Clonazepam Release from Core-shell Type Nanoparticles In Vitro

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AB-type amphiphilic copolymers (abbreviated as LE) composed of poly (L-leucine) (PLL) as the A component and poly (ethylene oxide) (PEO) as the B component were synthesized by the ring-opening polymerization of L-leucine N-carboxy-anhydride initiated by methoxy polyoxyethylene amine (Me-PEO-NH₂) and characterized. Core-shell type nanoparticles were prepared by the diafiltration method. Particle size distribution obtained by dynamic light scattering was dependent on PLL composition and the size for LE-1, LE-2 and LE-3 was 369.6±267, 523.4±410 and 561.2±364 nm, respectively. Shapes of the nanoparticles observed by transmission electron microscope (TEM) were almost spherical. The critical micelle concentration (CMC) of the nanoparticles determined by a fluorescence probe technique was dependent on the composition of hydrophobic PLL, and the CMC for LE-1, LE-2 and LE-3 was 2.0×10⁻⁵, 1.7×10⁻⁵ and 1.5×10⁻⁵ (mol/l), respectively. Clonazepam release from core-shell type nanoparticles in vitro was dependent on PLL composition and drug loading content.

Key words: Core-shell type nanoparticle, Diafiltration method, Critical micelle concentration, Amphiphilic copolymers

INTRODUCTION

Nanoparticles are solid colloidal particles ranging in size from about 10 to 1000 nm. They are widely employed in various fields of life science such as separation technologies, histological studies, clinical diagnostiic assays and drug delivery system (DDS) (1). Among DDSs, the nanoparticles were applied for site-specific drug carriers (2). Moghimi et al. prepared polystyrene nanoparticles coated with polyoxyethylene (POE)/polyoxypropylene (POP) block copolymers to control the rate of drainage from the subcutaneous injection site and to manipulate the lymphatic distribution (3). Maincent et al. reported that poly (hexyl cyanoacrylate) nanoparticles can be efficient for the treatment of cancers with dissemination of metastases in the abdominal cavity after intraperitoneal administration (4). However, these carriers have some drawbacks: rapid renal excretion, recognition by the reticuloendothelial system (RES) and too low stability in the physiological fluid. To overcome these problems, amphiphilic AB type block copolymer nanoparticles composed of hydrophilic and hydrophobic components, which have hydrophobic inner core and a hydrated hydrophilic outer shell in aqueous media were designed (5). These core-shell type nanoparticles hold separated functionalities. Hydrophobic components form inner core of the nanoparticle, which acts as a drug incorporation site and exhibits pharmacological activity. Hydrophobic drug may be easily incorporated in inner core of the polymeric carriers by hydrophobic interactions (5). Hydrophilic outer shell prevents interaction with various biocomponents such as protein and cell, which affects the pharmacokinetic properties (6) and biodistribution of the hydrophobic drug. The nanoparticles as vehicles to carry hydrophobic drugs have several advantages such as increased blood circulation time and reduced RES capture due to small size, solubilization of hydrophobic drug, high structural stability and high drug entrapment in the hydrophobic core (2,7). Recently, diafiltration method was developed for the preparation of nanoparticles based on polymeric micelles since it was simple and the nanoparticles were no-aggregation, small size, high yield, almost spherical shape and had biodegradation (8).

In this study, we wish to report nanoparticles preparation of diblock copolymers composed of poly (L-leucine) (PLL) as the hydrophobic block and poly
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Clonazepam Release from Core-shell Type Nanoparticles in vitro (ethylene oxide) (PEO) as the hydrophilic block by the diafiltration method in distilled water. PLL has been known as potentially biocompatible and biodegradable polypeptide (9). PEO is known as non-toxic, non-immunogenic water-soluble material and to reduce clearance of RES after intravenous injection (10-13). Anticonvulsant benzodiazepine, clonazepam (CZ), was selected as the hydrophobic model drug (water solubility: 14.7 µg/ml) because it had high interaction with protein in vivo. We have investigated physicochemical state of the nanoparticles and drug release from nanoparticles in vitro.

