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Cellular recognition of paclitaxel-loaded polymeric nanoparticles composed of poly(γ-benzyl L-glutamate) and poly(ethylene glycol) diblock copolymer endcapped with galactose moiety

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Abstract

Poly(γ-benzyl L-glutamate) (PBLG)/poly(ethylene glycol) (PEG) diblock copolymer endcapped with galactose moiety (abbreviated as GEG) was synthesized and characterized for study of liver-specific targeting. From dynamic light scattering measurement, particle sizes of copolymeric nanoparticles were decreased with an increase of PEG in the copolymer. The morphology of GEG-3 nanoparticles observed by transmission electron micrograph was observed as almost spherical shapes and ranged about 50–300 nm. From the structural characterization using ^{1}H nuclear magnetic resonance, both characteristic peaks of PBLG and PEG were visible in $CDCl_3$ but the characteristic peaks of PBLG were invisible in D_2O, indicating that GEG block copolymers are found to the core-shell type nanoparticles in water with PBLG innercore and PEG outershell, exposing that galactose moiety of GEG block copolymers are outerwards oriented on the nanoparticle surfaces. By galactose-specific aggregation test of particles using β-galactose specific lectin, and flow cytometry measurement, specific interaction between asialoglycoprotein receptors (ASGPR) of HepG2, human hepatoma cell line, and galactose moieties of the GEG nanoparticles was confirmed. From cell cytotoxicity test, HepG2 cells with ASGPR are more sensitive to paclitaxel (TX)-loaded nanoparticles than free TX whereas, P388 cells, murine leukemia cell line, and SK-Hep 01, human hepatoma cell line, without ASGPR is less sensitive to TX-loaded nanoparticles than free TX, suggesting that specific interaction between HepG2 cells and galactose moiety of the nanoparticles occurred.

Keywords: Nanoparticles; Liver-specific targeting; Paclitaxel; HepG2

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1. Introduction

Several approaches to effective drug delivery using polymeric drugs and prodrugs have been undertaken since Ringsdorf (1975) proposed the concept of missile drugs. These approaches include prodrugs (O'Mullane and Daw, 1991), colloidal carriers (Illum et al., 1982), polymeric micelles (Yokoyama et al., 1990b), nanoparticles (Alleman et al., 1993; Gref et al., 1994) and liposomes (Lasic, 1992). Especially, tissue targeting drug delivery system have been extensively proposed using macromolecular carriers with monoclonal antibodies (Blume et al., 1993) or carbohydrates (Duncan et al., 1983; Dragsten et al., 1987) as target signals. Selective delivery of drugs to target sites can be achieved by reducing non-specific interaction with normal tissues by increasing the affinity with the target sites. Among them, carbohydrate receptors in the liver, i.e., asialoglycoprotein receptors (ASGPR) in hepatocytes and mannose receptors in Kupffer and liver endothelial cells were reported by Ashwell and Harford (1982). From these findings, several researchers have developed more effective macromolecular drug carrier with galactose (Duncan et al., 1986; Goto et al., 1994) or mannose (Jansen et al., 1991) as targetable moiety for liver-specific chemical agent delivery (Meijer and Sluijs, 1989; Nishikawa et al., 1993). Among them, ASGPR on the hepatocytes has been proposed as a useful means to target liver-specific chemotherapy (Harford et al., 1982) and sugar-containing conjugates have been used as a target permitting organ specific therapy of liver diseases (Kopecek and Duncan, 1987). This selectivity is the best for antitumor drugs (O'Hare et al., 1989) due to their extreme cytotoxicity (Wu and Wu, 1988) because of their instability in the body fluid and tissues. Especially, hepatocellular carcinoma (HCC) is one of the most common and devastating human malignant tumors worldwide (Wands and Blum, 1991). Also, HCC is the most lethal cancer with a fatality rate higher than 94% (Skolnick, 1994). In recent years, important advances have been made in understanding the molecular pathogenesis of HCC, but only limited improvement has been achieved with respect to its early diagnosis and treatment. Therefore, development of liver-specific targetable drug carriers should be required for the treatment of liver cancer (O'Hare et al., 1989; Codde et al., 1993). However, most researches for liver-specific targetable carriers have been limited to the liposomes (Codde et al., 1993) or prodrugs (O'Hare et al., 1989) which have some disadvantages such as instability of carriers in the body fluid, rapid elimination by undesirable organs, difficulties in modifying macromolecular carriers, possibility of drug inactivation during chemical attachment, liberation rate of drug from the macromolecular-drug conjugates and biodegradation.

On the other hand, novel drug carrier systems of core-shell type nanoparticles (Gref et al., 1994; Peracchia et al., 1997), reported by Peracchia et al. or polymeric micelles reported by Yokoyama et al. (1990a, 1991) and Kwon et al. (1995), were attempted to solve the problems above mentioned. Nanoparticles based on core-shell structure or polymeric micelles have many advantages such as long circulation in the body, drug solubility, drug stability and high drug encapsulation. However, in spite of the extensive and excited studies, polymeric micelles or core-shell type nanoparticles are limited for application of specific drug targeting due to their lack in targetability and then the drug may be freely diffused throughout the body as well as other normal drug carriers.

