PVA samples had a degree of saponification >99%. Fig. 1 shows the chromatograms of PVA used. The estimated polydispersity was 2·07 (PVA-15), 3·29 (PVA-70), 4·05 (PVA-120), 4·54 (PVA-200), and 8·35 (PVA-430). PEG and gelatin samples with different molecular weights were supplied from Nippon Oil & Fats Co. Ltd, Tokyo, Japan, and Nitta Gelatin Co., Ltd, Osaka, Japan, respectively. 1,1′-Carbonyldiimidazole (CDI) and sodium pyrosulphite (SMS) were purchased from Nakalai Tesque, Kyoto, Japan. Tyramine was obtained from Wako Pure Chemical Industries, Ltd, Osaka, Japan. Mouse serum albumin and bovine serum albumin were obtained from Sigma Chemical Co., St Louis, MO. Na^{125}I (740 MBq mL^{-1}) solution and Dowex were purchased from NEN Research Products, Boston, MA and Dow Chemical Co., Ltd, Midland, MI, respectively. Other chemicals were of guaranteed grade and used without further purification.

In-vitro cell culture was carried out by use of the culture medium (RPMI-FCS) prepared by supplementing RPMI-1640 medium (Nissui Seiyaku Co., Ltd, Tokyo, Japan) with 10% foetal calf serum (FCS, M.A. Bioproducts, Walkersville, MD), 5 mM L-glutamine, and penicillin (100 units mL^{-1}), and buffered with 5 mM *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulphonic acid and $NaHCO_3$ to pH 7·4.

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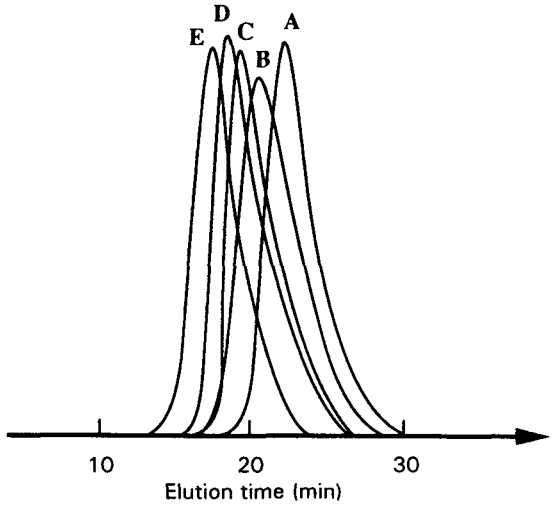

FIG. 1. Gel filtration chromatograms of PVA with different molecular weights. A. PVA-15, B. PVA-70, C. PVA-120, D. PVA-200, E. PVA-430.

Phosphate-buffered saline (PBS, pH 7·4) was also obtained from Nissui Seiyaku Co., Ltd, Tokyo, Japan.

Mice

Specific pathogen-free inbred female BALB/cCrSlc mice, 8–12 weeks old, were obtained from Shizuoka Animal Facility Center, Shizuoka, Japan.

^{125}I labelling of PVA

Tyramine residues were introduced to hydroxyl groups of PVA molecules by use of CDI (Beauchamp et al 1983). Two millilitres of dimethyl sulphoxide (DMSO) containing 7·4 mg CDI was added to 50 mL 0·4 wt% PVA solution in DMSO, followed by stirring for 30 min at 25°C. The reaction mixture was dialysed against distilled water for two days and further against 10 mM sodium borate buffer (pH 8·5) for 24 h. Then, 62·3 mg tyramine was added to the CDI-treated PVA and stirred for 48 h at 25°C to introduce tyramine residues to the CDI-treated PVA. The resulting tyramine-bound PVA was dialysed against distilled water for 2 days. The percentage of tyramine residues introduced to PVA, evaluated by measuring the UV absorbance of the sample solution at 280 nm on a spectrophotometer (type 200-20, Hitachi, Ltd, Tokyo Japan), was 0·17 (PVA-15), 0·15 (PVA-70), 0·20 (PVA-120), 0·15 (PVA-200), and 0·16 mol % (PVA-430).

