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Synthesis and biodistribution study of liver-specific prostaglandin E_1 polymeric conjugate

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Abstract

We synthesized a polymeric prodrug of prostaglandin E_1, (PGE_1) using galactosylated poly-(L-glutamic acid) (Gal-PLGA) as a biodegradable and targetable carrier to the liver parenchymal cells. PLGA was reacted with ethylenediamine followed by coupling with 2-imino-2-methoxyethyl-1-thiogalactosides to obtain Gal-PLGA. PGE_1 was activated with *N*,*N*′-carbonyldiimidazole (CDI) then the PGE_1 ester obtained was attached to Gal-PLGA. After intravenous injection in mice at a dose of 1 mg/kg PGE_1 conjugates with Gal-PLGA (PGE_1-PLGA-Gal) labeled with [^{111}In] or [^{3}H]PGE_1 rapidly accumulated in the liver up to 65 and 50% of the dose respectively. The hepatic uptake of [^{111}In]PGE_1-PLGA-Gal was remarkably inhibited by the co-administration of Gal-BSA indicating that the PGE_1 conjugates were taken up by the liver via the asialoglycoprotein receptor-mediated endocytosis. These findings suggest that PGE_1 can be effectively targeted to the liver parenchymal cells by covalently conjugating with Gal-PLGA.
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Keywords: Prostaglandin E_1; Galactosylated poly-(L-glutamic acid); Liver-specific drug carrier; Hepatitis; Conjugate; Asialoglycoprotein receptor

1. Introduction

PGE_1 is now clinically used for treating some diseases, such as peripheral vascular disturbance and skin ulcer. In addition, PGE_1 is also effective on fulminant or subfulminant viral hepatitis (Sinclair and Levy, 1991) by means of its cytoprotectivity (Stachura et al., 1981; Ueda et al., 1987; Beck et al., 1993; Helling et al., 1995). However, long-term administration of PGE_1 is required for the treatment of the hepatitis due to its low hydrophilicity and low physiological stability

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(Monkhouse et al., 1973; Younger and Szabo, 1986). An autoradiographic study demonstrated that [^{3}H]PGE_1 mainly distributed in the liver and kidneys (Hansson et al., 1965). However, it was reported as much as 80% of PGE_1 was metabolized and inactivated by β- or ω-oxidation during the first passage through the lungs (Porst, 1996). Therefore, a proper drug delivery system (DDS) should be developed for delivering PGE_1 to the liver in an intact form, for preventing side effects, and for solubilizing in an aqueous solution. Some DDS approaches for PGE_1 have been investigated using cyclodextrins (Uekama et al., 1992), lipid microspheres (Igarashi et al., 1992; Katoh et al., 1992; Mizushima et al., 1993), liposomes (Rossetti et al., 1994; Willerson et al., 1994) and heparin conjugate (Jacobs et al., 1986). However, there are few studies on the hepatic targeting of PGE_1, though the selective delivery of PGE_1 to the liver would increase the therapeutic availability of the drug against the hepatitis.

In the last few years, we have achieved the selective delivery of low-molecular weight drugs, proteins and polynucleotides to the liver via the sugar-recognizing receptors (Nishikawa et al., 1992, 1993, 1995a,b; Fujita et al., 1992a,b, Hirabayashi et al., 1996, Hashida et al., 1997; Mahato et al., 1997). Some galactosylated polymeric carriers synthesized using water-soluble polymers such as dextran, amylose and poly-(L-glutamic acid) (PLGA) were proved to be useful for delivering drugs to liver parenchymal cells after intravenous injection (Nishikawa et al., 1993; Hirabayashi et al., 1996; Hashida et al., 1997). Furthermore, the pharmacological activity of vitamin K_5 was enhanced by conjugating to galactosylated PLGA (Gal-PLGA) (Hashida et al., 1997).

In this study, therefore, we applied Gal-PLGA as a carrier for PGE_1 to selectively deliver to liver parenchymal cells. PGE_1 was covalently conjugated with Gal-PLGA by condensation, and the biodistribution characteristics were investigated in mice after intravenous injection of PGE_1 conjugate labeled with either [^{111}In] or [^{3}H]PGE_1.

2. Materials and methods

2.1. Chemicals

PLGA with an average molecular weight of approximately 15 000 was purchased from Sigma, St. Louis, MO. *D*-Galactose and ethylenediamine were obtained from Wako Pure Chemical, Osaka, Japan. Diethylenetriamine-*N*,*N*,*N*′,*N*″,*N*″-pentaacetic dianhydride (DTPA anhydride) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) were obtained from Dojindo Laboratory, Kumamoto, Japan. ^{111}Indium chloride was supplied from Nihon Medi-Physics, Takarazuka, Japan. Prostaglandin E_1 (PGE_1) was obtained from Ono Pharmaceutical, Osaka, Japan. [5,6(*n*)-^{3}H]PGE_1 was purchased from Amersham Japan, Tokyo. All other chemicals were reagent grade products obtained commercially.

2.2. Animals

Male ddY mice (25–28 g) were obtained from the Shizuoka Agricultural Cooperative Association for Laboratory Animals, Shizuoka, Japan.

