Graduate School of Pharmaceutical Sciences[1], Kyoto University, Kyoto and Faculty of Pharmaceutical Sciences[2], Toyama Medical and Pharmaceutical University, Toyama, Japan

Design of polymeric prodrugs of PGE1 for cell-specific hepatic targeting

M. HASHIDA[1], K. AKAMATSU[1], M. NISHIKAWA[1], F. YAMASHITA[1], H. YOSHIKAWA[2] and Y. TAKAKURA[1]

Based on the relationship between *in vivo* disposition of macromolecules and their physicochemical and biological characteristics obtained through clearance concept-based pharmacokinetic analysis, polymeric prodrugs of prostaglandin E1 (PGE1) were designed stepwise and evaluated on their targeting and therapeutic efficiencies. Although galactosylated poly-L-glutamic acid with a ethylene diamine (ED) spacer (Gal-ED-PLGA) showed good targeting efficacy in mice, its PGE1 conjugate synthesized by the carbonyldiimidazole method failed to show therapeutic effects probably due to inactivation of PGE1 during conjugation and lack of release in the tissue. In order to overcome these problems, PGE1 was conjugated to galactosylated poly-(L-glutamic acid) hydrazide (Gal-HZ-PLGA) via hydrazone bond. The PGE1-Gal-HZ-PLGA conjugate labeled with [^{111}In] or [^{3}H]PGE1 rapidly accumulated in the liver parenchymal cells after intravenous injection. In addition, PGE1 conjugate effectively inhibited the increase of GPT level in plasma, while free PGE1 indicated no therapeutic efficacy even at more than ten times higher doses, in carbon tetrachloride-induced hepatitis mice. These findings suggest potentials of polymeric targeting systems of PGE1 to hepatocyte utilizing galactose recognition.

1. Introduction

Drug delivery system are defined as tools to precisely control *in vivo* behavior of a drug aiming at optimization of its therapeutic efficacy. In this context, site-specific delivery is considered to be an approach to concentrate the drug on the specific site of the body through control of its biodistribution profile. Among various strategies for site-specific drug delivery, the use of polymer carriers would be formidable because of their high diversity and multiple functions. Successful application of polymers as drug carriers or therapeutic agents, however, requires a thorough understanding of the pharmacokinetic profiles of polymers at whole body, organ, and cellular levels.

In our series of investigations, systemic disposition of macromolecular drugs/carriers was explored in relation to their physicochemical and biological characteristics with the clearance concept-based pharmacokinetic analysis. A strategy for rational design of a targeting system was constructed [1]. This approach had been successfully applied to the design of macromolecular prodrugs of anticancer agents aiming at passive and monoclonal antibody-mediated active tumor targeting [2, 3]. However, absolute amounts of targeted drug were still limited due to restriction in extravasation and interstitial diffusion of macromolecules, as well as to the insufficient uptake capacity of the target cells. In contrast to solid tumors, the liver has some advantages for specific targeting in such as the discontinuous sinusoidal wall that enables macromolecules to gain free access to parenchymal cells and high and ligand-specific endocytic activity of parenchymal and non-parenchymal cells. By way of targeting to the liver, four types of biological recognition mechanisms such as 1) asialoglycoprotein receptor-mediated endocytosis by hepatocyte, 2) mannose receptor-mediated endocytosis by non-parenchymal cells, 3) scavenger receptor-mediated endocytosis of polyanions by nonparenchymal cells, and 4) universal electrostatic interaction of polycations were adopted in our study [4–6].

In this paper, the design of polymeric prodrugs of prostaglandin E1 (PGE1) with galactose moieties for cell-specific hepatic targeting will be discussed in relation to their pharmacokinetic and pharmacological characteristics. The discussion will be focused on the efficient introduction of a sugar moiety and the endowment of adequate pharmaceutical characteristics.

