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Synthesis and pharmacological activity of a novel water-soluble hepatocyte-specific polymeric prodrug of prostaglandin E_1 using lactosylated poly(L-glutamic hydrazide) as a carrier

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Abstract

A novel polymeric prodrug of prostaglandin E_1 (PGE_1) was synthesized using lactosylated poly(L-glutamic hydrazide) (Lac-NH-PLGA) as a targetable carrier to hepatocytes. Poly(L-glutamic hydrazide) (PLGA-HZ) was prepared by reacting poly(γ-benzyl-L-glutamate) with hydrazine monohydrate, followed by coupling with lactose via a hydrazone linkage. Then the lactosylated PLGA-HZ was reduced by sodium cyanoborohydride ($NaBH_3CN$) in order to make the linkage irreversible (Lac-NH-PLGA). Finally, PGE_1 was bound to hydrazide moieties remaining in Lac-NH-PLGA without any condensing agent under weakly acidic conditions (pH 5) where PGE_1 would be chemically most stable at room temperature (PGE_1 conjugate). The PGE_1 conjugate prepared was sufficiently water-soluble in spite of the hydrophobic nature of its backbone (-NH-CH-CO-) and PGE_1 itself. After intravenous injection in mice, the [^{111}In]PGE_1 conjugate rapidly accumulated in the liver, whereas [^{111}In]PLGA-HZ did not, suggesting the involvement of a galactose-specific mechanism in the uptake of the [^{111}In]PGE_1 conjugate. Fractionation of liver cells revealed that the [^{111}In]PGE_1 conjugate was preferentially taken up by liver parenchymal cells. The pharmacological activity was examined in mice with fulminant hepatitis induced by intraperitoneal injection of carbon tetrachloride. Intravenous injection of the PGE_1 conjugate at a dose of 1 mg (0.065 mg PGE_1)/kg effectively inhibited the increase in plasma glutamic pyruvic transaminase (GPT) activity compared with that of free PGE_1 at a dose of 0.065 or 0.65 mg/kg. These results suggest that the PGE_1 conjugate is an excellent prodrug for the treatment of fulminant hepatitis. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Prostaglandin E_1; Hepatitis; Hepatocyte-specific drug delivery; Lactose; Poly(L-glutamic hydrazide); Cytoprotection

1. Introduction

PGE_1 is well known to have cytoprotective activity and, therefore, it has been reported to be quite effective in fulminant hepatitis [1–5]. However, repetitive or persistent administration of PGE_1 is required for the treatment of hepatitis due to its low hydrophilicity and poor physiological stability [6,7]. Thus, the development of a suitable delivery system that achieves hepatic targeting of pharmacologically active PGE_1 would be very useful clinically.

Recently, several pharmaceutical approaches to the treatment of hepatitis, using PGE_1, have been investigated. The α-cycrodextrin–PGE_1 complex has also been used for this purpose, and it showed potential in rats in preventing acute hepatitis produced by dimethylnitrosamine, carbon tetrachloride (CCl_4), or *D*-galactosamine [8,9]. However, as mentioned above, persistent administration is required. Fortunately, we have been able to achieve sugar receptor-mediated selective targeting of various pharmaceutical agents to the liver [10–18]. We have now developed some liver-targeted PGE_1 prodrugs that have potential anti-hepatitis activity even after a single intravenous administration [19–21]. In a recent approach in the series described in this study, galactosylated PLGA-HZ was developed as a drug carrier [20,21]. This carrier macromolecule possesses both

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Abbreviations: PGE_1, prostaglandin E_1; PLGA-HZ, poly(L-glutamic hydrazide); Lac-NH-PLGA, lactosylated poly(L-glutamic hydrazide); PGE_1 conjugate, prostaglandin E_1 prodrug using Lac-NH-PLGA as a carrier; DTPA anhydride, diethylenetriamine-*N*,*N*,*N*′,*N*′′,*N*′′-pentaacetic dianhydride; GPT, glutamic pyruvic transaminase; AUC, area under the plasma concentration–time curve; ROS, reactive oxygen species; and EP receptor, receptor for prostaglandins expressed on a plasma membrane.