2.3. Synthesis and characterization of Gal-PLGA conjugate

2.3.1. Synthesis of 2-imino-2-methoxyethyl-1-thiogalactoside (IME-thiogalactoside)

IME-thiogalactoside was prepared as described previously (Nishikawa et al., 1995a). Briefly, cyanomethyl-1-thiogalactoside was treated with 0.01 M sodium methoxide methanolic solution at room temperature for 24 h. The solvent was evaporated in vacuo. Resultant syrup was dissolved in 4 ml of 0.4 M borate buffer (pH 10) (Solution 1).

2.3.2. Conjugation of PLGA and IME-thiogalactoside

PLGA (200 mg) was dissolved in 10 ml of distilled water. This was stirred in an ice bath at 0°C. Ethylenediamine (160 μl) was added to the above solution, and the pH of the mixture was adjusted to about 5.0 by addition of 1 N HCl.

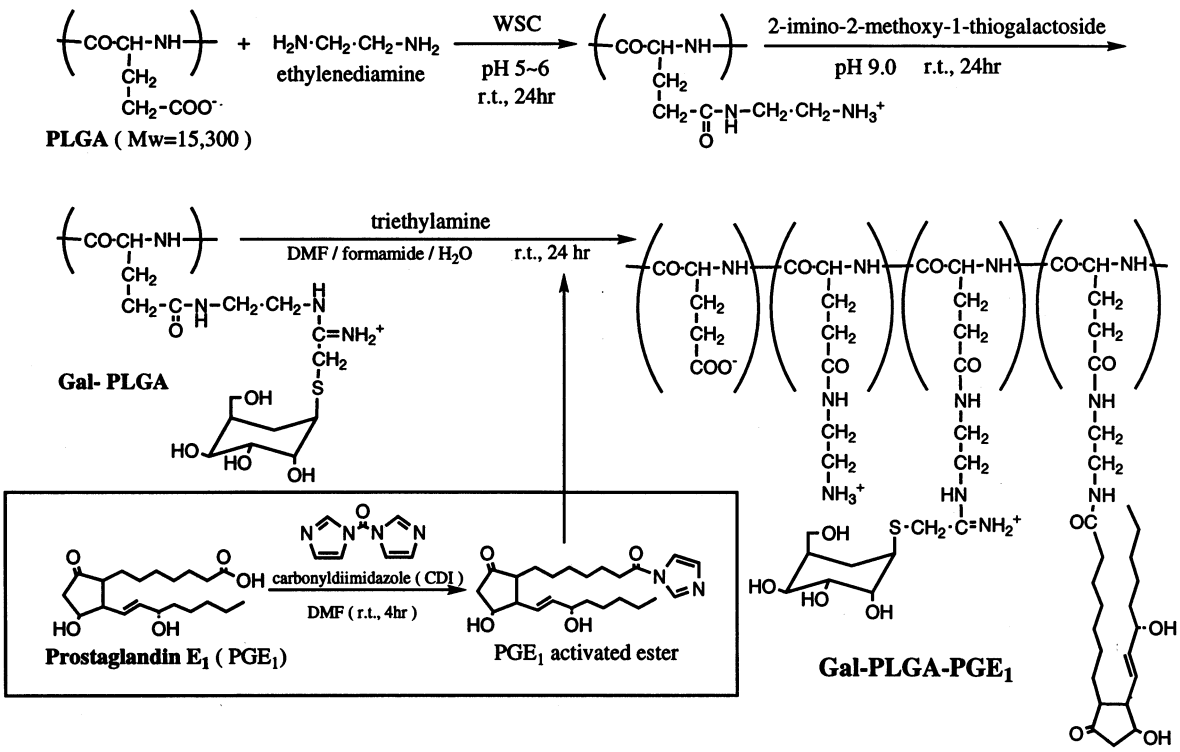

Fig. 1. Synthetic procedures of Gal-PLGA and PGE_1-PLGA-Gal.

Then, EDC aqueous solution (450 mg/2 ml) was added into the mixture. The pH of the reaction mixture was kept at 5.0. The reaction was allowed to proceed overnight with stirring. The solution was transferred to dialysis tubing (12 kDa cut-off) and dialyzed against distilled water for 24 h. The dialyzate was concentrated untifll 5 ml by ultrafiltration (10 kDa cut-off) (Solution 2).

Solution 1 was added dropwise into the Solution 2 and the pH of the mixture was maintained at about 10 by addition of 1 N HCl solution. After stirring for 10 h at room temperature, the reaction mixture was dialyzed against water for 48 h and the final solution was lyophilized (Gal-PLGA) (Fig. 1). The number of galactose residues in the derivative was determined by the anthrone-sulfuric acid method.

2.4. Synthesis of PGE_1-PLGA-Gal conjugate

2.4.1. Activation of PGE_1

PGE_1 activated ester was prepared by carbonyldiimidazole (CDI) method. PGE_1 (10 mg, 28.2 μmol) was dissolved in 0.5 ml of dry dimethylformamide (DMF) and chilled at 0°C. Then, CDI (5 mg, 30.8 μmol in 0.5 ml DMF) was added dropwise to the above solution with stirring. The reaction was maintained at 0°C for 10 min, and then at room temperature for 4 h. Radioactive PGE_1 activated ester was also prepared according to the same method using a trace amount of [^{3}H]PGE_1.