In a previous study, we reported simple preparation of biorecognition colloidal carriers for liver-specific drug targeting (Cho et al., 1997a, 1997b). Poly(γ-benzyl L-glutamate) (PBLG) or poly(L-lactic acid)(PLA) homopolymer nanoparticles coated with high density carbohydrate carrying polymers, poly(*N*-*p*-vinylbenzyl-*O*-β-D-galactopyranosyl-(1→4)-D-gluconamide) (PVLA), was prepared simply by a diafiltration method and this surface modified PBLG or PLA nanoparticles coated with lactose carrying polymer were recognized by hepatocytes.

In this study, we wish to report a novel type of targetable block copolymer composed of PBLG and poly(ethylene glycol) (PEG) endcapped with galactose moiety (abbreviated as GEG) for liver-specific drug delivery. PBLG as a hydrophobic part has biodgradability (Cho and Kim, 1988; Oh et al., 1995) and acts as a drug incorporation site. The PEG as a hydrophilic part is a non-toxic, non-immunogenic water soluble polymer and is known to prevent interactions with cells and proteins (Wang et al., 1997). Galactose moiety as a targetable site has a potential of liver-specific delivery (Duncan et al., 1986; Goto et al., 1994). Also, due to the amphiphilicity of this block copolymer, biorecognizable core-shell type nanoparticles with surface oriented sugar moiety prepared by a diafiltration method

will be expected as suitable site-specific carriers of paclitaxel (TX) (Kim et al., 1997; Jeong et al., 1998). TX represents a new class of anticancer agents and functions by promoting the assembly and stability of microtubules thereby causing mitotic arrest (Schiff and Horwitz, 1980). But new site-specific drug carriers for TX should be required owing to the side effects (Lorenz et al., 1977; Weiss et al., 1990), poor solubility in water, short half-life and high binding to albumin (Sonnichsen and Relling, 1994; Sorg et al., 1996).

2. Materials and methods

2.1. Materials

Diamine-terminated poly(ethylene glycol) (ATPEG = H_2N-PEG-NH_2) was supplied by Texaco Chem. Co. (Ballaire, Texas, USA). It has a number-average molecular weight of 4100. Paclitaxel (TX), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), RPMI (Roswell Park Memorial Institute) media 1640, Dulbecco's Modified Eagle's Medium (DMEM) and Minimum Essential Medium Eagle (MEM) were purchased from Sigma Co. (St. Louis, MO, USA). Tetrahydrofuran (THF), dimethylformamide (DMF) and dimethylsulfoxide (DMSO) as reagent grade were used without further purification.

2.2. Synthesis of PBLG/PEG-galactose (GEG)

GEG block copolymer was prepared as shown in Fig. 1. Lactonolactone (LL) (2) was prepared according to the method proposed by Kobayashi et al. (1985). Briefly, lactose-1 hydrate (12 g, 33 mM) (1) was dissolved in water (9 ml), diluted with methanol (25 ml), and added to an iodine (17.1 g) solution in methanol (240 ml) at 40 °C. At this temperature, 4% potassium hydroxide solution in methanol (400 ml) was added dropwise for 35 min with magnetic stirring until the color of iodine disappeared. After cooling down of the solution in an ice-bath, the precipitated crystalline product was filtered, washed with cold methanol, and then cold ether, and recrystallized from a mixture (800 ml) of methanol and water (9/1, v/v). The resulting potassium lactonate was then converted to the free acid by passing the aqueous solution through a column of Amberlite IR-120B. The acidic elute was collected and concentrated in a rotary evaporator. Repeated evaporation of methanol and ethanol solution converted the lactonic acid into LL (yield: 90%).

H_2N-PEG-LA (4): LL (2 g, 5.9 mM) was dissolved in refluxing methanol (30 ml) and diamine-terminated PEG (3.7 g, 3.7 mM) (3) in methanol solution (20 ml) was added. The mixed solution was refluxed for 5 h with magnetic stirring and allowed to stand at room temperature. The solvent was evaporated and the residue was dissolved in chloroform. The above solution was filtered to remove unreacted LL. The solvent was evaporated and the residual oily material was freeze-dried after dissolving it in water.

GEG block copolymer (6) was prepared by polymerization of γ-benzyl L-glutamate *N*-carboxyanhydride (BLG-NCA) (5) initiated with H_2N-PEG-LA in methylene chloride at a total concentration of BLG-NCA and H_2N-PEG-LA of 3 wt.% as previously reported (Cho et al., 1994). The reaction mixture was poured into a large excess of diethylether to precipitate the GEG copolymer. The resulting copolymer was washed with diethylether and then dried in vacuo.