Radioiodination of the tyramine-introduced PVA was carried out according to the chloramine T method (Greenwood et al 1963). Briefly, 2 μL $Na^{125}I$ solution was added to 150 μL 5 mg mL^{-1} tyramine-introduced PVA in 0·5 M potassium phosphate buffer (KPB, pH 7·5), followed by addition of 100 μL 0·2 mg mL^{-1} chloramine T in 0·05 M KPB (pH 7·2). After agitation for 2 min, 100 μL PBS containing 0·4 mg SMS was added to the solution. The reaction mixture was passed through the Dowex column to remove uncoupled ^{125}I molecules from the ^{125}I-labelled polymers. The radioiodination of the tyramine-introduced PVA and the separation of free ^{125}I molecules from the radioiodinated PVA were assured using gel filtration chromatography equipped with a radiodetection system (Radio HPLC system, Tosoh Co., Ltd). PVA was found to be radiolabelled without any change of the molecular weight during radioiodination. Labeling of other water-soluble polymers with ^{125}I was conducted by the same procedure as the above radioiodination.

Measurement of plasma concentration of ^{125}I-PVA after intravenous injection

Following injection of 100 μL ^{125}I-labelled PVA solution at a concentration of 0·1% in PBS via mouse tail vein, about 50 μL of the mouse blood was collected from the retro-orbital plexus by use of haematocrit glass capillaries at different time intervals. Radioactivity of the blood was measured by an Autowell gamma system (Aloka ARC-301B, Aloka Co., Ltd, Tokyo, Japan). Successive blood collection of more than six times was not conducted for one mouse because such frequent collection would cause an undesirable decrease of total blood volume to affect decrement patterns of ^{125}I-labelled PVA injected. The amount of ^{125}I-labelled PVA remaining in the whole blood was calculated from the volume of blood sample, the radioactivity, and the total volume of mouse blood, which was determined by a dilution method using mouse serum albumin, as will be described later.

Measurement of organ distribution of ^{125}I-PVA after intravenous injection

Following the intravenous injection of ^{125}I-labelled PVA by the same procedure described above, approximately 0·7 mL blood was collected from the heart by a heparinized syringe and the heart, lung, thymus, liver, spleen, kidney, gastrointestine, and thyroid gland were excised. The excised organs and the residual body, i.e. carcass, were rinsed twice with PBS and their radioactivity was measured. Since it was too difficult to completely exclude the blood from the organs and tissues even after vigorous rinsing, the radioactivity of the blood residing in organs was evaluated from the blood volume in the organs as described later. The experiments of organ distribution were carried out independently in triplicate and the data were expressed as mean ± s.d. In addition, the urine and faeces were collected to estimate the excreted radioactivity.

Volume measurement of the whole blood and blood residing in organs

The total volume of mouse blood and the blood volume in the organs was evaluated according to a dilution method (Yamaoka et al 1994). One hundred microlitres of 0·1 wt% ^{125}I-labelled mouse serum albumin (MSA) in PBS was administered to mice via the tail vein. Forty seconds after injection, the radioactivity of the blood was measured. The total volume was calculated from the comparison of the radioactivity concentration of the blood with that of original ^{125}I-labelled MSA solution. As the isogeneic albumin has a long half-life and there is low accumulation in organs and tissues, all the ^{125}I-labelled MSA are possibly present in the blood circulation without distributing into the organs and tissues, at least immediately after injection. Thus, the blood remaining in each organ can be estimated from the overall radioactivity of the organs and the radio-

activity concentration of the blood measured immediately after injection of ^{125}I-labelled MSA. The net radioactivity accumulated in the organ matrix was calculated by subtracting the radioactivity of the blood residing in the organ from the overall radioactivity of the organ. Such a treatment of experimental data permitted reduction of the percent recovery of radioactivity from more than 130% to about 100%, suggesting accuracy of this compensating method.

Pharmacokinetic analysis

To analyse decrement patterns of polymer concentration in plasma, a two-compartment model expressed by equation 1 was employed:

$$C(t) = Ae^{-\alpha t} + Be^{-\beta t} \quad (1)$$

The pharmacokinetic parameters, A, B, α and β, in equation 1 were calculated using a nonlinear minimum root square fitting program MULTI (Yamaoka et al 1981). The first-order transfer rate constant from a central compartment (CC) to a peripheral compartment (PC), k_{12}, the first-order transfer rate constant from the PC to the CC, k_{21}, the elimination constant from the CC, k_e, and the distribution volume V_{10} were calculated from parameters A, B, α, and β. The half-life of PVA at the β-phase of the plasma concentration–time curve was calculated using the parameter β.