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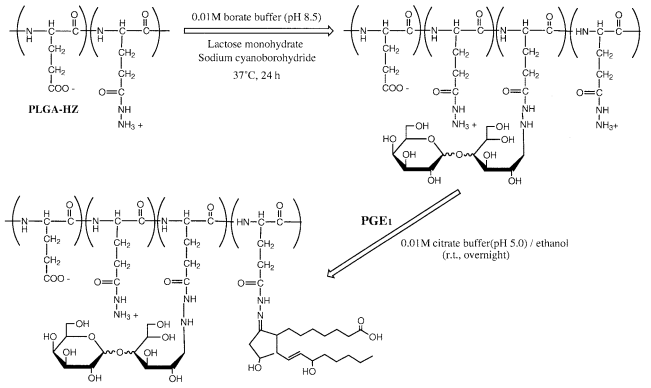

Fig. 1. Synthetic process of a novel polymeric prodrug of PGE_1

hepatocyte-targeting capabilities, i.e. numerous galactose molecules and hydrazine residues for the covalent binding of PGE_1. The PGE_1 polymeric conjugate using this carrier exhibited an excellent hepatitis-inhibiting effect even after bolus administration. Nevertheless, this conjugate tended to gradually aggregate, probably due to the reduced hydrophilicity of the conjugate following PGE_1 binding or intramolecular electric interactions. To combat these disadvantages, a new carrier with lactose instead of galactose residues was synthesized as a hepatocyte-targeting device. The biodistribution in mice of the PGE_1 conjugate using the Lac-NH-PLGA was determined, and then the pharmacological activity of the PGE_1 conjugate was examined in mice with carbon tetrachloride-induced fulminant hepatitis.

2. Materials and methods

2.1. Chemicals and animals

Poly(γ-benzyl-L-glutamate) with an average molecular weight of approximately 17,300 (degree of polymerization: 79) was purchased from the Sigma Chemical Co. Lactose monohydrate and hydrazine monohydrate were obtained from Wako Pure Chemicals. Sodium cyanoborohydride ($NaBH_3CN$) was obtained from the Aldrich Chemical Co. DTPA anhydride was obtained from Dojindo Laboratory. PGE_1 was obtained commercially and used without further purification. Male ddY mice (25–30 g) were obtained from the Shimizu Agricultural Cooperative Association for Laboratory Animals.

2.2. Synthesis of PGE

For the synthesis of the PGE_1 conjugate (Fig. 1), poly(γ-benzyl-L-glutamate) was prepared by the method of Hurwitz *et al.* [22]. Five hundred milligrams of poly(γ-benzyl-l-glutamate) was suspended in 10 mL of hydrazine monohydrate with stirring at room temperature for 3 hr to obtain a homogeneous solution. The reaction mixture obtained was transferred into dialysis tubing (3 kDa cut-off) followed by the addition of 40 mL of water (if a gel formed, aqueous HCl was added) and then dialyzed thoroughly against distilled water. The dialysate was concentrated by ultrafiltration (10 kDa cut-off) and lyophilized to obtain PLGA-HZ. The hydrazide group content of PLGA-HZ was measured photometrically using β-naphthoquinone-4-sulfonate [23].

PLGA-HZ (50 mg) was dissolved in 1 mL of 5 N NaOH solution and neutralized by adding 5 N HCl solution. Three milliliters of 0.1 M borate buffered solution (pH 8.5) was added to the PLGA-HZ solution to bring the pH to 8–9. Lactose monohydrate (143 mg) and sodium cyanoborohydride (50 mg) were added to the PLGA-HZ solution above and stirred overnight at 37°. The reaction mixture was transferred into dialysis tubing (3 kDa cut-off) and dialyzed thoroughly against distilled water. The dialysate obtained was concentrated by ultrafiltration (10 kDa cut-off) and then lyophilized to obtain reduced Lac-NH-PLGA. The number of lactose residues in the Lac-NH-PLGA was determined by the anthrone-sulfuric acid method and found to be 22 mol/mol of conjugate.

For binding PGE_1 to Lac-NH-PLGA, initially, PGE_1 (2.8 mg) was dissolved in 0.5 mL ethanol (or containing a trace amount of [^{3}H]PGE_1). The PGE_1 ethanolic solution was added slowly in drop-wise fashion to Lac-NH-PLGA (23 mg) dissolved in 5 mL of 0.01 M acetate-buffered solution (pH 5.0) with stirring overnight at room temperature. Purification of the reaction mixture was carried out as described above. The resulting product was water-soluble. The PGE_1 content of the conjugate was determined by counting the radioactivity of [^{3}H]PGE_1 and found to be 4 mol/mol of conjugate.