2.3. ^1H nuclear magnetic resonance (NMR) measurement

^1H NMR spectra of the copolymers were measured in $CDCl_3$ to estimate the copolymer compositions and the molecular weights of PBLG blocks, using a JEOL FX 90Q NMR spectrometer. As the number-average molecular weight (4100) of PEG is known, one can estimate the number-average molecular weight of the PBLG block and the copolymer composition calculated from the peak intensities in the spectrum assigned to both polymers, respectively (Cho et al., 1994; Jeong et al., 1998).

For investigation of the structural characteristics of nanoparticles of GEG block copolymer, ^1H NMR spectra were measured in $CDCl_3$ and D_2O.

2.4. Fluorescence labeling of GEG polymers

GEG-3 copolymer was fluorescently labeled with fluorescein isothiocyanate (FITC). To a solution of the polymer (100 mg) in 5 ml DMSO, 2.5 mg FITC, 100 μl of pyridine and 100 μg of dibutyltin dilaurate were added and allowed to stand for 1 h at 60 °C. The mixture was then added to an excess of ethanol to

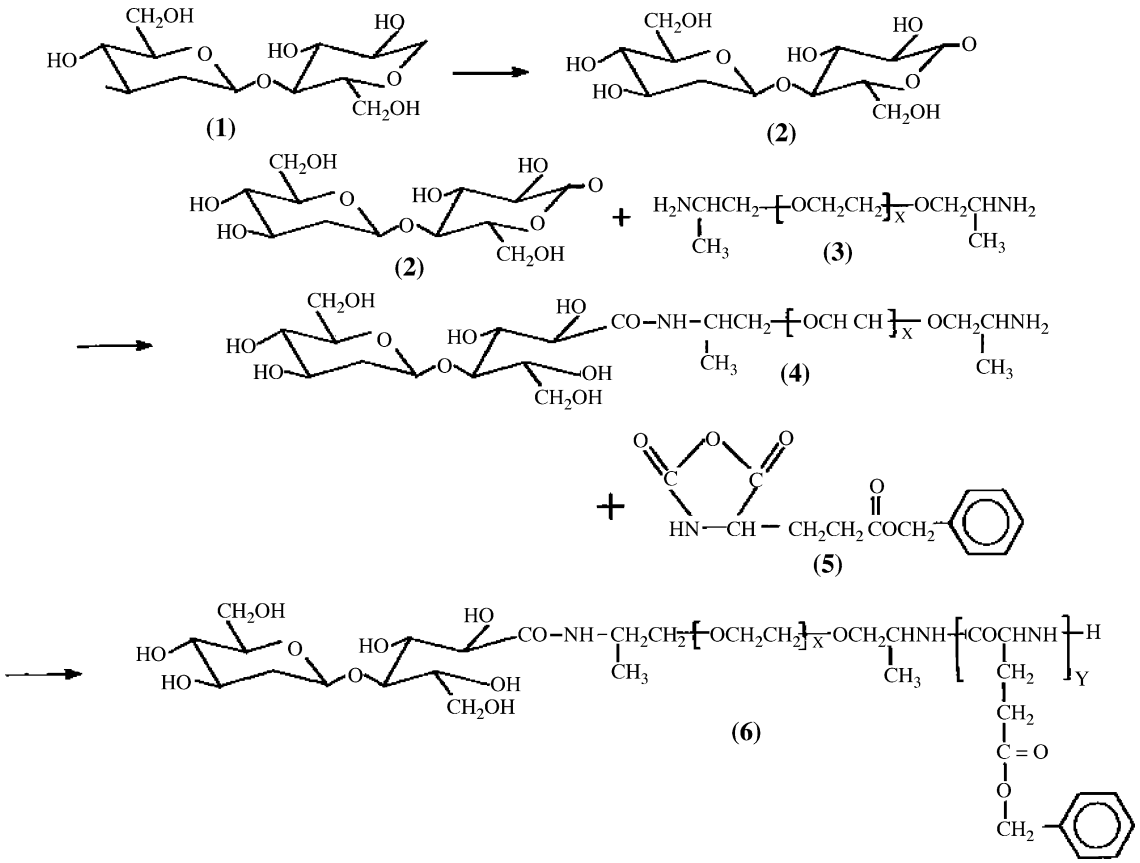

Fig. 1. Synthesis scheme of PBLG/PEG diblock copolymer endcapped with sugar moiety.

precipitate the polymer. The resulting polymer was purified twice by precipitation using DMSO and ethanol. The resulting solution was dialyzed with against 1 L of mild alkaline water (pH <8.0 with NaOH) using a molecular cut-off 2000 dialysis tube and then against distilled water for 3 days. The solution was freeze-dried.