2.4.2. Coupling of activated PGE_1 and Gal-PLGA

Fifty milligrams of Gal-PLGA was dissolved in 4 ml of DMF/formamide/H_2O (1:2:1 (v/v/v)) solution. Reaction mixture obtained above (PGE_1, activated ester) and triethylamine (5 μl) were slowly added to the solution. After stirring over night at 4°C the solution was dialyzed exhaustively against water for 48 h at 4°C followed by ultrafiltration and lyophilization (Fig. 1).

2.5. ^{111}In-labeling of PLGA derivatives

PLGA derivatives were radiolabeled with ^{111}In using DTPA anhydride as described previously (Nishikawa et al., 1995a). Each radiolabeled derivative was purified by gel-filtration chromatography using a Sephadex G-25 column (1.5×5.0 cm 0.1 M acetate buffer (pH 6.0) and determined to have a specific activity of approx. 37 MBq/mg.

2.6. Quantification of PGE_1 covalently bound to Gal-PLGA

The PGE_1 amount in the unlabeled PGE_1-PLGA-Gal conjugate was quantified for the PGE_1 to PGB_1 degradation. PGE_1 undergoes a base-catalyzed dehydration and rearrangement to form PGB_1 ($\lambda_{max} = 282$ nm, $\varepsilon = 20\,400/M$ per cm). PGE_1-PLGA-Gal aqueous solution (2 mg/ml) was prepared in a sample tube following addition of 0.6 ml of 5 M NaOH. After 15 min at room temperature the absorbance at 282 nm was measured on a UV spectrophotometer since there was no further increase in the absorbance when the solution was incubated for more than 15 min. The amount of [^{3}H]PGE_1 in [^{3}H]PGE_1-PLGA-Gal was estimated from the ^{3}H-radioactivity of the conjugate.

2.7. Biodistribution experiment

Mice received a 1 mg/kg dose of [^{111}In]PLGA, [^{111}In]Gal-PLGA, [^{3}H]PGE_1-PLGA-Gal or a 0.026 mg/kg dose of [^{3}H]PGE_1 in saline by tail vein injection and were housed in metabolic cages for urine collection. At appropriate intervals after injection, blood was collected from the vena cava under ether anesthesia and plasma was obtained by centrifugation. The heart, lungs liver, spleen, kidneys and muscle were excised, rinsed with saline, weighed, and examined for radioactivity. The amount of radioactivity in urine was determined by collecting urine both excreted and remaining in the bladder. ^{111}In radioactivity was counted by a well-type NaI scintillation counter (ARC-500, Aloka, Tokyo). And the radioactivity of ^{3}H was measured using a liquid scintillation counter (LSC-5000, Beckman, Tokyo) after dissolution with Soluene-350 (Packard, Netherlands) and added with a scintillation medium Clear-sol I (Nakalai Tesque, Tokyo). Radioactivity originating from the plasma in each tissue sample was corrected using the distribution data of [^{111}In]BSA at 10 min after intravenous injection (Nishikawa et al., 1995b), assuming that [^{111}In]BSA was not taken up by tissues during the 10-min period.

2.8. Pharmacokinetic analysis

Tissue distribution patterns of [^{111}In]PLGA derivatives were evaluated from the organ uptake clearance (CL_{org}) according to the method previously reported (Takakura et al., 1987). In the early period after injection, the efflux of ^{111}In radioactivity from organs is assumed to be negligible since the degradation products of ^{111}In-labeled ligands using DTPA cannot easily pass through biological membranes (Arano et al., 1994; Duncan et al., 1993). With the assumption described above, CL_{org} was calculated by dividing the amount of radioactivity in an organ at 10 min by the area under the plasma concentration-time curve (AUC) up to the same time point. AUC and the total-body clearance (CL_{total}), were calculated by fitting an equation derived from a linear one- or two-compartment open model to the plasma concentration data of the derivatives using the nonlinear least-square program MULTI (Yamaoka et al., 1981).

2.9. Simultaneous administration of PLGA derivatives and BSA derivatives

[^{111}In]Gal-PLGA or [^{111}In]PGE_1-PLGA-Gal was injected at a dose of 1 mg/kg with galactosylated bovine serum albumin (Gal-BSA) or succinylated BSA (Suc-BSA) at a dose of 10 mg/kg. At 10 min after injection, plasma and liver were sampled and subjected to assay for radioactivities. Differences in radioactivity distribution were statistically evaluated by Student's t-test.