2. Investigations, results and discussion

2.1. General biodistribution profiles of macromolecules

Fig. 1 summarizes hepatic and urinary excretion clearance values for macromolecules with various physicochemical and biological properties in mice. Macromolecules with molecular weights larger than 70,000 and weak anionic nature have small values in both parameters resulting in a prolonged retention in blood circulation and a large value of the area under the plasma concentration-time curves (AUC). On the other hand, strongly anionic or cationic macromolecules remarkably accumulate in the liver due to scavenger receptor-mediated endocytosis or electrostatic binding on the anionic tissue surface, respectively. Molecules with molecular weights of less than 30,000 are predominantly excreted into urine or taken up by the kidney after glomerular filtration. In the case of macromolecules having galactose or mannose moieties, selective uptake by parenchymal or non-parenchymal cells, respectively, due to receptor-mediated endocytosis was observed with large hepatic uptake clearance values.

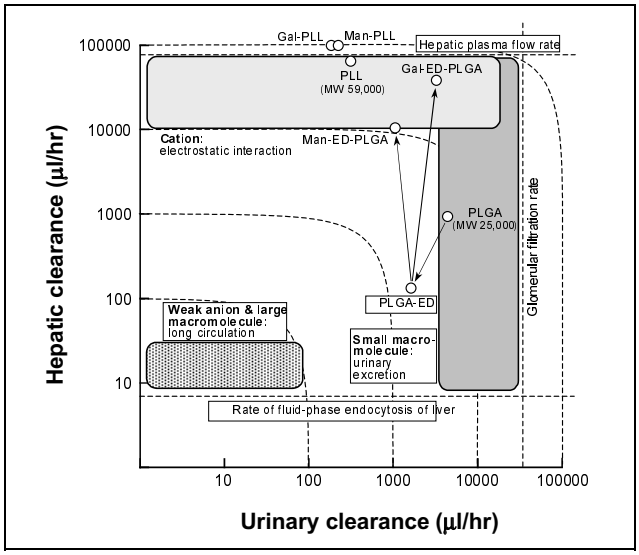

Fig. 1: General relationship between physicochemical properties such as molecular weight and electric charge of macromolecules and their hepatic uptake and urinary excretion clearances in mice. Parameters for polyamino acid derivatives are also plotted to show effect of introduction of sugar moieties aiming at cell specific targeting

2.2. Design of polymeric prodrugs of PGE1

Design of a polymeric prodrug is carried out with several steps such as; 1) choice of polymer backbone, 2) selection of conjugation method of a drug, 3) introduction of homing device, 4) control of physicochemical properties of the final products. The Table summarizes the criteria for choosing molecular species in the design of polymeric conjugates of PGE1. We have reported advantages of polyamino acids as drug carriers [7, 8] and the effectiveness of carbohydrate recognition systems as cell specific targeting mechanismS [9, 10]. Based on these observations, we developed novel polymeric prodrugs of PGE1 using poly-L-glutamic acid (PLGA) and poly-L-lysine (PLL) as water soluble and biodegradable polymer backbones and galactose moiety as a homing device to hepatocytes. Targeting efficacy and pharmacological activity of these compounds are discussed in relation to their molecular characteristics.

Fig. 2 explains rationale for delivering PGE1 to hepatocyte. PGE_1 is clinically used to treat peripheral vascular disturbances. In addition, PGE_1 is also effective on fulminant or subfulminant viral hepatitis [11] by means of its cytoprotective activity [12]. However, low solubility and stability of PGE1 leads its treatment to a long-term infusion [13] and more than 80% of intravenously administered PGE_1 is metabolized and inactivated by α- or β-oxidation during the first passage through the lungs, while an autoradiographic study demonstrated that radioactivity of $[^3H]PGE_1$ was mainly distributed in the liver and kidneys [14]. Furthermore, many side effects such as abdominal pain, diarrhea, hypotention and peripheral edema are suspected to appear during hepatitis therapy with PGE_1. Therefore, development of a delivery system that can focus PGE_1 to the liver attracts great interest. Recently, delivery systems for PGE_1 have been studied using cyclodextrins [15], lipid microspheres [16], liposomes [17] and heparin conjugate [18] but few studies have been carried out for cell-specific hepatic targeting aiming the treatment of hepatitis.