2.5. Preparation of TX-loaded GEG nanoparticles

Preparation of GEG nanoparticles and drug loading procedure were carried out by a diafiltration method as reported previously (Cho et al., 1995, 1997a, 1997b). Briefly, 20 mg of GEG block copolymer was dissolved in 10 ml of mixture of DMF and THF (3/7, v/v) and subsequently 5 mg of TX was added. The solution was stirred at room temperature to dissolve it entirely. To form TX-loaded nanoparticles and remove free-drugs, the solution was dialyzed using a molecular cut-off 12,000 dialysis tube against 1.0 L × 3 of distilled water for 3 h and then distilled water exchange at intervals of 3–4 h during 24 h. Then, the solution was freeze-dried.

For estimation of drug loading content, freeze-dried sample of GEG nanoparticles was suspended into methanol and vigorously stirred for 2 h and sonicated for 15 min. Resulting solution was centrifuged with 12,000 × *g* for 20 min and supernatant was taken for measurement of drug concentration using UV spectrophotometer (Shimadzu UV-1201, Japan) at 273 nm.

2.6. Differential scanning calorimetry (DSC) measurement

The melting temperature of TX itself and TX-loaded GEG-3 nanoparticles were measured with a PL DSC-30 (PL-Thermal Sci., UK). The measurement

was carried out in the range of 20–350 °C under nitrogen at a scanning rate of 10 °C/min.

2.7. Transmission electron microscope (TEM) measurement

A drop of GEG-3 nanoparticle suspension in ethanol was placed on a carbon film coated on a copper grid for TEM. Observation was done at 80 kV using TEM (JEOL JEM-2000 FX II, Japan).

2.8. Dynamic light scattering (DLS) measurement

DLS was measured with a S4700 (Malvern Instruments, UK) with an argon laser beam at a wavelength of 488 nm at 20 °C. The scattering angle of 90° was used. Nanoparticle solution prepared by a diafiltration method was used for DLS measurement (concentration: 0.1%, w/v) and measured without filtering.

2.9. Cell culture

P388 cells, murine leukemia cell line, were cultured in suspension in RPMI media 1640, supplemented with 10% heat inactivated fetal calf serum (FCS), 2β-mercaptoethanol (10 μM), L-glutamine (2 mM), and antibiotics. Two human hepatoma cell lines, SK-Hep 01 and HepG2, were cultured in DMEM and MEM, respectively, supplemented with 10% FCS. All cells were maintained to exponential growth phase in plastic dishes at 37 °C in a CO_2 (5 vol.%) incubator. The HepG2 and SK-Hep 01 cells were selected because the HepG2 cells have ASGPR whereas the SK-Hep 01 ones do not have ASGPR.

2.10. Interaction of HCC with GEG nanoparticles

A polystyrene dish (35 mm diameter) was treated with bovine serum albumin solution (Sigma Chem. Co., USA; 1 mg/ml Dulbeccos' PBS(+)) for 2 h to avoid cell adhesion to the dish surface. Then 8.0×10^5 cells in 1.5 ml of MEM (HepG2) or DMEM (SK-Hep 01) were placed in the dish. Nanoparticles prepared with FITC labelled GEG-3 were added to the dishes at a final concentration of 0.1 mg/ml and were incubated for 2 h at 37 °C in a humidified CO_2 (5 vol.%) incubator. After incubation, the cells were washed twice with Dulbeccos' PBS(+) at 4 °C by centrifugation and resuspension, and were analysed by a flow cytometer.

2.11. Cytotoxicity

For the cytotoxicity tests, cells were seeded at a density of 1×10^4 per 96-well plate with medium containing 10% FCS and incubated overnight in a CO_2 incubator (5 vol.% CO_2 at 37 °C). After that, PBS containing TX-loaded nanoparticles was added. After 48 h (P388 cells) or 72 h (SK-Hep 01 and HepG2), the cell viability assay was performed by MTT assay. After the incubation period, cells were exposed to 30 μl of MTT (5 mg/ml) for 4 h. Formazan crystals were then solubilized with DMSO and each well was read by a microplate reader using a test wavelength of 570 nm and a reference wavelength of 630 nm. Results were expressed as a percentage of the absorbance present in drug-treated cells compared to that in the control cells.

2.12. Flow cytometer measurement

All measurements were performed on FACSCAN (Becton and Dickinson). Propidium iodide (final concentration of 5 μg/ml) was added to each sample to stain and gate-off the dead cells. To test the interactions with nanoparticles prepared from FITC-labeled GEG-3 (0.1 mg/ml), SK-Hep 01 and HepG2 cells were incubated for 2 h at 37 °C. From each sample, 1×10^4 cells were analyzed with logarithmic amplification of fluorescence intensity. The fluorescence intensity of control cells (incubation without nanoparticles) and test cells were compared with each other.