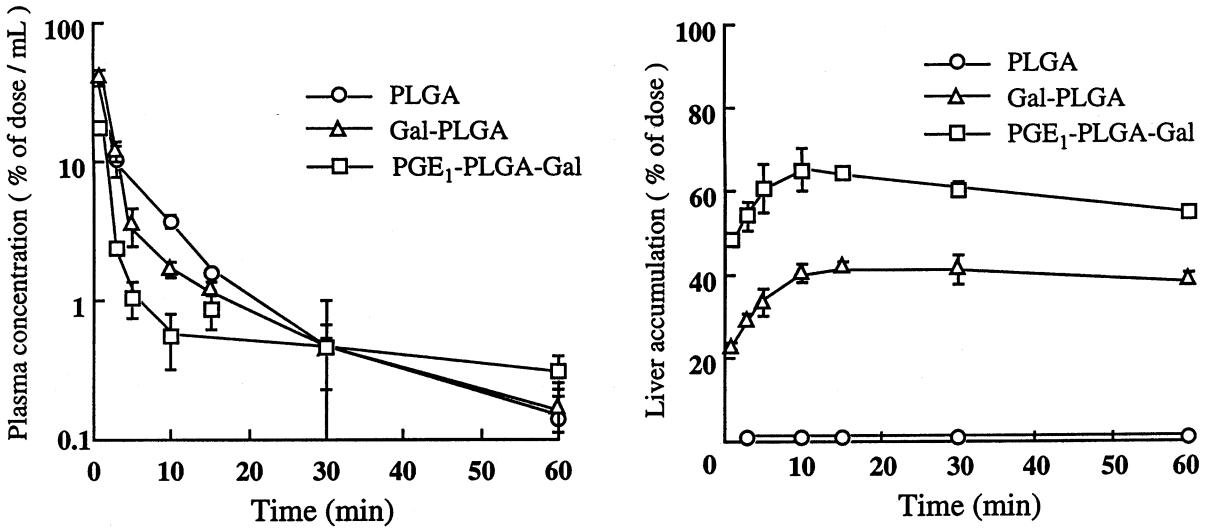

Fig. 2. Plasma concentration and liver accumulation-time courses of ^{111}In-labeled PLGA derivatives after intravenous injection in mice at a dose of 1 mg/kg. Results are expressed as the mean ± S.D. of three mice.

3. Results

3.1. Physicochemical characteristics of Gal-PLGA and PGE_1-PLGA-Gal

The synthesized Gal-PLGA contained 17 galactose residues per PLGA. This derivative was used for synthesizing PGE_1 conjugates. The numbers of PGE_1 in the conjugates were determined to be 1.6 and 0.032 per PGE_1-PLGA-Gal and [^{3}H]PGE_1-PLGA-Gal respectively.

3.2. Plasma clearance and tissue distribution of [^{111}In]PLGA, [^{111}In] Gal-PLGA and [^{111}In]PGE_1-PLGA-Gal

Fig. 2 shows the concentrations in the plasma and the amounts in the liver of [^{111}In]PLGA, [^{111}In]Gal-PLGA and [^{111}In]PGE_1-PLGA-Gal after intravenous injection in mice. All of them were rapidly cleared from the circulation and the amounts recovered in the liver were 1, 42 and 65% of the dose at 10 min after injection for [^{111}In]PLGA, [^{111}In]Gal-PLGA, and [^{111}In]PGE_1-PLGA-Gal, respectively. The amounts of [^{111}In]PGE_1-PLGA-Gal recovered in the liver were higher than those of [^{111}In]Gal-PLGA at any time points studied.

3.3. Plasma clearance and tissue distribution of [^{3}H]PGE_1 and [^{3}H]PGE_1-PLGA-Gal

Fig. 3 shows the concentration in plasma and the amounts in tissues of [^{3}H]PGE_1 and [^{3}H]PGE_1-PLGA-Gal after intravenous injection in mice. [^{3}H]PGE_1 was rapidly eliminated from the circulation and excreted in urine after accumulation in the kidneys and the liver. Although 35% of [^{3}H]PGE_1 administered was detected in the liver at 1 min after injection the radioactivity in the liver rapidly eliminated.

After intravenous injection [^{3}H]PGE_1-PLGA-Gal was also accumulated in the liver up to about 50% of the dose within 10 min. There were no significant recovery of radioactivity in other tissues sampled and urine except for the kidneys. The distribution characteristics of [^{3}H]PGE_1-PLGA-Gal were comparable to those of [^{111}In]Gal-PLGA indicating that PGE_1 would not be released from the carrier Gal-PLGA before delivered to the liver parenchymal cells.

3.4. Pharmacokinenic analysis of PLGA derivatives

Table 1 summarizes the clearance values for liver (CL_{liver}), kidney (CL_{kidney}), urine (CL_{urine}),