The affinity of PVA for various organs was determined as the influx rate of PVA to the organs (CL_{in}), calculated from equation 2 (Takakura et al 1990):

$$CL_{in} = \frac{A_{(t_1)}}{AUC_{0-t_1}} \quad (2)$$

where $A_{(t_1)}$ is the ratio of polymer accumulation in the tissue to the total injection dose at time t_1 after injection and AUC_{0-t_1} is the area under the plasma concentration–time curve up to time t_1.

Pinocytosis experiment of ^{125}I-labelled PVA by macrophages

Mouse peritoneal macrophages were isolated from the peritoneal cavity of BALB/cCrSlc mice which had been injected intraperitoneally with 2 mL thioglycollate broth (Brewer's Medium Difco Laboratories, Detroit, MI) (Ikada & Tabata 1986). Four days later, peritoneal exudate cells (PEC) were collected by syringe aspiration with cold RPMI-1640 medium and washed twice with the medium by centrifugation. Finally, 1 mL cell suspension including 1×10^6 macrophages was added to each well of 24-well multiwell culture plates (A/S Nunc, Kamstrup, Roskilde, Denmark). After 2-h incubation at 37°C in 5% CO_2–95% air, non-adherent cells were removed by rinsing with RPMI-1640 medium. More than 98% of the adherent cells had morphological and phagocytic properties as macrophages. ^{125}I-labelled PVA was added to the macrophage monolayer at a concentration of 1·0 mg mL^{-1} at 4 or 37°C. At different time intervals, macrophages were washed five times with medium and lysed with 2% sodium dodecyl sulphate/PBS solution. The radioactivity of the cell lysate was measured to determine the amount of PVA pinocytosed by macrophages.

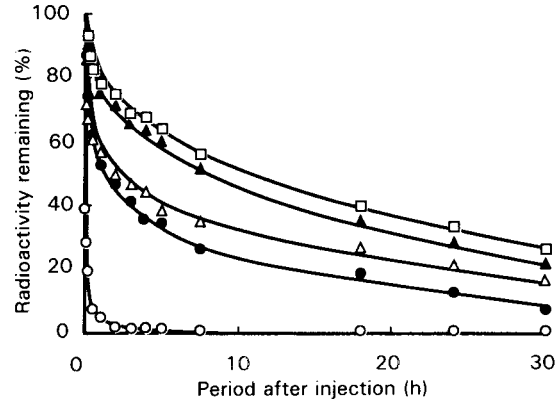

FIG. 2. Decrement patterns of ^{125}I-labelled PVA with different molecular weights after intravenous injection. ○ PVA-15, ● PVA-70, △ PVA-120, ▲ PVA-200, □ PVA-430.

Interaction of ^{125}I-labelled PVA with blood cells

Blood was taken from the heart 2 h after intravenous injection of ^{125}I-labelled PVA and fractionated by means of density gradient centrifugation using Ficoll-hyperque (Flow Laboratories, Irvine, UK) to prepare different blood components. After centrifugation at 3000 rev min^{-1} for 30 min at 25°C, the upper layer, the bottom layer, and the middle layer were collected. These layers consisted of plasma, red blood cells, and other types of cells, respectively.

Table 1. Pharmacokinetic parameters of PVA after intravenous injection.