3. Results and discussion

GEG block copolymer was prepared by polymerization of BLG-NCA initiated by the amine-terminated PEG endcapped with galactose moiety in methylene chloride as shown in Fig. 1. Since excess LL was added into H_2N-PEG-NH_2 solution, unreacted H_2N-PEG-NH_2 was not expected to exist in the product of H_2N-PEG-LA. Also, it may be expected that the primary amine of the H_2N-PEG-LA can dominantly initiate polymerization of BLG-NCA than the hydroxy groups of the sugar moiety in the H_2N-PEG-G (Cho et al., 1994). The GEG block copolymers were

Table 1
Characterization of GEG block copolymers

Sample	Contents of monomeric (mol%)		M_n
	PBLG	PEG	
GEG-1	79.0	21.0	81000
GEG-2	65.2	34.8	42600
GEG-3	14.5	85.5	7900

MW of PEG: 4100.

characterized as shown in Table 1. The molecular weight and composition of the copolymer were estimated by ^{1}H NMR. ^{1}H NMR of the block copolymers was measured in $CDCl_3$ to estimate the copolymer composition and the molecular weight of PBLG blocks. As the number-average molecular weight of PEG (4100) is known, one can estimate the number-average molecular weight of PBLG block and copolymer from the copolymer composition calculated from the peak intensities in the spectrum assigned to both polymers. The PEG content in the block copolymer was 21.0, 34.8 and 85.5 mol% for GEG-1, GEG-2 and GEG-3, respectively.

3.1. Morphology and size distribution of GEG nanoparticles

Fig. 2 shows TEM photograph of GEG-3. The shape of the nanoparticles was almost spherical and the sizes were ranged about 50–300 nm in diameter. Fig. 3 shows particle size distribution of GEG-3 nanoparticles by DLS. The result of DLS of GEG-3 copolymer nanoparticles revealed relatively narrow size distribution and particle sizes of GEG-3 copolymer were 104.1 ± 56.8 nm which is almost similar to TEM observation. The particle size distribution against PBLG chain length is shown in Table 2. The longer PBLG chain length, the bigger particle sizes. The results indicated that the particle sizes were dependent on the PBLG chain length. When TX was loaded in GEG-3 nanoparticles (drug loading contents: 17 wt.%), particle sizes were increased and fraction of big particles was relatively higher than non-loaded nanoparticles as shown in Table 2. After filtration with 0.45 μm syringe filter for sterilization, drug loading contents was decreased from 17.0 to 4.3 wt.% owing to increased particle sizes of drug-loaded nanoparticles.

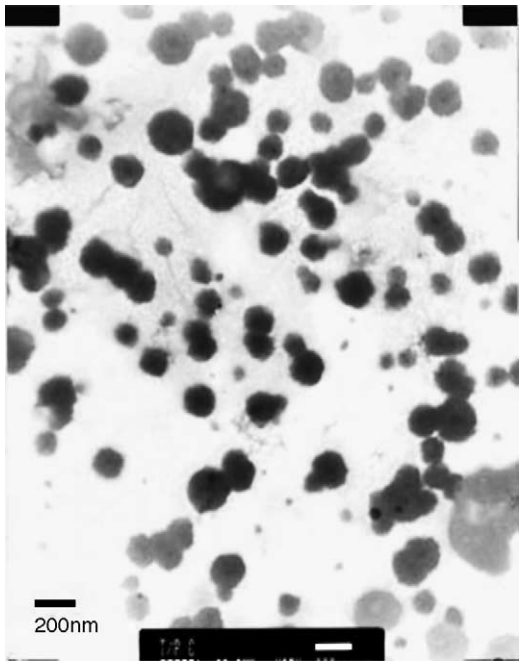

Fig. 2. TEM microphotograph of GEG-3 nanoparticles.

3.2. Structural characterization of GEG nanoparticles

Generally, block or graft copolymers form polymeric micelles (Yokoyama et al., 1990b; Kwon et al., 1995; Cho et al., 1995) or core-shell type nanoparticles (Cho and Kim, 1988; Gref et al., 1994) in

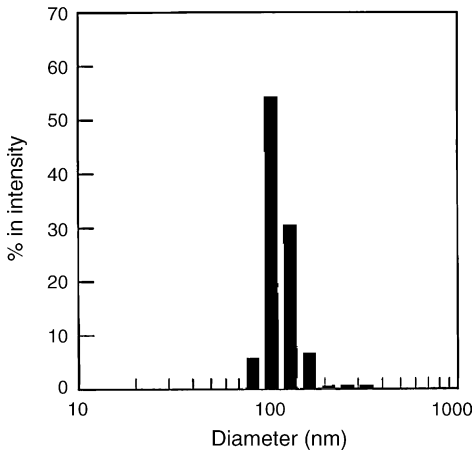

Fig. 3. Particle size distribution of GEG-3 nanoparticles by DLS.

