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Brain targeting with surface-modified poly(D,L-lactic-co-glycolic acid) nanoparticles delivered via carotid artery administration

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ABSTRACT

In this study, we investigated surface-modified nanoparticles (NP) formulated using a biodegradable polymer, poly(D,L-lactide-co-glycolide) (PLGA), for targeting central nervous system (CNS) diseases. Polysorbate 80 (P80), poloxamer 188 (P188), and chitosan (CS) were used to modify the surfaces of PLGA NP to improve the brain delivery of NP. Surface-modified PLGA NP were formulated using an emulsion solvent diffusion method. 6-Coumarin was used as a fluorescent label for NP. The different formulations of 6-coumarin-loaded PLGA NP were injected into rats via carotid arteries. NP remaining in the brain were evaluated quantitatively, and brain slices were observed using confocal laser scanning microscopy (CLSM). Carotid artery administration was more effective for delivering NP into the brain compared to intravenous administration. After administration, NP concentrations in the brain were increased by NP surface modification, especially CS- and P80-PLGA NP. CLSM observations indicated that P80-PLGA NP could cross the blood–brain barrier and thus serve as a drug delivery system for the CNS. These results indicate that surface-modified PLGA NP have a high potential for use in CNS delivery systems.

1. Introduction

Diseases of the central nervous system (CNS) are presently one of the five most serious diseases in humans. Typical CNS diseases include Alzheimer's disease, brain tumor, Parkinson's disease, white matter dystrophy symptom, Creutzfeldt–Jakob disease, meningitis, and encephalitis. For developing new medicines for CNS diseases, an obstacle is the dropout percentage during clinical trial phases is rather high in comparison with other drugs. The reason for this dropout is related to the unique structure of cerebral blood vessel such as the blood–brain barrier (BBB) that acts as a barrier for the absorption of drugs. Hence, material transport from blood to CNS is restricted. In general, the permeability of a substance into the brain is proportional to its partition coefficient. However, nutrients such as glucose, amino acids, and others are preferentially permeable, while cell toxic drugs such as anticancer drugs are less permeable, even though they are highly lipophilic. These phenomena are related to the specific transporter for uptake and efflux of an absorbed drug by a transporter in epithelial and endothelial cell membranes, such as P-glycoprotein. Because of this BBB drug transport restriction mechanism, drug delivery to the CNS is quite difficult.

Colloidal biodegradable polymeric particulate systems have been considered as candidates for CNS-targeted drug delivery. It has been reported that biodegradable polymeric poly(alkylcyanoacrylate) (PACA) nanoparticles (NP) achieve significant drug delivery to the brain in vivo [1]. In particular, surface modification of PACA NP by polysorbate 80 (P80) increased the effective drug delivery to the brain [2–5].

Among the biodegradable polymers, poly(lactide) (PLA) and poly(D,L-lactide-co-glycolide) (PLGA) have been approved by the FDA for certain human clinical uses. The degradation time of PLGA can be altered from days to years by varying the molecular weight and the lactic acid to glycolic acid ratio of the copolymers. PLGA NP have been suggested to be good drug delivery carriers because of their safety and property of achieving sustained release. We have successfully developed PLGA NP to improve peptide and gene delivery in vitro and in vivo [6–10]. In addition to the particle size, surface properties and composition also have a significant influence on particle stability and the pharmacodynamics of both the drug and carrier [11]. It was also reported that modifying drug (doxorubicin or loperamide)-loaded PLGA NP with pharmaceutical surfactants enabled the delivery of drugs into the brain [12].

In this study, surface-modified PLGA NP were examined as a brain drug delivery system in vivo. Drug delivery for brain

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targeting is usually carried out by injections into the tail vein. However, it has been reported that therapeutic effect against a brain tumor could be improved by injecting drug via the carotid artery [13]. Therefore, we evaluated the effect of route of administration on brain distribution for PLGA NP surface-modified with P80, P188, and CS using carotid artery administration.