2.3. Biodistribution experiment

To study the disposition of PLGA-HZ and the PGE_1 conjugate, they were radiolabeled with ^{111}In using DTPA anhydride as described previously [12]. 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 found to have a specific activity of approximately 37 MBq. Mice received a 1 or 10 mg/kg dose of [^{111}In]PLGA-HZ and [^{111}In]PGE_1 conjugate in saline by tail vein injection and were housed in metabolic cages to allow 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, and kidneys were excised, rinsed with saline, and weighed, and their radioactivity was determined. The amount of radioactivity in the urine was determined by collecting both the urine excreted and that remaining in the bladder. In addition, the distribution of these macromolecules in liver parenchymal and nonparenchymal cells was determined by separating these cells using collagenase after intravenous injection as previously reported [18]. ^{111}In radioactivity was counted in a well-type NaI scintillation counter (ARC-500, Aloka). Also, the ^{3}H radio-

activity was measured in a standard liquid scintillation counter (LSC-5000, Beckman), after dissolution in Soluene-350 (Packard) and addition of a scintillation medium, Clearsol I (Nakalai Tesque). Radioactivity originating from the plasma in each tissue sample was corrected using the distribution data for [^{111}In]BSA at 10 min after intravenous injection [13], assuming that [^{111}In]BSA is not taken up by tissues during the 10-min period.

2.4. 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 [24]. 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 cross biological membranes easily [25,26]. Using the assumption described above, the CL_{org} was calculated by dividing the amount of radioactivity in an organ at 10 min by the AUC up to the same time point. The AUC and 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-squares program MULTI [27].

2.5. In vivo pharmacological activity of the PGE_1 conjugate

The pharmacological activity of the PGE_1 conjugate was evaluated by measuring the GPT activity in the plasma of mice with fulminant hepatitis induced by CCl_4. Mice received an intraperitoneal injection of CCl_4 (2% solution in sesame oil) at a dose of 10 mL/kg. Immediately after CCl_4 administration, the PGE_1 conjugate (1 or 10 mg/kg) or free PGE_1 (0.065 or 0.65 mg/kg) solution was injected intravenously into the tail vein. The mice were then starved for 18 hr after CCl_4 administration, blood was collected from the vena cava under ether anesthesia, and plasma was obtained by centrifugation at 2000 *g* for 5 min at 4°. The GPT activity in the plasma sample was determined by the UV-rate method [28].

3. Results

3.1. Plasma clearance and tissue distribution of ^{111}In-labeled PLGA-HZ and PGE_1 conjugate

Figure 2 shows the time courses of the plasma concentration and tissue accumulation of PLGA-HZ and PGE_1 conjugate. The PGE_1 conjugate was eliminated rapidly from the plasma and accumulated mainly in the liver [up to 60% of a dose of 1 mg/kg within 10 min) by recognition of the galactose moieties by asialoglycoprotein receptors [10–18], whereas at a dose of 10 mg/kg up to only 35% of the dose

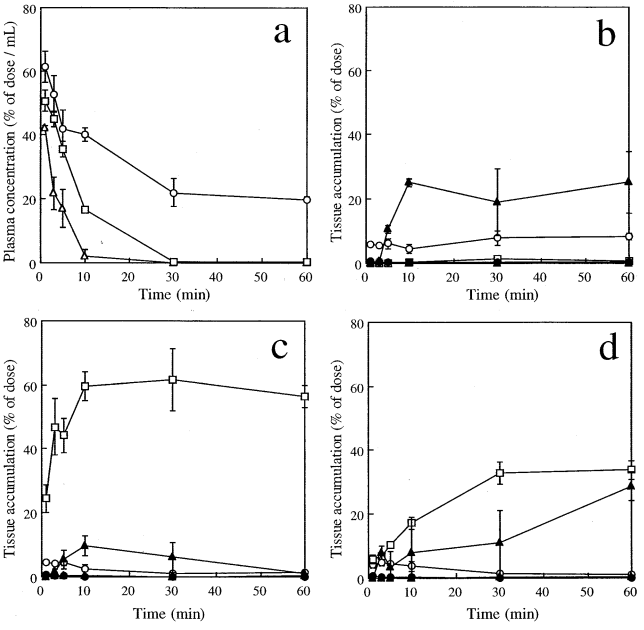

Fig. 2. Plasma concentration and tissue accumulation time courses of [^{111}In]PLGA-HZ and the [^{111}In]PGE_1 conjugate after intravenous injection in mice. Results are expressed as the means ± SD of three mice. (Panel a) plasma concentration–time courses of [^{111}In]PLGA-HZ at a dose of 1 mg/kg (❍), and the [^{111}In]PGE_1 conjugate at a dose of 1 mg/kg (0.065 mg of equivalent PGE_1/kg) (Δ) or 10 mg/kg (0.65 mg of equivalent PGE_1/kg) (❒). (Panels b–d) Tissue accumulation–time courses of (b) [^{111}In]PLGA-HZ at a dose of 1 mg/kg, (c) [^{111}In]PGE_1 conjugate at a dose of 1 mg/kg and (d) [^{111}In]PGE_1 conjugate at a dose of 10 mg/kg [kidney, (❍); spleen, (Δ), liver, (❒); lung, (●); and urine, (▲)].