Parameters	PVA-15	PVA-70	PVA-125	PVA-200	PVA-430
A	22·3	20·2	11·0	6·63	5·06
α	0·160	0·0338	0·0138	0·00621	0·0146
B	5·47	23·1	22·6	27·4	32·8
β	0·0131	0·0010	0·0005	0·0006	0·0006
V_{10} (mL)	3·60	2·31	2·98	2·94	2·64
k_{12} (min^{-1})	0·181	0·0181	0·0057	0·0025	0·0031
k_{21} (min^{-1})	0·0420	0·0185	0·0094	0·0051	0·0127
k_e (min^{-1})	0·0499	0·0018	0·0008	0·0007	0·0006
AUC (% h mL)	556	23400	40100	43600	54300
$t_{1/2\beta}$ (min)	52·7	684	1210	1070	1140

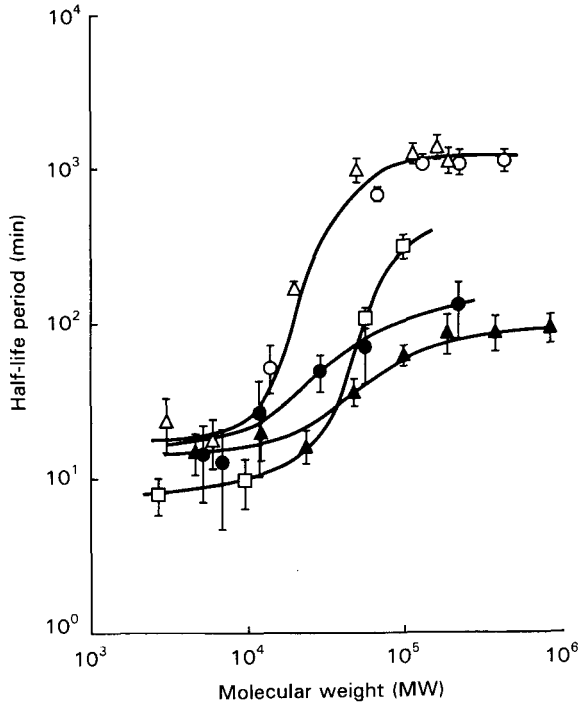

FIG. 3. Effect of molecular weight of various polymers on half-life in the blood. ○ PVA, △ PEG, □ gelatin, ● dextran, ▲ pullulan. Data are expressed as mean ± s.d.

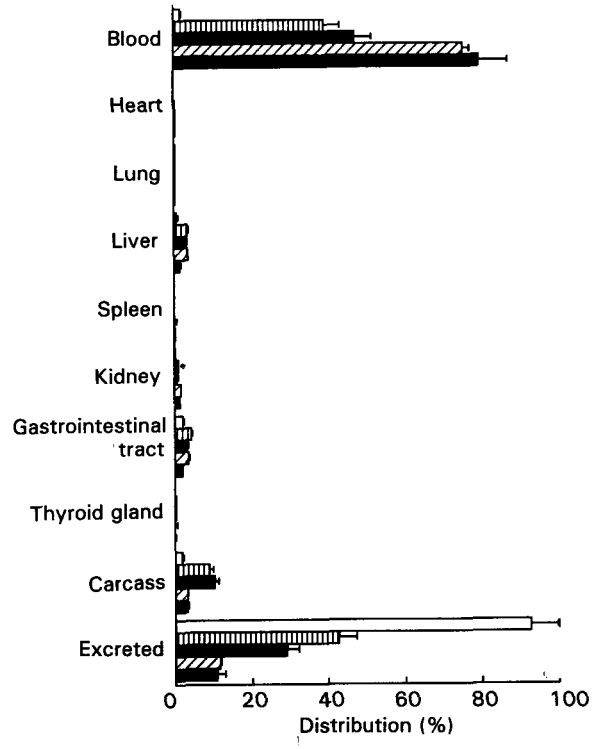

FIG. 4. Organ distribution of PVA with different molecular weights 4 h after intravenous injection. □ PVA-15, ▥ PVA-70, ■ PVA-120, ▨ PVA-200, ■ PVA-430, respectively. Data are expressed as mean ± s.d.

Their radioactivity was measured to estimate the PVA localization to each layer.

Results

Concentration profile of ^{125}I-labelled PVA in the circulation after intravenous injection

Decrement patterns of the radioactivity in the blood after intravenous injection of ^{125}I-labelled PVA are shown in Fig. 2. They greatly depended on the molecular weight of PVA; PVA with higher molecular weight remained in the blood at a higher concentration for a longer period than that with lower molecular weight. The pharmacokinetic parameters calculated from the results are summarized in Table 1. The rate constants k_{12} and k_{21} increased with a decrease in molecular weight, suggesting that smaller PVA were quickly diffused out into the extravascular space, reaching the equilibrium state between the interior and the exterior space of blood vessels.