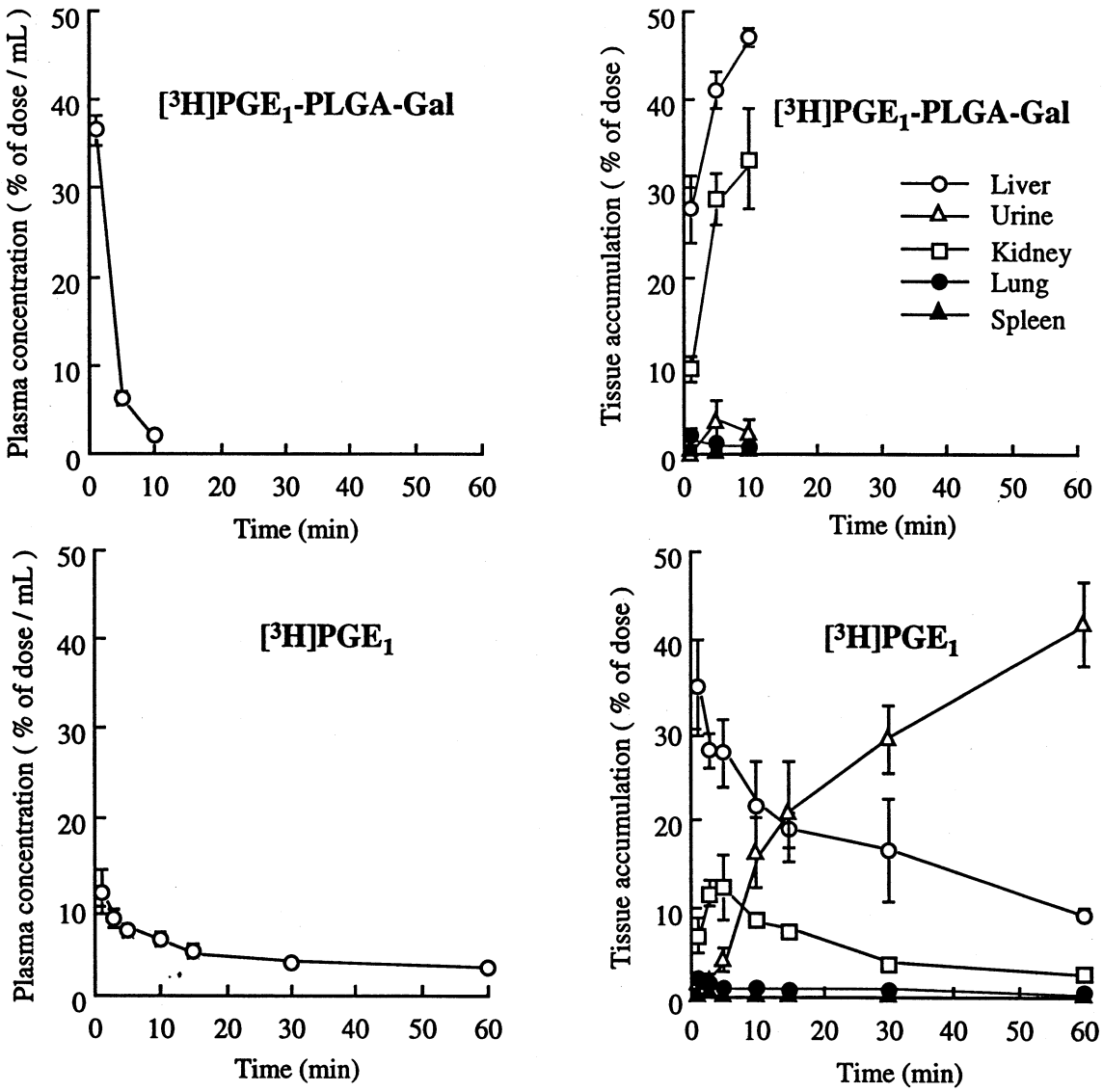

Fig. 3. Plasma concentration and tissue accumulation-time courses of ^{3}H-labeled PGE_1-PLGA-Gal and ^{3}H-labeled PGE_1 after intravenous injection in mice at a dose of 1 and 0.026 mg/kg respectively. Results are expressed as the mean ± S.D. of three mice.

spleen (CL_{spleen}), lung (CL_{lung}), CL_{total}, and the AUC of each compound calculated from the results of in vivo distribution experiments. [^{111}In]Gal-PLGA [^{111}In]PGE_1-PLGA-Gal and [^{3}H]PGE_1-PLGA-Gal showed high CL_{liver} but that of [^{111}In]PGE_1-PLGA-Gal was higher than those of other two derivatives. Most of pharmacokinetic parameters, such as CL_{liver}, CL_{kidney}, and CL_{total} of [^{3}H]PGE_1-PLGA-Gal were close to those of [^{111}In]Gal-PLGA.

3.5. Competitive inhibition of hepatic uptake of [^{111}In]PLGA derivatives by BSA derivatives

Table 2 summarizes the plasma concentration and liver accumulation of [^{111}In]Gal-PLGA and

Table 1
AUC and clearance for ^{111}In or ^{3}H-labeled PLGA derivatives after intravenous injection in mice at a dose of 1 mg/kg

Compounds	AUC (% of dose h/ml)	Clearance (μl/h)					
		CL_{total}	CL_{liver}	CL_{kidney}	CL_{urine}	CL_{spleen}	CL_{lung}
[^{111}In]PLGA	2.15	46 500	640	7870	18 000	6.0	83.0
[^{111}In]Gal-PLGA	1.97	50 900	21 300	15 800	1880	25.0	61.0
[^{111}In]PGE$_1$-PLGA-Gal	1.22	81 700	56 000	8450	5520	17.0	52.0
[^{3}H]PGE$_1$[a]	1.76	56 700	17 200	6890	12 900	192.0	786.0
[^{3}H]PGE$_1$-PLGA-Gal	2.24	44 700	21 500	15 100	1040	287.0	373.0

[a] PGE$_1$ was injected in mice at a dose of 0.026 mg/kg.

[^{111}In]PGE$_1$-PLGA-Gal administered with Gal-BSA or Suc-BSA. Co-administration of Gal-BSA reduced the amounts of [^{111}In]Gal-PLGA and [^{111}In]PGE$_1$-PLGA-Gal recovered in the liver from 44 to 19% and from 59 to 26% respectively. Both ^{111}In-labeled derivatives showed higher concentrations in plasma at 10 min after injection when administered with Gal-BSA probably due to the saturation of the hepatic uptake mediated by the asialoglycoprotein receptor. On the other hand the distribution patterns of both [^{111}In]Gal-PLGA and [^{111}In]PGE$_1$-PLGA-Gal were not affected by the simultaneous injection of Suc-BSA, a ligand taken up by the liver via the scavenger receptor-mediated endocytosis (Takakura et al., 1994).

