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Pharmacokinetics and Biodisposition of Poly(vinyl alcohol) in Rats and Mice

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Summary: Poly(vinyl alcohol) (PVA) of various molecular weight (MW = 10,560–116,600) was successfully labeled with fluorescein isothiocyanate isomer I (FITC) according to the method of de Belder and Granath. A high-performance size-exclusion chromatographic procedure was developed for the quantitative analysis of FITC-labeled poly(vinyl alcohol) (F-PVA) in biological samples. F-PVA (80 K) disappeared slowly from the blood circulation according to the first-order kinetics (t1/2 = 7 h) after intravenous injection to rats. A dose-independent behavior of F-PVA (80 K) was observed in the blood circulation, in the tissue distribution and in the urinary and fecal excretions. This suggested that PVAs are eliminated exclusively by the mechanisms that do not involve saturable transport processes. Furthermore, it was found that PVAs are very stable in the body because no degradation product was detected in the urine and feces. 125I-labeled poly(vinyl alcohol) (125I-PVA) was prepared by introducing tyramine residues to the hydroxyl groups of PVA molecules by the 1,1'-carbonyldiimidazole (CDI) activation method. 125I-PVA (80 K) was retained in the blood circulation for several days after intravenous injection to mice. Although the tissue distribution of PVAs was small, a significant accumulation into the liver and the spleen was observed. Fluorescence microscopic examination of paraffin section of the liver revealed that F-PVA (80 K) was endocytosed by the liver parenchymal cells. 125I-PVA (80 K) captured by liver was slowly transported via the bile canaliculi and gall bladder to the intestine and excreted in the feces. It was suggested, therefore, a long time is necessary for 125I-PVA (80 K) to be excreted perfectly from the body.

Key words: poly(vinyl alcohol); FITC; radioiodination; biodisposition; pharmacokinetics; high performance size exclusion chromatography; fluorescence microscopy; rat; mouse

Introduction

The idea of using drug carriers to improve the therapeutic efficacy of pharmacological agents is receiving increasing attention. The consequence of attachment of low molecular weight drugs to macromolecular carriers alters their rate of excretion from the body, changes their toxicity and immunogenicity and limits their uptake by cells via endocytosis, thus providing the opportunity to direct the drug to the particular cell type where its activity is needed.1–5) Poly(vinyl alcohol) (PVA) is a polymer which is synthesized by polymerizing not a vinyl alcohol monomer but a vinyl acetate monomer. This monomer is polymerized in to poly(vinyl acetate) and then hydrolyzed to produce PVA. Today it has become one of the most important polymers in industry and used in a variety of applications, including textile fibers, paper-coating agents, emulsion stabilizers, and biomedical applications.6) PVA's biocompatibility makes it an excellent material for use in medical applications such as soft contact lenses. Recently, PVA has been used for long-term implants, including a bioartificial pancreas, artificial cartilage, nonadhesive film, and esophagus or scleral buckling material.7) PVA also provide a potential targetable drug delivery system. Kojima and Maeda9) modified superoxide...
dismutase (SOD) by conjugating with PVA. The PVA-SOD exhibited a lower antigenicity and immunogenicity and enhanced therapeutic effect against ischemic edema of mouse foot pad. Davis et al. studied the photosensitizing and biodistribution characteristics of a photosensitizer conjugated to modified poly(vinyl alcohol). Arranz et al. synthesized a poly(vinyl alcohol)-nalidixic acid adduct and studied its controlled release behaviour.

Yamaoka et al. has first examined the body distribution of PVAs with various molecular weights. They have demonstrated that PVA was located in most organs but very small accumulation in the relatively short period of time. Tabata et al. examined the tumor accumulation of PVA with different molecular sizes after intravenous injection to the tumor-bearing mice. The use of natural macromolecules as drug carriers has received considerable attention. However, the use of soluble synthetic polymers has not been extensively studied so far. The aim of this paper is to investigate the basic biodisposition of PVA in experimental animals such as biodegradability, dose-dependency and the body distribution especially in the long period of time, as one of the most expected synthetic polymers for the soluble drug carrier system.