Table: Molecular design of macromolecular prodrugs of PGE1 for hepatic targeting: Criteria for choice of backbone, linkage, and homing device

1. The conjugate should have high targeting activity to hepatocyte
2. The conjugate should have adequate pharmaceutical properties
3. Sufficient amount of drug should be conjugated keeping pharmacological activity
4. The conjugated drug should retain its original activity until reached to the target
5. The conjugate should show minimum accumulation in the non-target site
6. Drug should be released at the target site and show its pharmacological activity

2.3. Synthesis of polymeric carriers and conjugation with PGE1

Gal-ED-PLGA, Gal-PLL, PGE1-Gal-ED-PLGA, and PGE1-Gal-HZ-PLGA have chemical structures shown in Fig. 3 [19]. All galactosylated polyamino acids have galactose moieties of 17–31 in one molecule and PGE1-Gal-ED-PLGA and PGE_1-Gal-HZ-PLGA molecules contain 1.6 and 5.0 molecules of PGE1, respectively.

2.4. In vivo disposition of glycosylated polyamino acids and polymeric prodrugs of PGE1

After intravenous injection in mice, [^{111}In] PLGA gradually disappeared from plasma and excreted into urine. [^{111}In]ED-PLGA showed a decrease in urinary excretion, but introduction of both galactose and mannose residues extremely enhanced disappearance from plasma and accumulation in the liver. And about 60% of the dose was recovered in the liver at 10 min after injection. On the other hand, all [^{111}In]PLL, [^{111}In]Gal-PLL, and [^{111}In]Man-PLL were rapidly eliminated from plasma and accumulated in the liver [20].

In Fig. 4, hepatic cellular localization of these macromolecules is compared. In the case of PLGA, introduction of galactose and mannose residues remarkably increased the uptake by parenchymal cells and nonparenchymal cells, respectively. In addition, the cell specific uptake of [^{111}In]Gal-ED-PLGA and [^{111}In]Gal-ED-PLGA was profoundly inhibited by co-administration of Gal-BSA and Man-BSA, respectively. These findings suggest that glycosylated PLGA can be used as a cell specific carrier for liver targeting. On the other hand, distribution patterns of [^{111}In]PLL, [^{111}In]Gal-PLL, and [^{111}In]Man-PLL between parenchymal and nonparenchymal cells are relatively similar suggesting that electostatic interaction plays an important role in them.

From biodistribution data, tissue uptake clearances were calculated for all compounds and hepatic uptake and urinary excretion clearance values are also plotted in Fig. 1. The effects of introduction of sugar moieties as cell specific recog-