Table 2
Particle size distribution of GEG nanoparticles by DLS

Sample	PBLG content (mol%)	Drug loading content (wt.%)	Particle size (nm)
GEG-1	79.0	0	320.0 ± 235.1
GEG-2	65.2	0	161.9 ± 100.2
	14.5	0	104.1 ± 56.8
GEG-3	14.5	17.0	157.8 ± 99.1 (50.5%)
			325.6 ± 210.9 (49.5%)

selective solvents by a self-assembling process. Various experimental apparatus such as small angle X-ray or neutron scattering, static or dynamic light scattering, fluorescence spectroscopy, TEM (Jeong et al., 1998), X-ray photoelectron spectroscopy (XPS) (Gref et al., 1994), and ^{1}H NMR (Hrkach et al., 1997) were used to examine the self-assembling properties and structural characteristics of polymeric micelles or core-shell type nanoparticles. Recently, Hrkach et al. (1997) reported core-shell type nanoparticles composed of poly (lactic-co-glycolic acid) (PLGA)-PEG diblock and PLA-PEG multiblock copolymers in aqueous solution which have a PLA core and an outer PEG shell. They reported that ^{1}H NMR was used to obtain direct evidence of the core-shell structure of these nanoparticles suspended in aqueous environment. In their results, when the ^{1}H NMR spectrum of nanoparticles in $CDCl_3$ was compared to that of a suspension of nanoparticles in D_2O, both characteristic peaks of PLGA block and PEG block were visible in $CDCl_3$ but the peaks of lactic acid and glycolic acid repeat units are not visible in D_2O, indication of solid core of PLGA block and surface oriented PEG block in aqueous solution but disappeared in organic solvent such as $CDCl_3$.

To investigate the structural characteristics and surface chemistry of GEG nanoparticles using ^{1}H NMR, freeze-dried GEG nanoparticles were dissolved and resuspended in $CDCl_3$ and D_2O, respectively. Since GEG block copolymers are composed of hydrophobic PBLG block and hydrophilic PEG block, core-shell type nanoparticles are expected in aqueous solution (Jeong et al., 1998). As shown in Fig. 4(a) of $CDCl_3$ where nanoparticle formation with core-shell structure is not expected, characteristic peaks of the protons of the benzyl group and the methylene protons adjacent to the benzyl group of the PBLG segment were shown in 7.2–7.4 and 5.0–5.2 ppm, respectively. In Fig. 4(b), these peaks disappeared in D_2O, indica-

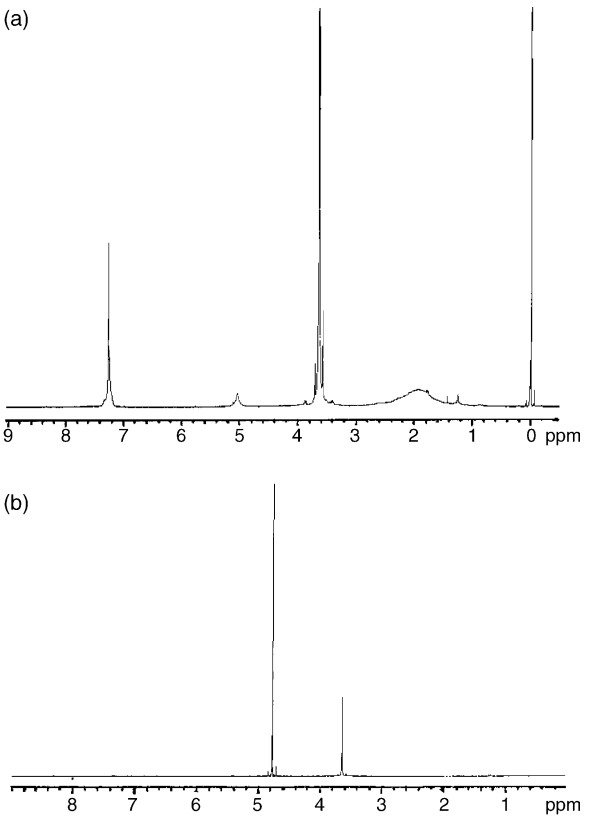

Fig. 4. ^{1}H NMR spectra of GEG nanoparticles: redissolved in $CDCl_3$ (a) and suspended in D_2O (b).

tion of restricted motions of these protons within the nanoparticle core and rigid structure of PBLG core of the GEG nanoparticles. This behavior of GEG nanoparticles is in contrast with low molecular amphiphiles and PEO-PPO-PEO block copolymers which typically exhibit liquidlike cores and relatively higher mobility. Also, structural characteristics of GEG nanoparticles are formed to core-shell type in aqueous solution which was similar results to Hrkach et al. (1997). From

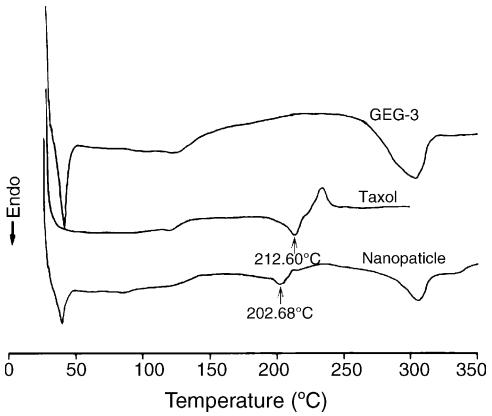

Fig. 5. DSC of GEG-3, TX and TX-loaded GEG-3 nanoparticles.