2. Materials and methods

2.1. Materials

PLGA (lactide:glycolide = 75:25, MW = 20,000) was purchased from Wako (Osaka, Japan). Poly(vinylalcohol) (PVA, MW = 25,000, 88% hydrolyzed) was purchased from Kuraray (Osaka, Japan). Chitosan (MW = 20,000; deacetylation degree 84.2%) was obtained from Katakurachikkarin (Tokyo, Japan). Polysorbate 80 (Tween® 80) was purchased from Kishida Chemical Co., Ltd. (Osaka, Japan). Poloxamer 188 (Pluronic® F-68) was purchased from Asahi Denka. Kogyo Co., Ltd. (Tokyo, Japan). The fluorescent dye coumarin 6 laser grade, [3-(2-benzothiazolyl)-7-(diethylamino) coumarin] (6-coumarin), was purchased from MP Biomedicals (Solon, OH, USA). All other chemicals were of the highest grade commercially available.

2.2. Preparation of surface-modified PLGA NP by emulsion solvent diffusion method

PLGA NP loaded with 6-coumarin as a fluorescent label were prepared by the previously reported emulsion solvent diffusion (ESD) method in aqueous solution [10]. PLGA (100 mg) and 6-coumarin (1 mg) were dissolved in 3 mL of an acetone/ethanol mixture (acetone:ethanol = 2:1). The resultant organic solution was poured into 50 mL of an aqueous PVA solution (2 w/v%, in distilled water) and stirred using a propeller-type agitator with three blades at room temperature. The entire dispersed system was then centrifuged (43,400*g* for 10 min), and the sediments were resuspended in distilled water. This process was repeated, and the resultant dispersion was freeze-dried. To prepare surface-modified PLGA NP, either a CS (0.25 w/v%, in 0.5 M acetate buffer, pH 4.4)-PVA (1%, w/v) or a surfactant (P80 or P188, 1 w/v%, in distilled water)-PVA (1 w/v%) solution was used as the dispersing phase for the ESD process.

2.3. Analysis of NP physicochemical properties

Particle size and zeta potential were determined using a Zetasizer 3000 HSA (Malvern Instruments Ltd., Malvern, UK). Particle size was measured by photon correlation spectroscopy. Zeta potential determinations were based on the electrophoretic mobility of the NP in aqueous medium.

2.4. Animal experiments

All animal experiments were performed according to the regulations of the Animal Care and Use Committee of Gifu Pharmaceutical University (Gifu, Japan). Male Wister rats (12-week-old) were anesthetized with diethyl ether before administering NP. NP were suspended in saline to a final concentration of 100 mg/mL in different volumes (0.05, 0.10, 0.25 mL). A suspension was administrated via the left carotid artery or left jugular vein. At different time points (0, 15, 60, 120, 240 min), animals were sacrificed by cervical dislocation. After blood was collected by exsanguination, brains were removed. A total of 3 mL of saline was added to 275 mg of tissue and homogenized for 2 min using a Polytron homogenizer (PCU-11, Kinematica, Littau/Luzern, Switzerland). The fluorescent dye (6-coumarin) was extracted from NP by mixing 1 mL of the homogenate with 5 mL of methanol/chloroform (1:1). To extract NP from collected blood, 100 μL of plasma was mixed with 5 mL of methanol/chloroform (1:1). The samples were centrifuged (1400*g* for 10 min), and 6-coumarin concentrations were measured with a fluorescence spectrophotometer (F-3010, Hitachi, Tokyo, Japan; excitation wavelength 490 nm; emission wavelength 520 nm).

2.5. CLSM observations

The brain was embedded in Tissue-Tek® O.C.T™ compound (Sakura Finetechnical, Tokyo, Japan) immediately after sacrifice. Serial sections (10 μm thick) were cut using a cryostat (CM1850; Leica, Wetzlar, Germany) at −20 °C and thaw-mounted onto glass slides. Brain slices were observed with an LSM 510 confocal laser scanning microscope (Carl Zeiss, Goettingen, Germany) equipped with a Zeiss Plan-Neofluar 40×/0.75 objective lens using an argon-ion laser (458–514 nm).