4. Discussion

PLGA can be a promising carrier for drugs since it is biodegradable, highly soluble in water and it has many carboxyl groups that are easily modified chemically, and low immunogenicity and low toxicity. The molecular weight of PLGA (15 kDa) was chosen for suppressing non-specific uptake by various tissues. Although the molecular weight of Gal-PLGA was not determined, a GPC analysis showed there was not a large difference in the molecular weights of PLGA and Gal-PLGA. In the previous study, the CL_{liver} of Gal-PLGAs were highly dependent on the number of galactose residues per PLGA molecule, suggesting that the hepatic targetability of the carrier could be controlled by its galactose content: 16 or more galactose residues were required for PLGA (25 kDa) to possess large CL_{liver} (Hirabayashi et al., 1996). Gal-PLGA used in this study, therefore, can be considered to have enough number or density of galactose residues to be targeted to liver parenchymal cells.

The CDI method would be a very effective approach to activate PGE$_1$ since CDI is highly reactive to the carboxyl group of PGE$_1$ even at low temperature. When PGE$_1$ was activated and conjugated to Gal-PLGA, inactive gaseous carbon dioxide and imidazole were generated as by-products (Anderson and Paul, 1958), which would promote the conjugation reaction as a base catalyst. The low [^{3}H]PGE$_1$ content of [^{3}H]PGE$_1$-PLGA-Gal (0.032 mol PGE$_1$ per mol conjugate) was probably caused by water contained in the [^{3}H]PGE$_1$ solution purchased (ethanol/water (7:3) solution).

After intravenous injection, [^{111}In]Gal-PLGA, [^{111}In]PGE$_1$-PLGA-Gal and [^{3}H]PGE$_1$-PLGA-Gal were rapidly accumulated in the liver (Fig. 2 and Fig. 3). The asialoglycoprotein receptor should be involved in their hepatic uptake since the amounts of both ^{111}In-labeled compounds were reduced by the co-injection of an excess amount of Gal-BSA, a well-known ligand for the receptor (Klantz et al., 1976; Nishikawa et al., 1992). [^{3}H]PGE$_1$-PLGA-Gal was also accumulated in the kidneys up to about 35% of the dose (Fig. 3). This distribution feature was also observed for [^{111}In]PLGA, [^{111}In]Gal-PLGA and [^{111}In]PGE$_1$-PLGA-Gal and would be explained by the reabsorption at the proximal tubules after the glomerular filtration (Hirabayashi et al., 1996; Mihara et al., 1994).

Table 2
Competitive inhibition of hepatic uptake of ^{111}In-labeled Gal-PLGA and PGE_1-PLGA-Gal by Gal-BSA or Suc-BSA[a]

Compounds (1 mg/kg)	Inhibitor (10 mg/kg)	Plasma concentration (% of dose/ml)	Liver accumulation (% of dose)
Gal-PLGA	None	0.59 ± 0.12[b]	43.9 ± 3.7
	Gal-BSA	2.61 ± 0.39*	18.7 ± 0.2*
	Suc-BSA	0.71 ± 0.02	43.1 ± 1.3
PGE_1-PLGA-Gal	None	0.76 ± 0.04	59.4 ± 1.2
	Gal-BSA	3.42 ± 1.02	26.2 ± 2.8*
	Suc-BSA	0.75 ± 0.20	59.6 ± 1.4

[a] Radiolabeled compound (1 mg/kg) was injected with an inhibitor (10 mg/kg), and the plasma concentration and liver accumulation of radiolabeled compound were assayed 10 min after injection.
[b] Results are expressed as the mean $\pm$ S.D. of three mice.
* Statistically significant difference based on Student's t-test ($p < 0.02$) as compared with each control.

Renal accumulation of PGE_1 conjugates could be suppressed by increasing the molecular weight of PLGA.

The CL_{liver} of [^{111}In]PGE_1-PLGA-Gal was higher than that of [^{111}In]Gal-PLGA (Table 2). This finding could be explained by two factors. One is the difference in their molecular weights. A GPC analysis showed that the peak start point of time of PGE_1-PLGA-Gal in its chromatogram was earlier than that of Gal-PLGA (data not shown). The other possibility is the effect of PGE_1 conjugated to Gal-PLGA. The conformation of Gal-PLGA chain could be changed by the conjugation of PGE_1. In addition the recognition of PGE_1 on Gal-PLGA by the PGE receptor on hepatocytes may play a role in increasing CL_{liver} (Smigel and Fleischer, 1974, 1977; Garrity et al., 1987). This is not the case for [^{3}H]PGE_1-PLGA-Gal since only 0.032 molecule of PGE_1 was attached to Gal-PLGA molecule when [^{3}H]PGE_1 was used in the conjugation.