accumulated in the liver following saturation of the sugar receptor (Fig. 2, panels a and c). At both doses, renal excretion seemed to be another elimination pathway for the conjugate, since a slight accumulation in the kidneys at the early phase and subsequent urinary excretion were observed. There was no significant accumulation of the PGE_1 conjugate in other tissues including the spleen and lungs. In contrast to the PGE_1 conjugate, PLGA-HZ without lactose (galactose) units hardly accumulated in the liver, but was excreted in the urine (Fig. 2, panels a and b). Also, the extremely small AUC and large hepatic clearance values of the PGE_1 conjugate reflected these results (Table 1).

3.2. Cellular distribution of ^{111}In-labeled PLGA-HZ and PGE_1 conjugate in liver

The hepatic cellular localization study of the carrier (Fig. 3) shows that it was selectively taken up by parenchymal cells. These data suggest that, using the polymeric drug carrier, potential PGE_1 should be successfully targeted to liver parenchymal cells without any metabolism following intravenous administration by this delivery system.

3.3. Therapeutic activity of the PGE_1 conjugate in mice with acute hepatitis

Figure 4 shows the GPT levels in the plasma of normal mice and mice with hepatitis induced by CC_{l4} and then

Table 1
AUC and clearances for [^{111}In]PLGA-HZ and [^{111}In]PGE_1 conjugate after intravenous injection in mice

Compounds[a]	Dose (mg/kg)	AUC[b,c] (% of dose · hr/mL)	Clearance (μL/hr)[c]					
			CL_{total}	CL_{liver}	CL_{kidney}	CL_{urine}	CL_{spleen}	CL_{lung}
[^{111}In]PLGA-HZ	1	250	410	0.80	18.0	110	0.13	1.6
[^{111}In]PGE_1 conjugate	1	8.7	30,000	19,000	730	3100	70	35
[^{111}In]PGE_1 conjugate	10	3.4	12,000	2800	2000	3200	8.0	130

[a] PGE_1 conjugate contains 0.065 mg PGE_1/mg of the conjugate.
[b] AUC: are as under the plasma concentration-time curve.
[c] AUC and clearance data are expressed as average values of three mice for 10 min after intravenous injection.

treated with PGE_1 or its polymeric conjugate. It can be seen that the PGE_1 polymeric conjugate strongly inhibited the increase in GPT concentration in the plasma; this inhibition was more effective than that of a bolus administration of free PGE_1.

4. Discussion

The Lac-NH-PLGA prepared in this study was more water-soluble than the galactosylated PLGA previously reported [21]. This is due to the increased number of OH moieties in the carrier due to the glucose units in lactose. It is generally important to increase the hydrophilicity of the carrier because most drugs, as well as PGE_1, have a relatively similar hydrophobicity. In addition, lactose and PGE_1 are not only readily bound to PLGA-HZ, but also the chemical structure of PGE_1 remains unchanged during the reaction (Fig. 1) because PGE_1 is chemically most stable around pH 4–5 [6]. Therefore, this lactosylated carrier will be applicable to any drugs that are stable under weakly acidic conditions having an aliphatic aldehyde or carbonyl moiety in their chemical structure.

The lactosylated (galactosylated) prodrug was targeted to the liver via the recognition of asialoglycoprotein receptors on the plasma membrane of the parenchymal cell [10–18]. However, a high concentration of the galactose should lead to saturation of the receptors. In the pharmacological activity experiment, a similar therapeutic effect was observed at lower (1 mg/kg) and higher (10 mg/mL) doses of the lactosylated PGE_1 conjugate (Fig. 4). Receptor saturation may be responsible for these results. If this were the case, a similar amount of the PGE_1 conjugate would be internalized in the liver parenchymal cells at both concentrations, which would result in similar pharmacological activities. Furthermore, the biodegradation rate of the carrier or the release