Materials and Methods

Materials: PVA samples with various molecular weights (10 K, MW = 10,560; 27 K, MW = 27,280; 46 K, MW = 46,200; 80 K, MW = 80,520; 116 K, MW = 116,600) were kindly supplied by Japan Vam&Poval Co., Ltd., Osaka, Japan. Fluorescein isothiocyanate isomer I (FITC) was obtained from Wako Pure Chemical, Osaka, Japan. Animals were purchased from Shimizu laboratory supplies. All other chemicals and reagents were of the highest grade commercially available.

Preparation of FITC-labeled PVA: FITC-labeled PVA (F-PVA) was prepared by the modified method of deBelder and Granath. PVA (300 mg) was dissolved in dimethyl sulfoxide (DMSO) (8 mL) containing 50 µL of pyridine. FITC (50 mg) was added, followed by dibutyltin dilaurate (20 mg), and the mixture was heated for 2 h at 95°C. After several precipitations in butanol to remove free dye, the fraction of F-PVA was dried in vacuo at 80°C. The F-PVA was further purified by size-exclusion chromatography on Sephadex G-25, and then freeze-dried. The F-PVA sample was dissolved in 25 mM borate buffer (pH 9.0), and the absorbance at 495 nm was measured. The fluorescein content of F-PVA was estimated using the calibration curve of fluorescein sodium (uranine).

Preparation of 125I-labeled poly(vinyl alcohol) (125I-PVA): Tyramine residues were introduced to the hydroxyl groups of PVA molecules by the 1,1'-cabonyldiimidazole (CDI) activation method. Two milliliters of DMSO containing CDI (7.4 mg) was added to 200 mg of PVA (80 K) dissolved in 50 mL of DMSO, followed by stirring for 30 min at room temperature. The reaction mixture was dialyzed against distilled water for 48 h at 4°C and further against 10 mM sodium borate buffer (pH 8.5) for 24 h. Then, tyramine (62.3 mg) was added to the CDI-activated PVA (80 K) and stirred for 48 h at 25°C. The resulting tyramine-bound PVA (80 K) was dialyzed against distilled water for 48 h at 4°C and the macromolecular fraction was lyophilized. Tyramine-bound PVA (80 K) was yielded as a white powder (180 mg) which was found to contain 1 w/w% of tyramine by the measurement of the absorbance at 280 nm.

The tyramine-bound PVA (80 K) (200 µg) was labeled with 0.25 mCi of 125I iodine (Amersham Pharmacia Biotech, Tokyo, Japan) by the chloramine T method. Unreacted 125I was removed by chromatography on a PD-10 column (Amersham Pharmacia Biotech).

Plasma level: Male Wistar rats weighing 200–300 g were fasted for 16 h prior to the experiments. Rats were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg) and were kept on a 40°C plate. Rats were injected with a single dose of F-PVA (80 K) in 0.2 mL of saline through the jugular vein. Blood samples (0.3 mL) were obtained periodically through a cannulated femoral artery after the drug administration. The samples were centrifuged in a microcentrifuge for 5 min, and the resultant serum was harvested. At the end of the experiment, liver was excised and treated as described later.

Tissue distribution: Rats were injected with a single dose of F-PVA (80 K) in 0.2 mL of saline through the tail vein. At 6 h after the administration, blood was collected from the vena cava under ether anesthesia and various organs such as liver, kidney, spleen, lung, heart, stomach, large intestine, small intestine and brain were excised and weighed. Each organ was homogenized on ice with a Potter-Elvehjem-type teflon homogenizer using a 3-fold volume of 0.1 M phosphate buffer (pH 7.4). F-PVA (80 K) in biological samples was analyzed by high-performance size-exclusion chromatography described later.

Mice were injected with 125I-PVA (80 K) (6 mg/kg) in 0.2 mL of saline through the tail vein. Aliquots of the sample solution were stored for the calibration of the disintegration of radioiodine. At appropriate times after the administration, blood was collected from the vena cava under ether anesthesia and various organs such as liver, kidney, spleen, and lung were excised and weighed. The radioactivity was determined with a gamma counter (Aloka 301).