Fig. 3 shows the half-life of PVA in the blood plotted as a function of molecular weight, together with that of other water-soluble polymers reported previously (Yamaoka et al 1993, 1994). The half-life of PVA depended on the molecular weight. PVA injected intravenously was retained in the blood circulation for a period as long as PEG. The molecular weight dependence of its half-life was equivalent to that of PEG over the molecular weight range studied but was much longer than that of dextran, pullulan, and gelatin when compared at a same molecular weight. For example, the half-life was about 1 day for PVA with MW > 100 000 Da and was longer with the increasing molecular weight, rapidly changing around ~MW 30 000 Da, irrespective of the type of polymers studied.

Organ distribution of PVA after intravenous injection

Fig. 4 shows the organ distribution of PVA with different molecular weights. The extent of PVA accumulation to each organ was very small. It is well recognized that free ^{125}I molecules are accumulated in the thyroid gland following their injection (Alazraki et al 1971). In the present experiment, 7·5% of total radioactivity injected was detected in the thyroid gland (data not shown). On the other hand, little

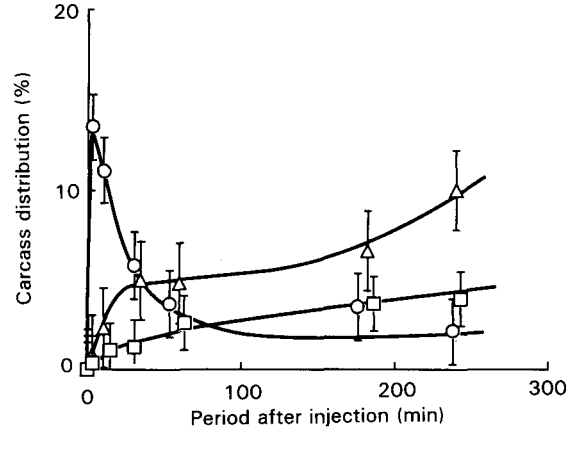

FIG. 5. Time course of carcass distribution of PVA with different molecular weights after intravenous injection. ○ PVA-15, △ PVA-70, □ PVA-200. Data are expressed as mean ± s.d.

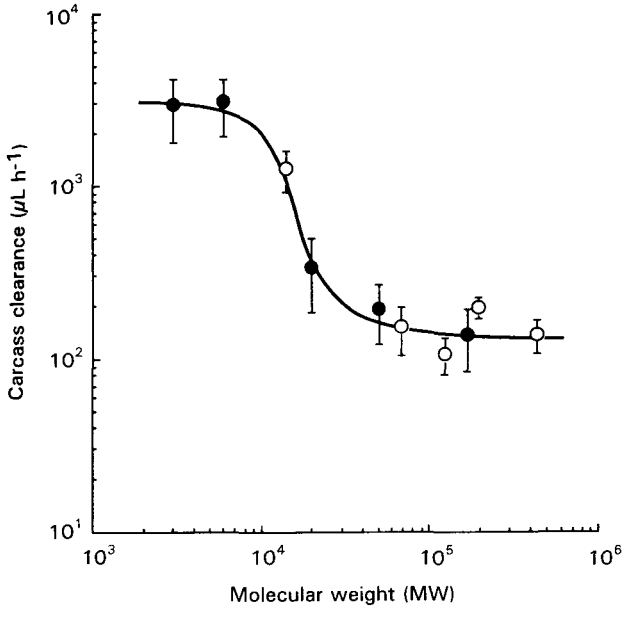

FIG. 6. Dependence of the carcass clearance on molecular weight of PVA (○) and PEG (●). Data are expressed as mean ± s.d.

radioactivity was detected in the thyroid gland when ^{125}I-labelled PVA was injected. This finding indicates that ^{125}I-labelled PVA was stable in the body during the distribution experiment and the amount of free ^{125}I released was negligibly small.