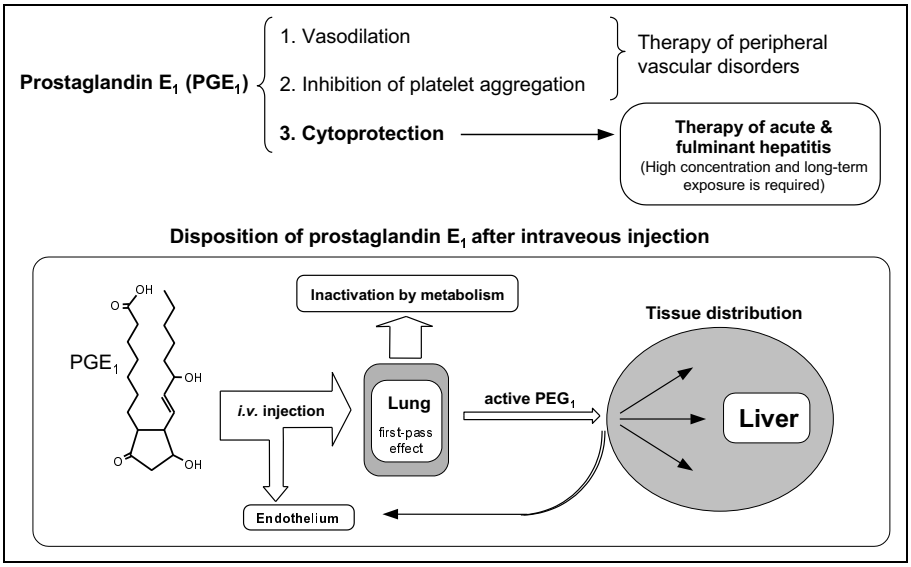

Fig. 2:
The rationale for cell-specific hepatic targeting of PGE1

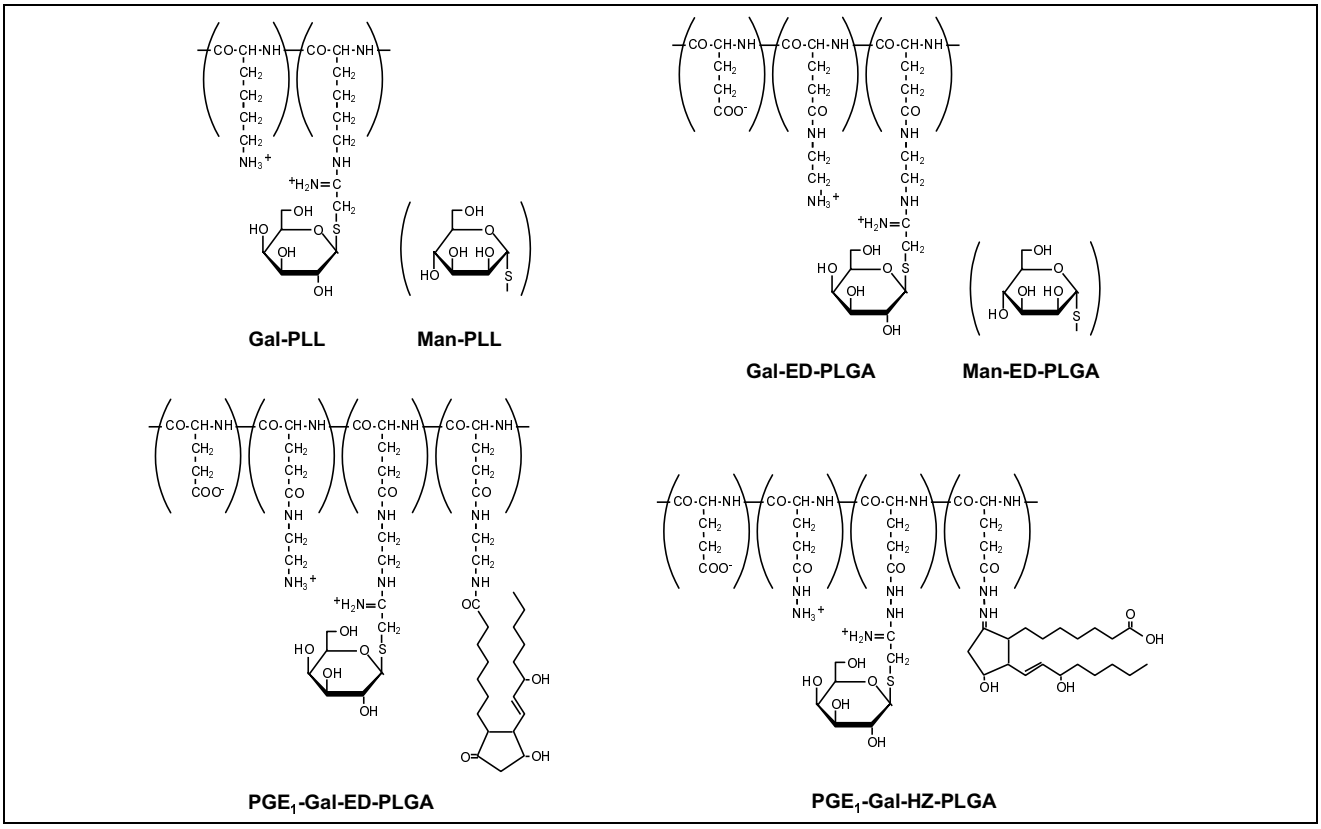

Fig. 3: Chemical structures of PLL and PLGA derivatives developed in this study for cell specific hepatic targeting of PGE1