3. Results

3.1. Physicochemical properties of surface-modified PLGA NP

The physicochemical properties of the surface-modified PLGA NP are summarized in Table 1. PLGA NP were prepared by ESD in water and modified with three types of polymers; CS, P80, and P188. PLGA NP sizes ranged from 250 to 400 nm, depending on the type of surface modifier used. Freeze-dried NP were readily dispersed in aqueous medium with shaking by hand, giving nearly the same particle diameter as that before drying. Unmodified PLGA NP (Non-PLGA NP), prepared by using only the nonionic polymer PVA as an NP stabilizer, possessed a negative zeta potential (−21.2 mV) due to the dissociation of the carboxyl group of PLGA in distilled water. CS-PLGA NP had a positive zeta potential (+6.1 mV) in distilled water due to protonation of the amino group. Zeta potentials of surfactant (P80 and P188)-modified PLGA NP were nearly the same as Non-PLGA NP.

3.2. Effect of administration route on NP brain distribution

After injection via the left carotid artery, all types of NP (Fig. 1A) showed higher brain distribution percentages than those after jugular vein injection (Fig. 1B). NP concentrations in the brain were increased by P80 and CS modification compared to Non-PLGA NP. In contrast, the elimination rate of P188-PLGA NP was faster than Non-PLGA NP for the carotid artery injection. The differences of NP distributions between the right and left side of the brain after carotid artery administration were examined (data not shown). The left brain side was passaged first by NP after administration because NP were injected via the left carotid artery. Thus, NP concentration remaining in the left brain side was much higher than in the right brain side.

Table 1
Physicochemical properties of surface-modified 6-coumarin-PLGA NP. Results are the means ± SD (n = 3).

	Particle size (nm)		Zeta potential (mV)
	Before freeze-drying	After freeze-drying	
Non-PLGA NP	263.3 ± 6.1	269.3 ± 31.6	−21.2 ± 1.3
CS-PLGA NP	294.2 ± 13.7	396.2 ± 14.4	6.1 ± 0.3
P80-PLGA NP	214.9 ± 6.7	231.7 ± 9.1	−20.3 ± 1.1
P188-PLGA NP	232.4 ± 9.9	252.2 ± 7.3	−19.0 ± 0.5

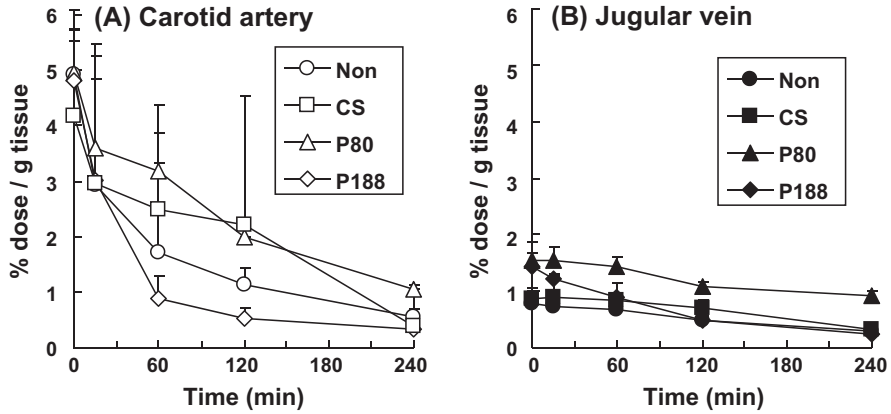

Fig. 1. Effect of administration route on brain distribution for surface-modified PLGA NP. (A) Carotid artery and (B) Jugular vein administration. Results are the means ± SD (n = 3–6).