The PVA concentration in the blood increased with the increasing molecular weight, but the opposite molecular weight dependence was observed for excretion. PVA was hardly accumulated in the organs other than liver, gastrointestine and carcass. PVA of middle molecular weight tended to accumulate more remarkably in the above three organs than that of higher or lower molecular weight. Fig. 5 shows the time course of the carcass distribution of ^{125}I-labelled PVA after intravenous injection. The smallest PVA-15 accumulated in the highest amount during the first 3 min after injection, followed by a rapid decrease, whereas the larger PVA-70 and PVA-200 more slowly accumulated

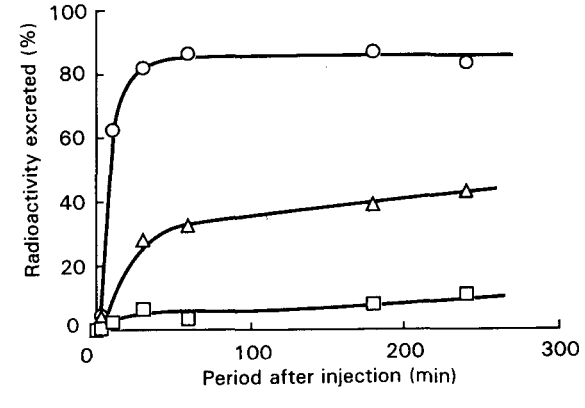

FIG. 7. Time course of excretion of PVA with different molecular weights after intravenous injection. ○ PVA-15, △ PVA-70, □ PVA-200.

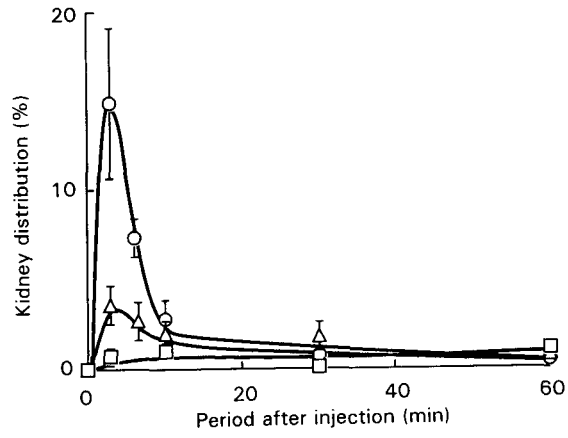

FIG. 8. Time course of kidney distribution of PVA with different molecular weights after intravenous injection. ○ PVA-15, △ PVA-70, □ PVA-200. Data are expressed as mean ± s.d.

in the carcass with time. A similar molecular weight dependence was observed in other organs such as kidney, gastrointestine, and heart (data not shown). Fig. 6 shows the molecular weight dependence of the carcass clearance for PVA and PEG, which was identical. The clearance value was accelerated with a decrease in molecular weight.

Fig. 7 shows the time course of urinary excretion of PVA after intravenous injection. PVA of lower molecular weight was more rapidly excreted than that of higher molecular weight. Almost 80% of PVA-15 was excreted within the first 30 min, while a smaller amount of PVA-70 and PVA-200 was excreted during the first hour, followed by gradual excretion thereafter. The time course of PVA distribution to the kidneys is shown in Fig. 8. Similar to the time profile of carcass accumulation, PVA-15 showed an accumulation peak around 3 min after injection. This suggests that PVA may be rapidly excreted from the kidneys into the urine without residing there. Fig. 9 shows the effect of molecular

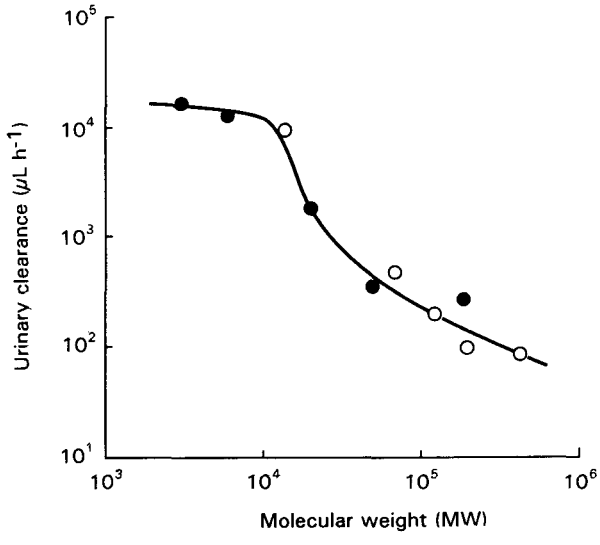

FIG. 9. Dependence of the urinary clearance on the molecular weight of PVA (○) and PEG (●).