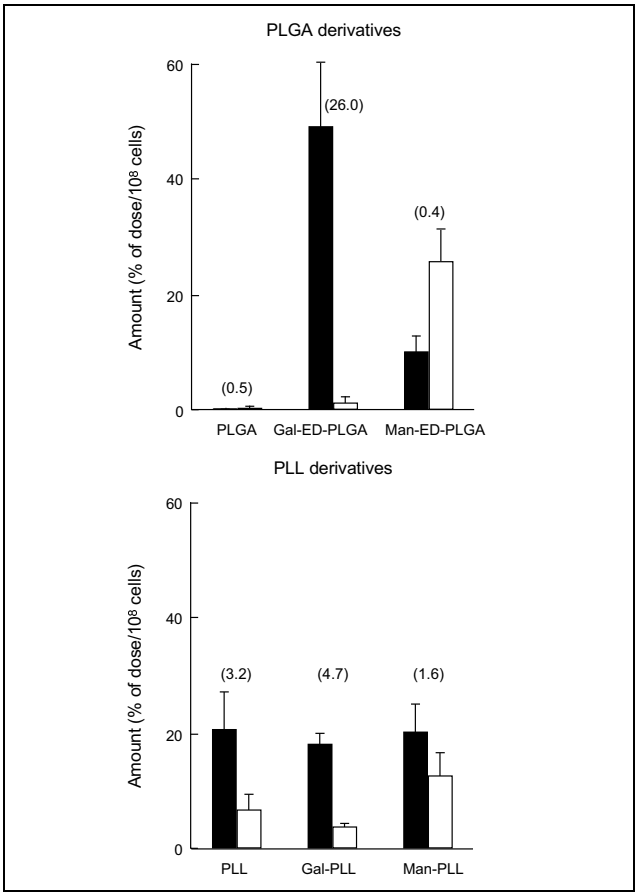

Fig. 4: Hepatic cellular localization of ^{111}In-labeled PLGA and PLL derivatives with sugar moieties at 30 min after intravenous injection in mice at a dose of 1 mg/kg. Amount of radioactivities in 10^8 cells are shown for parenchymal ("black") and non parenchymal ("white") cells and their ratios shown in parenthesis

nition sites to PLGA are clearly demonstrated in this figure. Since the biodistribution pattern of PLL is decided by its polycationic nature, the introduction of sugar moieties shows little effect on PLL. Based on these findings, PLGA was selected as a carrier backbone in our series of experiment.

Fig. 5 shows plasma and tissue concentrations of [^{3}H]PGE1 at 1 and 60 min after intravenous injection in mice with free or polymer conjugate forms. PGE_1 was rapidly eliminated from the circulation and excreted in the urine. Although 35% of the administered dose of PGE_1 was recovered in the liver 1 min after injection, it decreased rapidly. In addition, these radioactivity seems to mostly represent PGE1 metabolites since PGE1 has an extensive first-pass effect in the lung after intravenous injection. On the other hand, PGE_1-Gal-ED-PLGA showed prolonged retention in plasma and relatively specific accumulation in the liver. Thus, tissue distribution of [^{3}H]PGE_1-Gal-ED-PLGA is basically similar to that of [^{111}In]Gal-ED-PLGA which suggests that Gal-ED-PLGA is accompanied by PGE_1 molecules to the liver without separation. Furthermore, [^{3}H]PGE1-Gal-HZ-PLGA showed an extreme increase in liver uptake which reached about 60–80% of the dose and continued for a long period. These results suggest a superior activity of the Gal-HZ-PLGA carrier for specific targeting of PGE1 to the liver.

2.5. Degradation of [^{111}In]PLGA and PLL derivatives in the liver

Biodegradability of [^{111}In]PLGA and [^{111}In]PLL derivatives in the liver homogenate was evaluated based on the gel filtration elution profiles of ^{111}In-radioactivity. In the cases of [^{111}In]Gal-ED-PLGA and [^{111}In]Man-ED-PLGA, most radioactivity was eluted in low-molecular weight fractions, suggesting that they are rapidly digested in the liver within 30 min after intravenous injection. The degra-