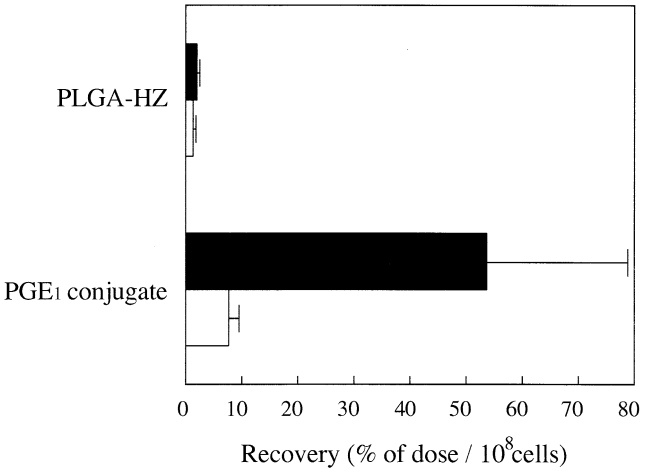

Fig. 3. Hepatic cellular localization of radioactivity after intravenous injection of [^{111}In]PLGA-HZ and the [^{111}In]PGE_1 conjugate in mice at a dose of 1 mg/kg. The unit of recovery was expressed as percent of dose per 10^8 cells, considering that the numbers of hepatic parenchymal cells (■) and nonparenchymal cells (❒) in a mouse were estimated to be 1.25×10^6 and 0.65×10^6 cells/g liver, respectively [29]. Results are expressed as the means ± SD of three mice.

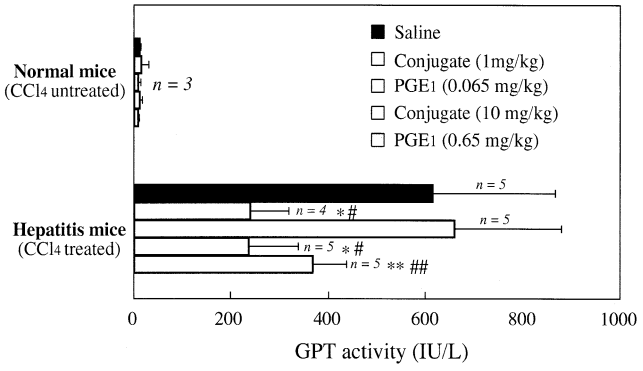

Fig. 4. GPT levels in the plasma of normal or hepatitis mice treated by PGE_1 or the PGE_1 conjugate (4 mol PGE_1/mol conjugate). Statistically significant differences were determined based on ANOVA (Student-Newman-Keuls multiple comparisons test). Key: (*, **) compared with saline-treated mice (*: $P < 0.01$, **: $P < 0.05$), and (#, ##) compared with PGE_1-treated mice (#: $P < 0.01$; ##: $P < 0.05$). Results are expressed as the means ± SD of at least three mice. Hepatitis was induced in mice by intraperitoneal injection of CCl_4 (2% solution in sesame oil) at a dose of 10 mL/kg. In this experiment, four kinds of drug solution were prepared: saline, 0.9% NaCl; PGE_1, 0.2 mg/mL of polyethyleneglycol solution; PGE_1 conjugate, 0.1 or 1 mg/mL of aqueous solution. Each solution of PGE_1 conjugate was injected intravenously into the tail vein at a dose of 1 or 10 mg/kg, respectively. GPT activity in the plasma samples, obtained from the vena cava, was assessed 18 hr after collection and was measured by the UV-rate method [28]. The order of the symbols in the key represents the order of the bars in the figure.

rate of the PGE_1 molecule from the carrier may, in part, affect the pharmacological efficacy. At a higher dose, these rates should be also reduced.

Our method for the estimation of the therapeutic activity may prove clinically impractical: CCl_4-induced fulminant or acute hepatitis is unlikely to occur in humans, and is almost irreversible, and the PGE_1 conjugate was administered immediately after CCl_4 injection. However, if the PGE_1 conjugate is injected several hours after CCl_4 administration, it should be no more effective. In addition, free PGE_1 would not be released from the carrier until the carrier molecules binding PGE_1 are digested in the hepatic parenchymal cells. Taking the delay time into account, clinically the drug should be administered as soon as possible after poisoning.