Tissue distribution of [^{3}H]PGE_1-PLGA-Gal was very similar to that of [^{111}In]GalPLGA (Fig. 2 and Fig. 3). In addition pharmacokinetic parameters of [^{3}H]PGE_1-PLGA-Gal were comparable to those of the carrier molecule (Table 1). These results suggest that the in vivo fate of PGE_1 is controlled by the distribution properties of the carrier Gal-PLGA and that PGE_1 can be efficiently delivered to the liver before released from the carrier. Although free [^{3}H]PGE_1 was also rapidly distributed to the liver after intravenous injection most of PGE_1 should be metabolized on passage through the pulmonary circulation (Porst, 1996). The PGE_1 conjugated to Gal-PLGA could be prevented from the enzymatic metabolism in the lungs because of the steric hindrance of the carrier molecule. And, therefore, it could reach the liver prior to its degradation. Although the fate of PGE_1-PLGA-Gal after being taken up by the liver parenchymal cells has not clarified yet the degradation characteristic of Gal-PLGA would help PGE_1 to be released from the carrier molecule. PGE_1 will probably show its cytoprotective activity through the binding to its receptor. Therefore, it should be released from the carrier after being delivered to the liver parenchymal cells. Although the release of PGE_1 from Gal-PLGA would help PGE_1 to be released from the carrier as PGE_1- or PGE_1-amino acid derivative. PGE_1 released should be metabolized in the cells as injected in a free form but the increased amount of PGE_1 could enhance its cytoprotectivity against several liver failures. Anyway, the intracellular behavior of conjugate and the release of PGE_1 as well as the stability of PGE_1 conjugates must be investigated thoroughly.

5. Conclusions

A PGE_1 polymeric conjugate was successfully synthesized by CDI method using Gal-PLGA as a targetable carrier to liver parenchymal cells. PGE_1

could be targeted to the liver via the asialoglycoprotein receptor-mediated endocytosis before released from the carrier. These findings will support the possibility of PGE_1-PLGA-Gal as a therapeutic agent for fulminant viral hepatitis.

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References

Anderson, G.W., Paul, R., 1958. *N,N′*-carbonyldiimidazole, a new reagent for peptide synthesis. J. Am. Chem. Soc. 80, 4423.

Arano, Y., Mukai, T., Uezono, T., Wakisaka, K., Motonari, H., Akizawa, H., Taoka, Y., Yokoyama, A., 1994. A biological method to evaluate bifunctional chelating agents to label antibodies with metallic radionuclides. J. Nucl. Med. 35, 890–898.

Beck, P.L., McKnight, G.W., Kelly, J.K., Wallace, J.L., Lee, S.S., 1993. Hepatic and gastric cytoprotective effects of long-term prostaglandin E_1 administration in cirrhotic rats. Gastroenterology 105, 1483–1489.

Duncan, J.R., Welch, M.J., 1993. Intracellular metabolism of indium-111-DTPA-labeled receptor targeted proteins. J. Nucl. Med. 34, 1728–1738.

Fujita, T., Nishikawa, M., Tamaki, C., Takakura, Y., Hashida, M., Sezaki, H., 1992a. Targeted delivery of human reconbinant superoxide dismutase by chemical modification with mono- and polysaccharide derivatives. J. Pharmacol. Exp. Ther. 263, 971–978.

Fujita, T., Furitsu, H., Nishikawa, M., Takakura, Y., Sezaki, H., Hashida, M., 1992b. Therapeutic effect of superoxide dismutase derivatives modified with mono- or polysaccharides on hepatic injury induced by ischemia/reperfusion. Biochem. Biophys. Res. Commun. 189, 191–196.

Garrity, M.J., Brass, E.P., 1987. Fasting-induced changes in prostaglandin binding in isolated hepatocytes and liver plasma membranes. Endocrinology 120, 1134–1139.

Hansson, E., Samuelsson, B., 1965. Autoradiographic distribution studies of ^3H-labeled prostaglandin E_1 in mice prostaglandins and related factors. Biochim. Biophys. Acta 106, 379–385.

Hashida, M., Hirabayashi, H., Nishikawa, M., Takakura, Y., 1997. Targeted delivery drugs and proteins to the liver via receptor-mediated endocytosis. J. Control. Rel., 129–137.

Helling, T.S., Wogahn, B.M., Olson, S.A., Evans, L.S., Reddy, B.R., VanWay, C.III., 1995. The effect of prostaglandin E_1 on liver adenine nucleotides and cytoplasmic enzymes in a porcine model of normothermic hepatic ischemia. Hepatology 22, 1554–1559.

Hirabayashi, H., Nishikawa, M., Takakura, Y., Hashida, M., 1996. Development and pharmacokinetics of galactosylated poly-L-glutamic acid as a biodegradable carrier for liver-specific drug delivery. Pharm. Res. 13, 880–884.

Igarashi, R., Mizushima, Y., Takenaga, M., Matsumoto, K., Morizawa, Y., Yasuda, A., 1992. A stable PGE_1 prodrug for targeting therapy. J. Control. Rel. 20, 37–46.

Jacobs, H., Kim, S.W., 1986. In vitro bioactivity of a synthesized prostaglandin E_1-heparin conjugate. J. Pharm. Sci. 75, 172–175.

Katoh, K., Kawai, T., Narita, M., Uemura, J., Tani, K., Okubo, T., 1992. Use of prostaglandin E_1 (lipo-PGE_1) to treat Raynaud's phenomenon associated with connective tissue disease: thermographic and subjective assessment. J. Pharm. Pharmacol. 44, 442–444.