3.3. NP remaining in the blood after carotid artery injection

The elimination rates from the blood of surface-modified PLGA NP were evaluated after NP injections (Fig. 2). The concentration of P80-PLGA NP in the blood after carotid artery injection was much higher than the other NP that were evaluated. P80-PLGA NP had a prolonged circulation in the blood compared to the other NP.

3.4. Effect of dose on NP brain distribution

The effect of injected dose on NP brain distribution was examined after 60 min, as shown in Fig. 3. PLGA NP suspensions of 0.05, 0.10, and 0.25 mL were injected via the carotid artery. The uptake levels in the brain of Non- and P188-PLGA NP were lower compared to P80- and CS-PLGA NP. This suggested that the interactions between Non- and P188-PLGA NP and vascular endothelial cells of the brain were weak. The differences due to different doses of CS-PLGA NP were small compared to the other surface modifiers. In contrast, for P80-PLGA NP, the remaining concentration of NP in the brain was dependent on dose.

3.5. CLSM observations

Brain cryostat microtome sections after the administration of different 6-coumarin-loaded PLGA NP via carotid artery were observed by CLSM (Fig. 4). The possibility for these NP to overcome the BBB was studied by an in vivo approach in rats. The brain blood vessels in these pictures are shown as the areas surrounding the red line. CS-PLGA NP were only adsorbed on endothelial cells and not transferred to brain tissue (Fig. 4C). In contrast, 6-coumarin fluorescence for P80-PLGA NP could be observed in the parenchyma beyond the cerebral blood vessel endothelial cells (Fig. 4D). Non- and P188-PLGA NP mostly remained in the cerebral blood vessel (Fig. 4B and E).

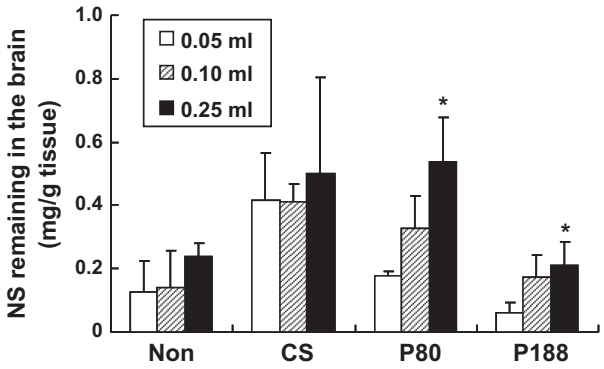

Fig. 3. Effect of injected dose of PLGA NP on NP brain distribution remaining in the brain 60 min after NP injection via carotid artery. Results are means ± SD of six experiments. Results are the means ± SD (n = 3–6), $^{*}p < 0.05$, significantly different compared with 0.05 mL.

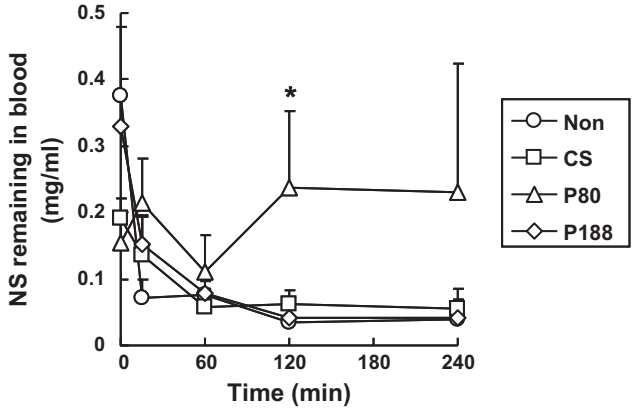

Fig. 2. Effect of surface modifier on the time course of PLGA NP concentration in the blood after injection via carotid artery. Results are the means ± SD (n = 3–6), $^{*}p < 0.05$, significantly different compared with Non-PLGA NP.