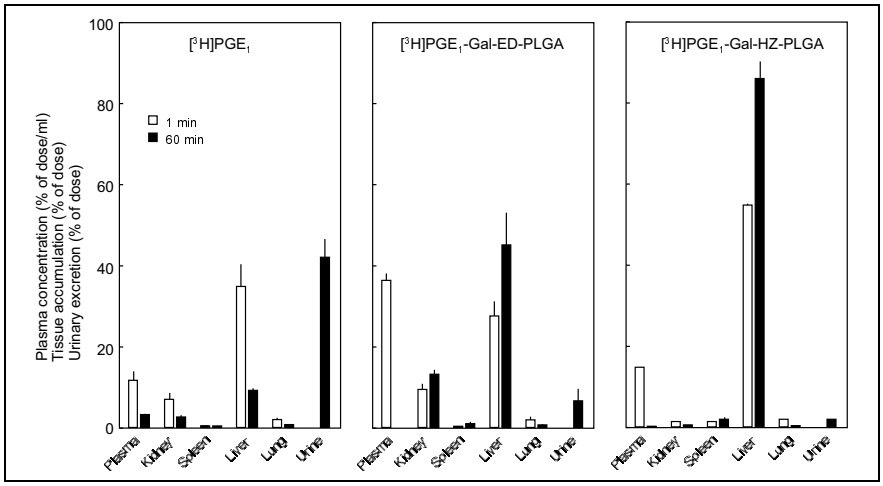

Fig. 5:
Biodistribution patterns of [^{3}H]PGE1 injected in a free or polymeric prodrug forms in mice

dation of [^{111}In]Gal-PLL and [^{111}In]Man-PLL were quite similar to those of [^{111}In]glycosylated PLGAs.

2.6. Release of PGE_1 radioactivity from its polymeric conjugate in the liver homogenate

In the case of [^{3}H]PGE1-Gal-ED-PLGA, the release of radioactivity in the mouse liver homogenate was very slow. On the contrary, radioactivity of a free form of [^{3}H]PGE_1 gradually increased with incubation time and approximately 30% of the initially conjugated total radioactivity was recovered up to 24 h as a free form in the case of [^{3}H]PGE1-Gal-HZ-PLGA. Moreover, the analysis of radioactive components by TLC suggested that PGE_1 polymeric conjugate was degraded in the liver and released PGE_1 or its related compound with the moderate release rate.

2.7. Therapeutic activity of PGE1 polymeric conjugate against acute hepatitis

Anti-hepatitis efficacy of PGE_1 polymeric conjugates was evaluated by measuring GPT activity in plasma of hepatitis mice prepared by intraperitoneal administration of CCl_4 [21]. PGE1 showed no effect at doses of 0.05 and 2.0 mg/kg. PGE_1-Gal-ED-PLGA also hardly decreased GPT value in plasma at a dose of 0.05 mg/kg. On the other hand, PGE1-Gal-HZ-PLGA showed a significant effect on GPT value in plasma suggesting its superior therapeutic effect on CCl_4-induced hepatitis. Therefore, it is concluded that the PGE_1 conjugate would show a significant preventing effect against hepatitis at a low dose probably through hepatocyte targeting via a galactose receptor-mediated endocytosis mechanism [22].

We reported successful targeting of recombinant human superoxide dismutase (SOD) to the liver parenchymal and nonparenchymal cells by introducing galactose and mannose residues, respectively [23]. In this case, mannosylated SOD showed a good inhibitory effect against hepatic injury caused by ischemia-reperfusion treatment. This finding and the present results suggest that the target cells in the treatment of hepatitis may change depending on the mechanism of drug action and it is very important to line up various approaches which enable us to deliver the drug with diverse spacial and temporal patterns. In this context, the strategies for a rational design of targeted delivery systems established through the pharmacokinetic considerations in our investigations should contribute a lot.

2.8. Conclusion

Galactosylated PLGA carriers developed in this study showed a remarkable targeting ability to hepatocytes. Among the tested compounds, PGE1-Gal-HZ-PLGA showed a gradual release of PGE1 and a superior therapeutic activity against hepatic damage caused by CCl4. These results suggest usefulness of the present approach in developing cell-specific targeting systems for the treatment of diseases of the liver such as hepatitis.

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Prof. Dr. Mitsuru Hashida
Graduate School of Pharmaceutical Sciences
Kyoto University
46–29 Yoshidashimmoadachi-cho, Sakyo-ku
Kyoto 606–8501
Japan
hashidam@pharm.kyoto-u.ac.jp