Urinary and fecal excretions: Urine and feces sam-
samples were collected for 72 h in rats after the administration of various doses of F-PVA (80 K) through the tail vein. Urine samples were filtered and diluted appropriately. Feces were homogenized with a Waring Blender-type homogenizer using 9-fold volume of the buffer. The homogenate was centrifuged at 2500 rpm and the supernatant was filtered.

Urine and feces samples were also collected for 8 weeks in mice after the administration of 125I-PVA (80 K) (6 mg/kg) through the tail vein.

**Fluorescence microscopic examination:** Mice were injected with F-PVA (80 K) (120 mg/kg) in 0.2 mL of saline through the tail vein. The liver was excised periodically under ether anesthesia. The liver tissues were fixed overnight in 10% formaldehyde in neutral phosphate buffer in the dark at room temperature. After dehydration in EtOH, the tissues were embedded in paraffin for the preparation of thin sections. Specimens were examined with a Hitachi transmitted light fluorescence microscope.

**High-performance size-exclusion chromatography (HPSEC):** F-PVA (80 K) in biological samples was analyzed by HPSEC. A hundred microliters of H2O and 80 μL of 30% (w/v) trichloroacetic acid were added to 100 μL of plasma. The mixture was vortexed and then centrifuged at 14000 rpm for 5 min. The supernatant (100 μL) was neutralized by addition of 15 μL of 11% (w/v) of NaOH and was diluted with 85 μL of a mobile phase to make 200 μL. After filtration, 50 μL of the sample solution was injected into the HPLC system. In biological samples other than plasma, 200 μL of the sample solution was used without addition of 100 μL of H2O.

HPSEC was carried out using a Tosoh HPLC system (CCPD; Tokyo, Japan) equipped with a variable-wavelength fluorescent detector (RF-530, Shimadzu, Kyoto, Japan). The excitation and emission wavelengths were set at 495 nm and 520 nm, respectively. A 7.8 × 300 mm, TSKgel G4000PWXL column (Tosoh) was used at ambient temperature. The mobile phase was 0.2 M NaCl in 0.05 M phosphate buffer, pH 7.0 and the flow rate was 1.0 mL/min.

**Fluorometric determination:** The amount of F-PVA (80 K) in the urine and feces samples was also determined fluorometrically. The filtrate (100 μL) of the homogenate supernatant was added to 1.9 mL of a 0.5 M Tris-HCl buffer (pH 8.0) containing 0.1% of sodium dodecylsulfate. After thorough mixing, the fluorescence at 520 nm was determined by the excitation at 495 nm.

**Results**

**FITC-labeling and chromatographic analysis of F-PVAs:** PVAs with five different molecular weights of 10 K, 27 K, 46 K, 80 K, and 116 K were labeled with FITC by the modified method of deBelder and Granath. The proposed structure of F-PVA is depicted in Fig. 1. The degree of substitution by FITC was estimated to be 1.37–2.07 × 10−3 FITC mol/OH mol; on the average 480–730 vinyl alcohol groups were found to have one FITC molecule.

Size-exclusion chromatography of each F-PVA which was monitored by fluorescence detection (λex = 495 nm, λem = 520 nm) yielded a single peak as shown in Fig. 2. A strong linear relationship was observed between the retention time of F-PVAs and the logarithm of their molecular weight in the range 10,000–116,000 (Fig. 3). The same retention time was observed in the case of
non-FITC-labeled PVAs which were determined by HPSEC with a differential refractometer, suggesting that the FITC-substitution has no influence on the molecular weight estimation.

On the basis of a 50 \mu L-injection volume, a linear relationship was observed between the peak area of F-PVA (80 K) and their concentrations in serum or other biological specimen. The recovery ratios of F-PVA (80 K) from the serum and the liver homogenate were 91% and 92%, respectively. The sensitivity of the assay was close to 1.5 ng/mL (1.9 \times 10^{-8} M).

**Tissue distribution of F-PVA (80 K) in rats:** Figure 4 shows the plasma levels of F-PVA (80 K) after intravenous injection to rats at various doses. The plasma concentration-time curves were linear on a semilogarithmic scale and the blood persistence of F-PVA (80 K) was independent of the dose. An apparent biological half life and volume of distribution estimated from each linear regression line were almost identical, being 7 h and 11 mL, respectively.