Klantz, M.J., Holtzman, N.A., Stowell, C.P., Lee, Y.C., 1976. Attachment of thioglycosides to proteins: Enhancement of liver membrane binding. Biochemistry 15, 3963–3968.

Mahato, R.I., Takemura, S., Akamatsu, K., Nishikawa, M., Takakura, Y., Hashida, M., 1997. Physicochemical and disposition characteristics of antisense oligonucleotides complexed with glycosylated poly (L-lysine). Biochem. Pharmacol. 53, 887–895.

Mihara, K., Sawai, K., Takakura, Y., Hashida, M., 1994. Manipulation of renal disposition of human recombinant superoxide dismutase by chemical modification. Biol. Pharm. Bull. 17, 296–301.

Mizushima, Y., Hoshi, K., 1993. Recent advances in lipid microsphere technology for targeting prostaglandin delivery. J. Drug Targeting 1, 93–100.

Monkhouse, D.C., Campen, L.V., Aguiar, A.J., 1973. Kinetics of dehydration and isomerization of prostaglandin E_1 and E_2. J. Pharm. Sci. 62, 576–580.

Nishikawa, M., Miyazaki, C., Yamashita, F., Takakura, Y., Hashida, M., 1995a. Galactosylated proteins are recognized by the liver according to the surface density of galactose moieties. Am. J. Physiol. 268, G849–G856.

Nishikawa, M., Hirabayashi, H., Takakura, Y., Hashida, M., 1995b. Design for cell-specific targeting of proteins utilizing sugar-recognition mechanism: Effect of molecular weight of proteins on targeting efficacy. Pharm. Res. 12, 209–214.

Nishikawa, M., Kamijo, A., Fujita, T., Takakura, Y., Sezaki, H., Hashida, M., 1993. Synthesis and pharmacokinetics of a new liver-specific carrier, glycosylated carboxymethyldextran, and its application to drug targeting. Pharm. Res. 10, 1253–1261.

Nishikawa, M., Ohtsubo, Y., Ohno, J., Fujita, T., Koyama, Y., Yamashita, F., Hashida, M., Sezaki, H., 1992. Phamacokinetics of receptor-mediated hepatic uptake of glycosylated albumin in mice. Int. J. Pharm. 85, 75–85.

Porst, H., 1996. The rationale for prostaglandin E_1 in erectile failure: A survey of worldwide experience. J. Urol. 155, 802–815.

Rossetti, R.G., Brathwaite, K., Zurier, R.B., 1994. Suppression of acute inflammation with liposome associated prostaglandin E_1. Prostaglandins 48, 187–195.

Sinclair, S.B., Levy, G.A., 1991. Treatment of fulminant viral hepatic failure with prostaglandin E. Dig. Dis. Sci. 36, 791–800.

Smigel, M., Fleischer, S., 1974. Characterization and localization of prostaglandin E_1 receptors in rat liver plasma membranes. Biochim. Biophys. Acta 332, 358–373.

Smigel, M., Fleischer, S., 1977. Characterization of Triton X-100-solubilized prostaglandin binding protein of rat liver plasma membranes. J. Biol. Chem. 252, 3689–3696.

Stachura, J., Tarnawski, A., Ivey, K.J., Mach, T., Bogdal, J., Szczudrawa, J., Klimczyk, B., 1981. Prostaglandin protection of carbon tetrachloride-induced liver cell necrosis in the rat. Gastroenterology 81, 211–217.

Takakura, Y., Fujita, T., Furitsu, H., Nishikawa, M., Sezaki, H., Hashida, M., 1994. Pharmacokinetics of succinylated proteins and dextran sulfate in mice: Implications for hepatic targeting of protein drugs by direct succinylation via scavenger receptors. Int. J. Pharm. 105, 19–29.

Takakura, Y., Takagi, A., Hashida, M., Sezaki, H., 1987. Disposition and tumor localization of mitomycin C-dextran conjugates in mice. Pharm. Res. 4, 293–300.

Ueda, Y., Matsuo, K., Kamei, T., Ono, H., Kayashima, K., Tobimatsu, M. and Konomi, K., 1987. Prostaglandin E_1 but not E_2 is cytoprotective of energy metabolism and reticuloendothelial function in the ischemic canine liver. Transplant Proc. XIX, 1329-1330.

Uekama, K., Adach, H., Irei, T., Yano, T., Saita, M., Noda, K., 1992. Improved transdermal delivery of prostaglandin E, through hairless mouse skin: combined use of carboxymethyl-ethyl-*B*-cyclodextrin and penetration enhancers. J. Pharm. Pharmacol. 44, 119–121.

Willerson, J.T., Yao, S.-K., McNatt, J., Cui, K., Anderson, H.V., Swensen, C., Ostro, M., Buja, L.M., 1994. Liposome-bound prostaglandin E_1 often prevents cyclic flow variations in stenosed and endothelium-injured canine coronary arteries. Circulation 89, 1786–1791.

Yamaoka, K., Tanigawara, Y., Tanaka, H., Uno, Y., 1981. A pharmacokinetic analysis program (MULTI) for microcomputer. J. Pharmacobio-Dyn 4, 879–885.

Younger, E.W., Szabo, R.M., 1986. The stability of prostaglandin E_1 in dilute physiological solutions at 37°C. Prostaglandins 31, 923–927.