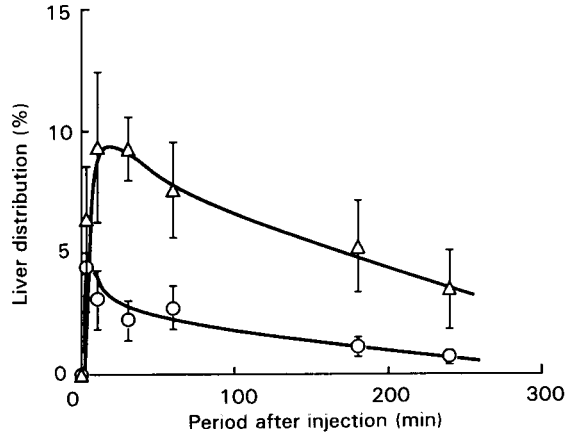

FIG. 10. Time course of liver distribution of PVA with different molecular weights after intravenous injection. ○ PVA-15, △ PVA-200. Data are expressed as mean ± s.d.

weight of PVA on the urinary clearance together with that of PEG. Clearly, a similar molecular weight dependence on the excretion rate was observed between the two polymers. The excretion rate became lower with an increase in molecular weight. This indicates that the permeability of PVA and PEG at the glomeruli is determined only by their molecular weight, as they have no biospecific moieties in the molecule.

Fig. 10 shows the time course of liver distribution of PVA after intravenous injection. The amount of PVA accumulated in the liver decreased with time and passing through a maximum, gradually decreased. To evaluate the interaction of PVA with the scavenger cells, pinocytosis of PVA by

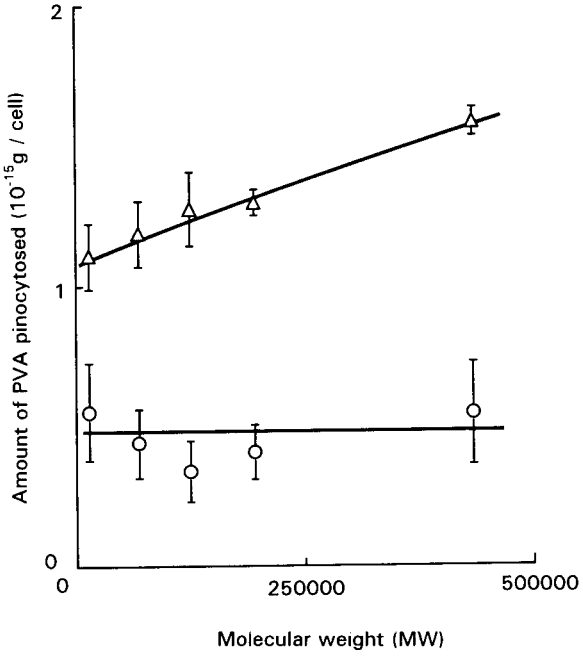

FIG. 11. Effect of molecular weight of PVA on its pinocytosis by mouse peritoneal macrophages in-vitro after 3 (○) and 24 h (△) incubation. Data are expressed as mean ± s.d.

Table 2. Amounts of polymer pinocytosed by mouse peritoneal macrophages in-vitro.

Polymer	Incubation time (h)	Molecular weight (Da)	Polymer pinocytosed per cell (10^{-15}g)
BSA	2	66 000	60·1
PEG	24	50 000	1·3
PVA	24	68 000	1·2

mouse peritoneal macrophages was studied under in-vitro conditions (Fig. 11). Macrophage uptake of PVA was slightly dependent on the molecular weight for a short period of incubation but after 24-h incubation, tended to increase with an increase in the molecular weight. Table 2 shows the amount of PVA and PEG ingested by macrophages together with that of BSA (bovine serum albumin), which is known to interact weakly with macrophages. These polymers have a similar molecular weight around 60 000 Da. The amount of PVA and PEG ingested by macrophages was much less than that of BSA, although macrophages were incubated with PVA and PEG for a period twelve times longer than with BSA.