Figure 5 shows the tissue distribution of F-PVA (80 K) at 6 h after intravenous injection to rats at various doses. Although the relatively high levels of F-PVA (80 K) were observed in the plasma as shown in Figure 4, it was slightly distributed into each organ but not detected in the kidney, muscle and brain (Figure 5). No dose-dependency was found in the tissue distribution.

**Excretion of F-PVA (80 K) into urine and feces in rats:** Time profiles of cumulative urinary and fecal excretions of F-PVA (80 K) after intravenous injection to rats at various doses are illustrated in Figure 6. The urinary excretion was almost terminated in 72 h and 25% of the dose was excreted totally in the urine. The amount of F-PVA (80 K) excreted in the feces was 2–3% of the dose at 72 h after injection, but continued to increase gradually. No dose-dependency was also found in the urinary and fecal excretions.

F-PVA (80 K) was excreted as an intact form both in the urine and feces. As shown in Figure 7, the two different measurements both by the HPSEC method and the fluorometric method gave the same results.

**Biodisposition of \textsuperscript{125}I-PVA (80 K) in mice:** Mice were injected intravenously with \textsuperscript{125}I-PVA (80 K) (6 mg/kg). At appropriate times until 56 d (8 w), the radioactivities in a variety of organs were determined with a gamma counter. Figure 8 shows the time profile of the
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tissue distribution of $^{125}$I-PVA (80 K) after intravenous injection to mice. It was found that $^{125}$I-PVA (80 K) was retained in the blood circulation for several days and was gradually distributed into the liver and the spleen.

Time profiles of cumulative urinary and fecal excretions of $^{125}$I-PVA (80 K) after intravenous injection to mice are illustrated in Fig. 9. The urinary excretion was almost terminated in 28 d and 40% of the dose was excreted totally in the urine. The amount of $^{125}$I-PVA excreted in the feces was 18% of the dose at 56 d after injection, and was continued to increase slightly. Figure 10 shows the comprehensive biodistribution of $^{125}$I-PVA (80 K) after intravenous injection to mice. Totally 58% of $^{125}$I-PVA (80 K) was excreted in the urine and feces, and 3% of the dose was still remained in the liver even 56 d after injection. Furthermore, 10% of the radioactivity was detected in the carcass at the end of the experiment (56 d). Therefore, over 70% of the dose was recovered in this experiment.

**Fluorescence microscopic examination:** Fluorescence microscopic examination of paraffin section of the...
Fig. 10. Time profile of biodisposition of $^{125}$I-PVA (80 K) after intravenous injection (6 mg/kg) to mice. (●) urine, (●) feces, (▲) liver, (▼) spleen, (○) plasma.

Fig. 11. Fluorescence microscopic examination of paraffin section of mouse liver at 1 week after intravenous injection of F-PVA(80 K)(120 mg/kg). The bar represents the length of 50 μm.

Fig. 12. Fluorescence microscopic examination of paraffin section of mouse liver at 30 min after intravenous injection of FITC-labeled arabinogalactan (120 mg/kg). The bar represents the length of 50 μm.

Discussion

A high-performance size-exclusion chromatographic procedure for the quantitative analysis of FITC-labeled dextrans in biological media was first developed by Kurtzhals et al. We have demonstrated the pharmacokinetics and biodisposition of fluorescein-labeled arabinogalactan in rats by the same method.

In this study, we have successfully labeled PVAs by FITC according to the method of deBelder and Granath. Then, a specific and sensitive HPSEC method has been established to measure the concentrations of F-PVAs in serial tissues, urine and feces. No significant release of fluorescein was seen in the biological specimen or in the treatment with trichloroacetic acid, indicating that the thiocarbamoyl linkage between the fluorescein and the PVA molecule is stable under these experimental conditions. It was reported that no significant change was seen in either the retention time or the peak area of the FITC-labeled dextrans for more than three days at 37°C in all biological media. Furthermore, a good linear relationship was observed between the retention time of F-PVAs and the logarithm of their molecular weights. Therefore, the method is useful to determine the molecular weight change of F-PVAs in the body based on the retention time analysis.