4. Discussion

Colloidal polymeric NP systems have been used and researched as brain-targeting delivery systems. In particular, P80-modified PACA NP have been successfully used [1]. The purpose of this study was to investigate the capability of surface-modified PLGA NP to deliver drugs to the brain. Three types of materials were used as surface modifier such as P80, P188, and CS. P80 and P188 are nonionic surfactant, and it has been reported that surfactant-modified polymeric NP were good carriers for use in a brain-targeting system [12]. Further, we have found that the cellular uptake of PLGA NP increased after surface modification with CS and that this was due to an electrostatic interaction with the cell membrane [11]. Among to these aspects, we considered that these materials could be candidates for the surface modification of PLGA NP for use in a brain-targeting drug delivery system.

6-Coumarin-loaded PLGA NP were prepared by an ESD method (Table 1). 6-Coumarin was chosen as a model drug and fluorescent

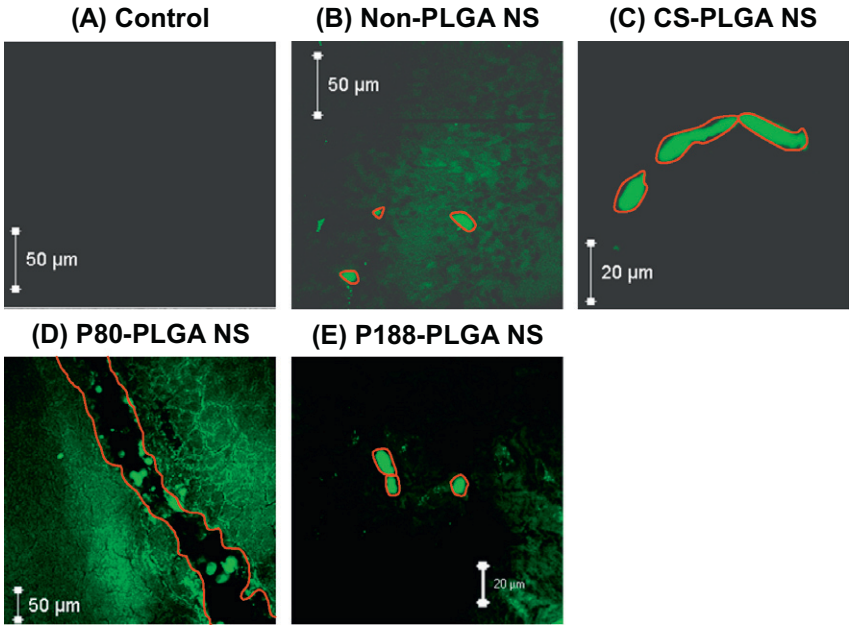

Fig. 4. Confocal laser microscope images of rat brain slices with surface-modified PLGA NP at 60 min after injection via carotid artery. Brain blood vessels in these pictures are shown as the areas surrounding the red line. (A) Control, (B) Non-PLGA NP, (C) CS-PLGA NP, (D) P80-PLGA NP, and (E) P188-PLGA NP.

label. ESD method is a technically simple process using mild conditions which has several advantages as follows: it produces uniformly sized NP, the reproducibility of NP production is good, and large-scale production and surface modification of NP are easily accomplished, which are important considerations for NP in biomedical applications. Surface-modified PLGA NP can be prepared by simple process that is adding a solution of surface modifier into the outer phase with a PVA solution.

For PVA, a nonionic polymer and requirement material as cryoprotectant, particle size of the Non-PLGA NP was 250–300 nm, and the zeta potential was a negative charge after the dissociation of the PLGA carboxyl group in distilled water. Particle sizes of CS-PLGA NP increased because adsorbed molecular layers of CS formed on the surfaces of PLGA NP. Whereas, zeta potentials of surfactant (P80 and P188)-modified PLGA NP were very similar to that of Non-PLGA NP; thus, it was difficult to confirm NP modification based on physicochemical properties.