Interaction of ^{125}I-labelled PVA with blood components

The distribution of PVA-15, PVA-70, and PVA-200 into blood cell components was evaluated from their radioactivity. As is shown in Table 3, the overall radioactivity remaining in the blood after intravenous injection was clearly dependent upon the molecular weight of PVA. This is in accordance with the fact that the decay pattern of PVA from the blood circulation was greatly affected by the molecular weight. On the other hand, no difference in the localization was observed among the cell fractions for all PVA samples. More than 80% of the injected dose was detected in the liquid fraction, while very little PVA was associated with blood fractions, suggesting a weak interaction of PVA with blood cells.

Discussion

Various peptides and proteins have been modified with monomethoxy PEG, having one functional group, to prolong the in-vivo life of drugs. Our body-distribution studies of PEG injected intravenously revealed its low interaction with tissues and organs and a large dependence of its body

Table 3. Radioactivity detected in blood fractions 2 h after injection of ^{125}I-labelled PVA.

Fraction	Radioactivity (%)		
	PVA-15	PVA-70	PVA-200
Plasma	1·94 ± 0·15 (84·2 ± 6·3)[a]	42·45 ± 4·30 (87·9 ± 8·9)	65·40 ± 5·24 (88·6 ± 7·1)
Red blood cells	0·26 ± 0·05 (11·3 ± 2·1)	3·43 ± 0·92 (7·1 ± 1·9)	6·27 ± 2·29 (8·5 ± 3·1)
Other cells	0·10 ± 0·04 (4·5 ± 1·8)	2·42 ± 0·97 (5·0 ± 2·0)	2·13 ± 0·96 (2·9 ± 1·3)

[a] Percentage of total blood radioactivity.

distribution pattern on the molecular weight (Yamaoka et al 1994). PEG modification permits the preparation of drug conjugates in which one drug is coupled to several PEG molecules when the drugs themselves have multiple functional groups usable for the coupling reaction. However, when the drug molecule has only one or a few functional groups, PEG is not appropriate for drug modification. Thus, some other water-soluble polymers having many functional groups in the molecule have been investigated as drug carriers. They include dextran, poly(styrene-co-maleic acid), pyran copolymer and PVA (Sherwood et al 1977; Przybylski et al 1978a, b; Schacht et al 1985; Matsumoto et al 1986; Maeda et al 1988). The present study was undertaken to compare the profile of body distribution between water-soluble polymers such as PVA which permit coupling to many drugs and PEG currently used as a drug carrier.

The biological fate of materials injected intravenously can be divided into two routes after diffusing out from the blood stream and the half-life of any biomaterial is determined by both rates; the excretion from the kidney and the accumulation in some organs. When PVA, a non-degradable polymer, is used as a drug carrier, the accumulation in tissues or organs should be avoided, because the long stay in the body may cause toxicity. Thus, it is desirable for a drug carrier to be excreted as soon as possible after reaching high blood concentrations. The molecular weight dependence of the urinary clearance and the organ distribution profiles indicated that the amount of PVA accumulated in the organs was too small to affect the biological fate. These findings suggest that PVA is excreted to the same extent as PEG, supporting the safety of PVA.

The weak interaction with various cells is also an excellent feature of PVA. Because PVA circulates in the blood stream without interacting with the blood cells and weakly interacts with the cells residing in the organs, it can pass through the vascular wall depending on its size and can flow back again into the blood stream from the extravascular space of the organs in which PVA accumulated on the first pass. This would account for the appearance of a peak in the carcass and kidney accumulation of PVA. The decay of the large molecule in the liver may be due to biliary excretion as reported previously (Kagawa & Tomizawa 1980; Hashida et al 1990).

However, since even a small amount of carrier polymers may cause complications if accumulated in organs for a longer period, their disappearance from the body through excretion should be checked. Seiler et al (1983) reported the uptake of PVA by macrophages in the glomerular mesangium while Michael et al (1980) reviewed the uptake and processing of a variety of macromolecules by the glomerular mesangium. Although a very small amount of PVA was found to accumulate in the kidney after elimination from the blood in the present study, the exact fate of PVA located in organs should be examined for much longer periods of time.

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