The cytoprotective effects of PGE_1, PGE_2, and their chemically stable analogs (PGEs) have been studied using mainly hepatocytes, gastrointestinal cells, spermatocytes, and Chinese hamster ovary (CHO) K1 cells *in vivo* or *in vitro* in terms of a variety of stimulating factors, e.g. ethanol [30–32], nonsteroidal anti-inflammatory drugs (NSAIDs) [33], ROS [34,35], deoxycholate [36], actinomycin C [37], *D*-galactosamine [9], and ionizing radiation [38] as well as CCl_4. Thus, PGEs generally defend cells against cell death, i.e. apoptosis or necrosis, as an endpoint. In the case of agents that cause damage to DNA, such as actinomycin C, ROS, or ionizing radiation, the cytoprotective effects of PGEs may be due to their ability to promote DNA repair, which could be triggered by the binding of EP_2 receptor subtypes expressed on the plasma membrane to the ligand [38], while their protective effects against agents that do not cause damage to DNA, e.g. ethanol, are thought to involve the EP_1 receptor subtype [30]. The effect against other stimulators, such as CCl_4, remains unknown. Thus, the cytoprotective mechanisms of PGEs are complex and unclear at present. Nevertheless, we have designed the novel polymeric prodrug of PGE_1 for the treatment of hepatitis in order to increase the overall hepatic uptake of active PGE_1, using sugar receptor-mediated internalization into hepatocytes.

The present study has demonstrated that the novel PGE_1 polymeric prodrug not only is delivered into hepatocytes effectively but also exhibits greater cytoprotective activity than free PGE_1. Although the detailed mechanism for its pharmacological activity is unclear, we believe that free PGE_1 released from the conjugate is responsible for its effect. A previous study [21] has confirmed that PGE_1 (or its derivatives) is released from the carrier, galactosylated PLGA-HZ, in liver homogenate but not in phosphate-buffered saline (pH 7.4 at 37°), suggesting that certain enzymes in the liver may be needed for the release of PGE_1. It is unlikely that PGE_1 conjugated with the polymeric carrier is active because the interacting site in the PGE_1 molecule for the EP receptor is blocked by its hydrazone linkage (Fig. 1). PGE_1 released from the carrier in hepatocytes may bind to EP receptors on the cell or neighboring hepatocytes passing through the plasma. However, in accordance with a few reports [30,38], interaction of PGE_1 with EP receptors should play an important role in its pharmacological activity, although no other sites of action for PGE_1 have been reported. Hence, the advantage of the delivery system for PGE_1 is that it allows active PGE_1 molecules to be released gradually within and in the vicinity of hepatocytes. This characteristic avoids down-regulation of the EP receptors induced by bolus administration.

Hepatic injury produced by CCl_4 can be induced not only by direct plasma membrane oxidation in the parenchymal cells but also by cytotoxic cytokines and tumor necrosis factor-α produced from activated Kupffer cells. Kayano *et al.* [39] have demonstrated that PGE_1 can suppress the activation of Kupffer cells. Although most PGE_1 molecules would be internalized into liver parenchymal cells using the polymeric carrier, part of the PGE_1 passing through the plasma membrane might have an effect on Kupffer cells.

In the CCl_4-induced fulminant hepatitis model, bolus injection of 1 mg/kg (0.065 mg PGE_1/kg) of the PGE_1 conjugate immediately after CCl_4 administration was more cytoprotective than that of 0.65 mg/kg of free PGE_1 (Fig. 4), suggesting that the conjugate was 10-fold more effective than free PGE_1 in the model. This result implies that the chemical structure of the drug or its pharmacological activity has been maintained before internalization into the hepatocytes following intravenous administration, while free PGE_1 should be metabolized very fast, particularly in the lungs [40]. Consequently, the pharmacological efficiency of PGE_1 as an anti-hepatitis drug was improved markedly by using the hepatocyte-specific carrier system. To assess the practical relevance of the conjugate, further studies should be carried out based on hepatitis models other than the CCl_4-induced model.

In conclusion, we have developed a novel water-soluble polymeric prodrug of PGE_1 targetable to hepatocytes. The PGE_1 polymeric prodrug may be useful as a therapeutic agent for inflammatory liver diseases including hepatitis. The agent could also be a powerful tool for clarifying the mechanism of the therapeutic effect of PGE_1 against hepatitis induced by CCl_4.

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References

[1] Sinclair SB, Levy GA. Treatment of fulminant viral hepatic failure with prostaglandin E. Dig Dis Sci 2000;36:791–800.
[2] Stachura J, Tarnawski A, Ivey KJ, Mach T, Bogdal J, Szczudrawa J, Klimczyk B. Prostaglandin protection of carbon tetrachloride-induced liver cell necrosis in the rat. Gastroenterology 1981;81:211–7.