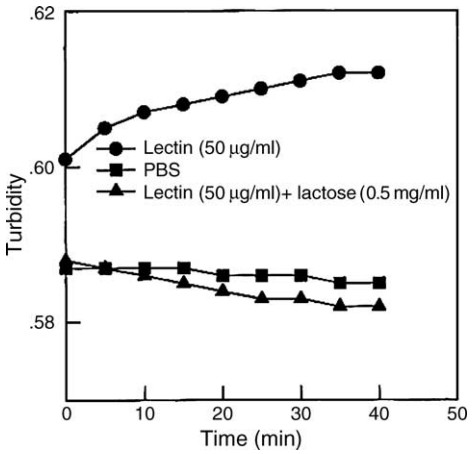

Fig. 6. Lectin-induced aggregation of GEG-3 nanoparticles. Nanoparticles + lectin (●); nanoparticle suspension in PBS (0.25 mg/ml) (■) and nanoparticle suspension + lectin + Allo A lectin (50 μg/ml) (▲).

the above results, it is expected that galactose moiety of GEG block copolymers is outerwards oriented on the nanoparticle surfaces. The surface oriented galactose moiety of GEG nanoparticles will be applicable to the recognition of liver cells for liver-specific drug targeting.

3.3. Thermal analysis of GEG-3, TX and TX-loaded GEG-3 nanoparticles

Fig. 5 shows DSC measurement of GEG-3, TX and TX-loaded GEG-3 nanoparticles (loading contents: 17 wt.%). From the DSC results, it was found that melting temperature (202.7 °C) of TX in the TX-loaded GEG-3 nanoparticles was shifted to lower temperature than that of TX itself (212.6 °C) due to the amorphous state of TX in the nanoparticles. The melting temperature of hydrophobic drug in the innercore of the core-shell type nanoparticles was generally shifted to lower temperature due to the amorphous state of drug in the nanoparticles (Gref et al., 1994). However, melting temperature of PEG (42.6 °C) and PBLG (303.1 °C) in the TX-loaded nanoparticles is similar to that of GEG although enthalpy of melting for the TX-loaded nanoparticles decreased when compared with that of GEG.

3.4. Lectin-induced aggregation of GEG nanoparticles

To confirm the surface oriented galactose moiety of the GEG nanoparticles, galactose-specific aggregation of particles was examined using β-galactose specific lectin, Allo A lectin. Fig. 6 shows turbidity change in the absence or presence of the lectin using UV spectrometer. When lectin was added, turbidity of the nanoparticle solution was increased whereas turbidity was not increased in the absence of lectin or addition of lactose. The results showed that the aggregation of GEG was inhibited by the addition of lactose, β(1–4)-galactosylglucose, indicating that the aggregation was due to specific crosslinking of β-galactosyl residues by lectin. Therefore, it can be said that sugar moiety was oriented on the nanoparticle surface, and recognized by carbohydrate binding proteins.

3.5. Cell cytotoxicity

P388, SK-Hep 01 and HepG2 cells were incubated with TX and TX-loaded GEG-3 nanoparticles to compare cell cytotoxicity. For cell cytotoxicity, TX-loaded nanoparticles were filtered with 0.45 μm syringe filter and drug loading content of filtered TX-loaded nanoparticles was 4.3 wt.%.

Table 3 shows cytotoxicity of P388 cells against TX and TX-loaded GEG-3 nanoparticles. The results indicated that high cytotoxicity of P388 cells appeared at low concentration of TX itself, whereas low cell cytotoxicity appeared below 0.25 μg/ml concentration of TX for TX-loaded GEG-3 nanoparticles. It is

Table 3
Cell cytotoxicity of P388 cells (cell density: 10^4 cells/well) by TX and TX-loaded GEG-3 nanoparticles after 48 h exposure

Concentration of TX (μg/ml)	Cytotoxicity (%)	
	TX	TX-loaded GEG-3 nanoparticles
0.125	91.8	16.9
0.25	98.5	21.5
0.5	98.8	78.9
5	98.7	–

thought that slow release of TX from TX-loaded GEG-3 nanoparticles affected low cytotoxicity of the P388 cells.

Cytotoxicity of SK-Hep 01 cells without ASGPR and HepG2 cells with ASGPR was compared in Fig. 7. It was found that cytotoxicity was more sensitive to HepG2 cells against concentration of TX for the TX-loaded GEG-3 nanoparticles than SK-Hep 01 cells whereas not much difference of cytotoxicity between HepG2 and SK-Hep 01 cells was found for the TX itself. The results suggest that TX-loaded nanoparticles may be actively delivered to the HepG2 cells with ASGPR through receptor-mediated mechanism whereas TX-loaded nanoparticles were passively delivered to the SK-Hep 01 cells without ASGPR.