In this ESD system, only small amounts of surface modifier were adsorbed on the NP surface, because by any excess surface modifier not adsorbed onto the NP surface was removed the centrifugation process on PLGA NP preparation. In general, cytotoxicity using a surfactant is caused at concentrations higher than the critical micelle concentration. Surface-modified PLGA NP did not form micelles. Therefore, no cytotoxic effects were caused [11].

Other reports have chosen intravenous administration for brain targeting, and several other routes of administration are not yet examined. Particularly, most of small particulate carrier should be uptaken and eliminated by the reticuloendothelial system (RES) during blood circulation after injection. Here, we tried administering PLGA NP via carotid artery for delivery to the brain in vivo. Irrespective of PLGA NP types, carotid artery administration was more effective than intravenous administration (Fig. 1). PLGA NP via the carotid artery could be delivered directly to the brain before circulation to other organs.

Based on this study, Non- and P188-PLGA NP might not interact with brain endothelial cells, as shown in Fig. 1A. In contrast, after injection into the carotid artery, P80-PLGA NP showed a higher brain distribution percentage compared to other modified NP. Till date, drugs that have been successfully delivered into the brain using P80-coated PACA NP include the hexapeptide dalargin, the dipeptide kyotorphin, loperamide, tubocuranine, the NMDA receptor antagonist MRZ 2/576, and doxorubicin [1]. It has been hypothesized that P80-PACA NP adsorb apolipoproteins from the bloodstream and therefore, could undergo receptor-mediated endocytosis into brain capillary endothelial cells, as with low-density lipoproteins (LDL) via the LDL receptor family [4]. In addition, P80-PLGA NP showed a prolonged blood circulation time in the body, which might be one factor for increasing the brain distribution by avoiding uptake by RES, as shown in Fig. 2.

Despite the rapid clearance of CS-PLGA NP from the blood circulation (Fig 2), the concentration of CS-PLGA NP in the brain was increased (Fig. 1). Moreover, CS-PLGA NP remaining in the brain were not changed by the injection dose (Fig. 3). These results suggested that CS-PLGA NP adsorption on vascular endothelial cells reached to saturate under these injection dose. CS-PLGA NP appeared to adsorb onto the cerebral blood vessel by adhering to endothelial cells due to electrostatic interactions, whereas unabsorbed particles were eliminated rapidly from blood circulation due to uptake by the RES. From CLSM studies, CS-PLGA NP were observed on only vascular endothelial cells, but did not transfer to the brain tissues (Fig. 4C). CS has the ability to open intracellular tight junctions [8]. Therefore, if CS-PLGA NP are used for drug loading, the drug may be also transported to the brain via a paracellular route. Additional investigations to study the pharmacological effects of drug-loaded CS-PLGA NP will be needed.

In this study, PLGA NP fate in the brain were examined using CLSM (Fig. 4). P80-PLGA NP could be observed in the tissue over cerebral blood vessel. This suggested that P80-PLGA NP could not only adsorb to the endothelial membrane of cerebral blood vessel, but could also be internalized by endothelial cells and cross the BBB. Portions of Non- and P188-PLGA NP also entered the parenchyma, but they mostly remained in the blood vessels. The amounts of Non- and P188-PLGA NP remaining in the brain were small in comparison with other modifiers in quantitative analysis (Figs. 1 and 3). Therefore, P80-PLGA NP might be the most effective drug carrier for CNS delivery in this study.

The detail BBB transport mechanism of PLGA NP was not elucidated in this study. However, Kreuter reported that for in vivo studies carried out using these surfactants, adsorption of apolipoprotein E (apo E) on the surface of P80-NP is observed, while no adsorption is observed on those coated with P188 or on unmodified NP after incubation for five min in plasma [4]. It was suggested that receptor-mediated endocytosis via apo E adsorption could participate in NP transport into the brain.

5. Conclusion

PLGA NP modified with surfactants can be used for the delivery of drugs across the BBB. Present results indicate that colloidal polymeric systems represent a promising strategy to overcome the BBB. However, additional investigations are required in order to identify the mechanisms for a brain delivery system using PLGA NP.

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