EXPERIMENTAL

Material

Methoxy polyoxyethylene amine (Me-PEO-NH₂, M. W. ca. 12,500) was kindly provided by Japan Oil and Fat Co., Dimethyl sulfoxide (DMSO), methylene dichloride and diethyl ether were purchased from Sigma Chemical Co. and used without further purification. Clonazepam (CZ) was obtained from Roche, Switzerland.

Synthesis of LE diblock copolymers

PLL/PEO (abbreviated as LE) diblock copolymers were prepared by ring-opening polymerization of LL-NCA initiated with Me-PEO-NH₂ as similar method reported previously (14,15). The reaction mixture was poured into a large excess of diethyl ether to precipitate the PLL/PEO diblock copolymers. The resulting copolymer was washed with diethyl ether and then dried in vacuo.

Measurement of ¹H NMR spectroscopy

¹H NMR spectroscopy of the LE diblock copolymers was measured to estimate the composition and molecular weight of the block copolymers, (JEOL FX 90Q NMR spectrometer) in deuterated trifluoroacetic acid (CF₃COOD). As the number-average molecular weight (12,500) of PEO is known, the number-average molecular weight of the PLL block of the block copolymer can be estimated from the copolymer composition via the peak intensities assigned to both polymer blocks.

Measurement of infrared (IR) spectroscopy

IR spectra of samples prepared by KBr method were measured with Bruker IFS-66 FTIR spectrometer between 4,000 and 400 cm⁻¹.

Preparation of nanoparticles and CZ-loaded nanoparticles

The 20 mg of PLL/PEO block copolymers was entirely dissolved in 4 ml of dimethyl sulfoxide (DMSO) and subsequently CZ was added. The solution was stirred at room temperature for 12 hrs. The resulting solution was dialyzed using molecular weight cut-off 12,000 g/mol dialysis membrane with distilled water for 24 hrs and freeze-dried (16).

Measurement of fluorescence spectroscopy

Fluorescence measurements were carried out using CZ as a probe to estimate critical micelle concentration (CMC) of LE nanoparticles in doubly distilled water. Emission spectra were measured with varying copolymer concentration by a fluorometer (Shimadzu RF-5,000) at room temperature. The CMC of the block copolymers was estimated from fluorescence emission spectra with excitation wavelength of 306 nm.

Measurement of transmission electron microscope (TEM)

A drop of nanoparticles suspension in ethyl alcohol was placed on a copper grid coated with carbon film for observation of nanoparticle shape using TEM (JEOL JEN-2,000 FX II). The specimen on the copper grid was not stained. The accelerating voltage of the TEM was 80 kV.

Dynamic light scattering (DLS) measurement

Dynamic light scattering was measured for particle size distribution using a 54,700 (Malvern Instruments, England) with an argon laser beam at a wavelength of 488 nm and value is expressed in weight-averaged scales as unimode. The scattering angle of 90° was used. The lyophilized sample was sonicated for 1 min in deionized water (concentration: 0.1 w/v%) and measured without filtering.

In vitro release test

The release test of CZ from LE nanoparticles was carried out as followed: CZ loaded nanoparticles and 1 ml phosphate buffered saline (PBS, pH=7.4) were put into dialysis membrane and then dialysis membrane was introduced into vial with 10 ml PBS in a shaking incubator at 37°C and 1 ml aliquot was taken and replaced with fresh PBS at specific time points. The concentration of the released CZ was determined by a UV spectrophotometer (Shimadzu, the model of UV-1,201) at 306 nm and expressed by the total release amount of CZ (w/w %).

RESULTS AND DISCUSSION

LE diblock copolymers were prepared by ring-opening polymerization of LL-NCA initiated with Me-PEO-NH₂ in a methylene dichloride solution as scheme shown in Fig 1. It is assumed that the polymerization