In the previous work, we reported that the blood persistence of polysaccharides such as dextrans and pullulans was increased with an increase in dose. It was found that the marked dose-dependency in the non-linear pharmacokinetics of dextrans and pullulans was mainly due to the saturation of hepatic uptake of these polysaccharides. As can be seen in Figs. 4–6, a dose-dependent behavior of F-PVA (80 K) was found neither in the blood circulation, nor in the tissue distribution, nor in the urinary and fecal excretions. Therefore, it was suggested that PVAs are eliminated exclusively by the mechanisms that do not involve saturable transport processes. Furthermore, it was found that PVAs are very stable in the body because no degradation product was detected in the urine and feces.
(Fig. 7).

Ravin et al. used $^{131}$I-labelled and $[^{14}C]poly$(vinylpyrrolidone) (PVP) to determine whether or not PVP can be metabolized by the body and the long-term fate of the polymer. It has been shown that PVP is not metabolized to any significant degree by the rat, dog or man. The reticuloendothelial system retains PVP with a molecular weight in excess of 110,000 to 120,000 for a long time, probably years.

The half-life of $^{125}$I-PVA in the blood circulation largely depends on the molecular weight. The cut-off molecular weight of PVAs, which is consists of nonionic, random-coiled chain molecules, for the glomerular permeability was reported to be ~30,000. Mean residence time of $^{125}$I-PVA (80 K) for the elimination phase in the mice plasma was estimated to be 12.9 d (Fig. 10).

Although the tissue distribution of PVAs was small, a significant accumulation into liver and spleen was observed (Fig. 8). It was reported that $^{125}$I-PVP was taken up by non-specific fluid-phase endocytosis in rat liver and spleen. The uptake clearances of $^{125}$I-PVA (80 K) by liver and spleen were estimated to be 0.005–0.018 mL/h/g organ and 0.006–0.009 mL/h/g organ, respectively, which were almost similar to those of $^{125}$I-PVP reported.

The participation of parenchymal, Kupffer, and endothelial liver cells was suggested in the clearance of PVAs by the reticuloendothelial system after intravenous injection in mice. Although the specific rate of uptake by parenchymal cells was always smaller than that shown by both Kupffer and endothelial cells, the total contribution of parenchymal cells to the clearance of PVAs was suggested to be even greater than that of the other cell classes. This was confirmed by the fluorescence microscopic examination of paraffin section of the liver at 1 w after intravenous injection to mice, showing that F-PVA (80 K) was endocytosed by the liver parenchymal cells (Fig. 11).

Mean residence times of $^{125}$I-PVA (80 K) in the liver and spleen were estimated to be 72.9 d and 64.3 d, respectively (Fig. 10). The amount of $^{125}$I-PVA excreted in the feces was 18% of the dose at 56 d after injection, and was continued to increase slightly (Fig. 10). $^{125}$I-PVA (80 K) captured by the liver was slowly transported via the bile canaliculi and gall bladder to the intestine and excreted in the feces. It was suggested, therefore, a long time is necessary for $^{125}$I-PVA (80 K) to be excreted perfectly from the body.

**Conclusion**

Water-soluble nonionic polymers such as PVAs are usually characterized by relatively low toxicity. When administered systemically these polymers are often removed from the body without degradation. The rates and mechanisms of clearance of the polymers are dependent on their molecular weight. The use of polymers displaying a molecular mass above the renal threshold limit permits the polymers to escape renal clearance, shifting the primary route of blood clearance to elimination through extravasation into tissues.

If very large polymer molecules are needed for drug delivery, one can produce such molecules by cross-linking smaller smaller nondegradable polymers via biodegradable links. The cleavage of those linkages in the body will result in the release of the smaller polymers, which can be eventually removed through the renal clearance route.

Although PVAs are candidate for a useful drug carrier, our results demonstrated here must be kept in mind in the future design of drug delivery system with such synthetic polymers.

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**References**