[3] Ueda Y, Matsuo K, Kamei T, Ono H, Kayashima K, Tobimatsu M, Konomi K. Prostaglandin E_1 but not E_2 is cytoprotective of energy metabolism and reticuloendothelial function in the ischemic canine liver. Transplant Proc 1987;XIX:1329–30.
[4] Beck PL, McKnight GW, Kelly JK, Wallace JL, Lee SS. Hepatic and gastric cytoprotective effects of long-term prostaglandin E_1 administration in cirrhotic rats. Gastroenterology 1993;105:1483–9.
[5] Helling TS, Wogahn BM, Olson SA, Evans LS, Reddy BR, Van Way C. The effect of prostaglandin E_1 on liver adenine nucleotides and cytoplasmic enzymes in a porcine model of normothermic hepatic ischemia. Hepatology 1995;22:1554–9.
[6] Monkhouse DC, Campen LV, Aguiar AJ. Kinetics of dehydration and isomerization of prostaglandin E_1 and E_2. J Pharm Sci 1973;62:576–80.
[7] Younger EW, Szabo RM. The stability of prostaglandin E_1 in dilute physiological solutions at 37 degree C. Prostaglandins 1986;31:923–7.
[8] Suzuki A, Hagino M, Yasuda N, Sagawa K, Terawaki T, Ogawa M, Kondo K, Hamanaka N, Tanaka M, Aze Y. Inhibitory effects of prostaglandin E_1·α-cyclodextrin (PGE_1·CD) on dimethylnitrosamine-induced acute liver damage in rats. Folia Pharmacol Jpn 1995;105:221–9.
[9] Suzuki A, Katayama T, Hagino M, Ogawa M, Kondo K, Hamanaka N, Tanaka M, Aishita H. Inhibitory effects of prostaglandin E_1·β-cyclodextrin (PGE_1·CD) on carbon terachloride or *D*-galactosamine-induced acute liver damage in rats. Gendai Iryo 1995;27:735–42.
[10] Nishikawa M, Ohtsubo Y, Ohno J, Fujita T, Koyama Y, Yamashita F, Hashida M, Sezaki H. Pharmacokinetics of receptor-mediated hepatic uptake of glycosylated albumin in mice. Int J Pharm 1992;85:75–85.
[11] Nishikawa M, Kamijo A, Fujita T, Takakura Y, Sezaki H, Hashida M. Synthesis and pharmacokinetics of a new liver-specific carrier, glycosylated carboxymethyl-dextran, and its application to drug targeting. Pharm Res 1993;10:1253–61.
[12] Nishikawa M, Miyazaki C, Yamashita F, Takakura Y, Hashida M. Galactosylated proteins are recognized by the liver according to the surface density of galactose moieties. Am J Physiol 1995;268:G849–56.
[13] Nishikawa M, Hirabayashi H, Takakura Y, Hashida M. Design for cell-specific targeting of proteins utilizing sugar-recognition mechanism: effect of molecular weight of proteins on targeting efficacy. Pharm Res 1995;12:209–14.
[14] Fujita T, Furitsu M, Nishikawa M, Takakura Y, Sezaki H, Hashida M. Therapeutic effects of superoxide dismutase derivatives modified with mono- or polysaccharides on hepatic injury induced by ischemia/reperfusion. Biochem Biophys Res Commun 1992;189:191–6.
[15] Fujita T, Nishikawa M, Tamaki C, Takakura Y, Hashida M, Sezaki H. Targeted drug delivery of human recombinant superoxide dismutase by chemical modification with mono- and polysaccharide derivatives. J Pharmacol Exp Ther 1992;263:971–8.
[16] Hirabayashi H, Nishikawa M, Takakura Y, Hashida M. Development and pharmacokinetics of galactosylated poly-L-glutamic acid as a biodegradable carrier for liver-specific drug delivery. Pharm Res 1996;13:880–4.
[17] Hashida M, Hirabayashi H, Nishikawa M, Takakura Y. Targeted delivery of drugs and proteins to the liver via receptor-mediated endocytosis. J Control Release 1997;46:129–37.
[18] Mahato RI, Takemura S, Akamatsu K, Nishikawa M, Takakura Y, Hashida M. Physicochemical and disposition characteristics of antisense oligonucleotide complexed with glycosylated poly(L-lysine). Biochem Pharmacol 1997;53:887–95.
[19] Akamatsu K, Nishikawa M, Takakura Y, Hashida M. Synthesis and biodistribution study of liver-specific prostaglandin E_1 polymeric prodrug. Int J Pharm 1997;155:65–74.
[20] Hashida M, Akamatsu K, Nishikawa M, Yamashita F, Takakura Y. Design of polymeric prodrugs of prostaglandin E_1 having galactose residue for hepatocyte targeting. J Control Release 1999;62:253–62.