From flow cytometry results as shown in Fig. 8, fluorescence intensity of HepG2 cells incubated with FITC-GEG nanoparticles (c) was stronger than that of SK-Hep 01 cells with FITC-GEG ones (b) or control (a), indicating that the GEG nanoparticles were specifically recognized by HepG2 cells.

In conclusion, PBLG/PEG diblock copolymer endcapped with galactose moiety was synthesized and characterized for application of liver-specific targeting. From DLS measurement, particle sizes of copolymeric nanoparticles decreased with an increase

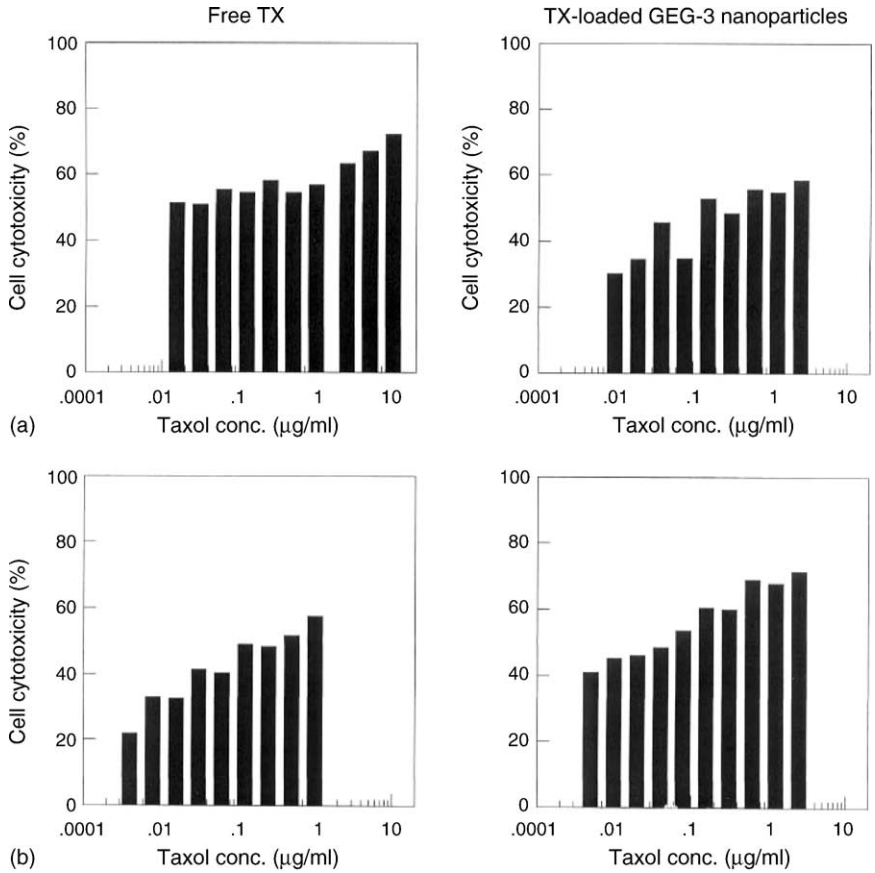

Fig. 7. Cell cytotoxicity of free TX and TX-loaded GEG-3 nanoparticles against SK-Hep 01 cells (a) and HepG2 cells (b).

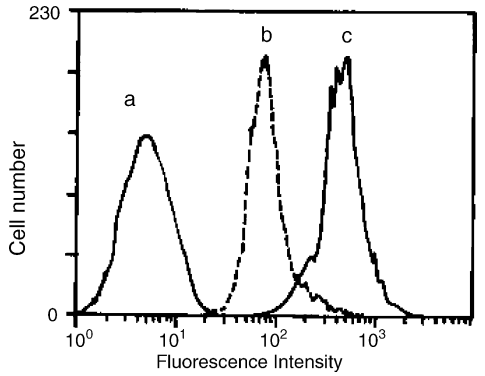

Fig. 8. Flow cytometry of HepG2 cells themselves (a); SK-Hep 01 with GEG-3 nanoparticles (b); and HepG2 cells with GEG-3 nanoparticles (c). SK-Hep 01 and HepG2 cells were incubated with FITC labelled GEG-3 nanoparticles for 2 h at 37 °C. After washing, cells were analyzed by flow cytometer.

of PEG content in the copolymer. From ^{1}H NMR measurement, GEG block copolymers are assembled to core-shell type nanoparticles in water with PBLG innercore and PEG outershell. By sugar-specific aggregation of particles using β-galactose specific lectin, surface oriented galactose moiety of the GEG-3 nanoparticles was confirmed. In cell cytotoxicity, P388 cells were more sensitive to the free TX than TX-loaded GEG-3 nanoparticles. Cytotoxicity was more sensitive to HepG2 cells for the TX-loaded GEG-3 nanoparticles than SK-Hep 01 cells due to active delivery of TX-loaded GEG-3 nanoparticles to HepG2 cells through receptor-mediated mechanism.

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