[21] Akamatsu K, Yamasaki Y, Nishikawa M, Takakura Y, Hashida M. Development of hepatocyte-specific prostaglandin E_1 polymeric prodrug and its potential for preventing carbon tetrachloride-induced fulminant hepatitis in mice. J Pharmacol Exp Ther 1999;290:1242–9.
[22] Hurwitz E, Wilchek M, Pitha J. Soluble macromolecules as carrier for daunorubicin. J Appl Biochem 1980;2:25–35.
[23] Pratt EL. Colorimetric estimation of isonicotinic acid hydrazide. Anal Chem 1953;25:814–6.
[24] Takakura Y, Takagi A, Hashida M, Sezaki H. Disposition and tumor localization of mitomycin C-dextran conjugate in mice. Pharm Res 1987;4:293–300.
[25] Arano Y, Mukai T, Uezono T, Wakisaka K, Motonari H, Akizawa H, Taoka Y, Yokoyama A. A biological method to evaluate bifunctional chelating agents to label antibodies with metallic radionuclides. J Nucl Med 1994;35:890–8.
[26] Duncan JR, Welch MJ. Intracellular metabolism of indium 111-labeled receptor targeted proteins. J Nucl Med 1993;34:1728–38.
[27] Yamaoka K, Tanigawara Y, Nakagawa T, Uno T. A pharmacokinetic analysis program (MULTI) for microcomputer. J Pharmacobiodyn 1981;4:879–85.
[28] Wroblewski F, La Due JS. Serum glutamic pyruvic transaminase in cardiac and hepatic disease. Proc Soc Exp Biol Med 1956;91:569–71.
[29] Blomhoff R, Blomhoff HK, Tolleshaug H, Christensen TB, Berg T. Uptake and degradation of bovine testes β-galactosidase by parenchymal and non-parenchymal rat liver cells. Int J Biochem 1985;17:1321–8.
[30] Araki H, Ukawa H, Sugawa Y, Yagi K, Suzuki K, Takeuchi K. The roles of prostaglandin E receptor subtypes in the cytoprotective action of prostaglandin E_2 in rat stomach. Aliment Pharmacol Ther 2000;14 (Suppl 1):116–24.
[31] Banan A, Smith GS, Kokoska ER, Miller TA. Role of actin cytoskeleton in prostaglandin-induced protection against ethanol in an intestinal epithelial cell line. J Surg Res 2000;88:104–13.
[32] Ko JK, Cho CH. Co-regulation of mucosal nitric oxide and prostaglandin in gastric adaptive cytoprotection. Inflamm Res 1999;48:471–8.
[33] Turner PV, Albassam MA, Percy DH, Lillie LE, Macallum GE. Role of cytoprotectants and nitric oxide inhibition in nonsteroidal anti-inflammatory drug-induced gastroduodenal injury in the rat. Comp Med 2000;50:140–6.
[34] Kishimoto S, Sakon M, Umeshita K, Miyoshi H, Taniguchi K, Meng W, Nagano H, Dono K, Ariyosi H, Nakamori S, Kawasaki T, Gotoh M, Monden M, Imajoh-Ohmi S. The inhibitory effect of prostaglandin E_1 on oxidative stress-induced hepatocyte injury evaluated by calpain-μ activation. Transplantation 2000;69:2314–9.
[35] Sugawara Y, Kubota K, Ogura T, Esumi H, Inoue K, Takayama T, Makuuchi M. Protective effect of prostaglandin E_1 against ischemia/reperfusion-induced liver injury: results of a protective, randomized study in cirrhotic patients undergoing subsegmentectomy. J Hepatol 1998;29:969–76.
[36] Kokoska ER, Smith GS, Rieckenberg CL, Deshpande Y, Banan A, Miller TA. Adaptive cytoprotection against deoxycholate-induced injury in human gastric cells *in vitro*: is there a role for endogeneous prostaglandins? Dig Dis Sci 1998;43:806–15.
[37] Dymond JB, Kalmus GW. The cytoprotective properties of prostaglandin E_2 against the toxic effects of actinomycin C on embryonic neural retina cells. Prostaglandins 1992;44:129–34.
[38] van Buul PPW, van Duyn-Goedhart A, Sankaranarayanan K. *In vivo* and *in vitro* radioprotective effects of the prostaglandin E_1 analogue misoprostol in DNA repair-proficient and -deficient rodent cell systems. Radiat Res 1999;152:398–403.
[39] Kayano K, Sakaida I, Kubota M, Yasunaga M, Okita K. Functional differences between activated and normal rat liver macrophages: LPS uptake capacity by flow cytometric analysis in contrast with TNF-α release. Liver 1995;15:253–9.
[40] Porst H. The rationale for prostaglandin E1 in erectile failure: a survey of worldwide experience. J Urol 1996;155;802–15.