International Journal of Pharmaceutics 386 (2010) 268–274

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Self-assembled drug delivery systems. Part 5: Self-assemblies of a bolaamphiphilic prodrug containing dual zidovudine

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ARTICLE INFO

Article history:
Received 22 August 2009
Received in revised form
17 November 2009
Accepted 18 November 2009
Available online 24 November 2009

Keywords:
Anti-HIV
Bolaamphiphilic
Macrophage
Nanotechnology
Prodrug
Molecular self-assembly
Zidovudine

ABSTRACT

A bolaamphiphilic prodrug containing dual zidovudine, pentadecanedioyl dizidovudine (PDDZ), was prepared. The vesicular self-assemblies were formed in aqueous media through injecting the methanol solution of PDDZ into water. Hydrophobic interaction between lipid chains should drive molecular self-assembly. The nonionic surfactant, Tween 20, was used to increase the physical stability of self-assemblies because the surfactant micelles could prevent the assemblies from aggregating. The doping hydroxylpropylmethylcellulose (HPMC) slowed down the degradation of prodrugs due to adsorption. The self-assemblies were nanoscale with the mean particle size of 156 nm. Degradation of PDDZ was very slow in buffered solutions, but very rapid in enzyme and plasma, and the parent drug zidovudine (AZT) was the unique product. PDDZ self-assemblies showed strong anti-HIV activity on MT4 cell model. The 50% effective concentration (EC_{50}) of PDDZ was 5 nM, equal to that of AZT. PDDZ was rapidly eliminated from circulation and mainly distributed into liver, spleen and testis followed by the rapid production of AZT after intravenous administration of the self-assemblies to rabbits. Macrophages in liver, spleen and testis are the reservoir of HIV so that the macrophage targeting effect of PDDZ self-assemblies would benefit to anti-HIV therapy. The self-assemblies composed of bolaamphiphilic PDDZ are a promising self-assembled drug delivery system (SADDS).

1. Introduction

Self-assembled drug delivery systems (SADDSs) are defined as the self-assemblies of amphiphilic prodrugs (Jin, 2008; Jin et al., 2006). In the previous researches, we designed and prepared several amphiphilic prodrugs of nucleoside antivirals and the anti-tuberculosis agent isoniazid (Jin et al., 2008b,c, 2005, 2009b). Most of the amphiphilic prodrug molecules can associate into nanoscale self-assemblies of various morphologies through injecting their solution in organic solvents into water. The self-assemblies have macrophage targeting effect followed by the controlled *in situ* degradation of prodrugs after intravenously (i.v.) administration (Jin et al., 2009a,b, 2006). Some SADDSs show the antiviral activity on the *in vitro* cell model depending on the degradation rates of prodrugs. An optimal SADDS may be prepared based on two important standards: stable self-assemblies and suitable degradation of prodrugs. Therefore, the rational design of amphiphilic prodrugs is the key of SADDS research (Jin et al., 2009a). Nevertheless, compared with traditional drug carriers, SADDS have the unique advantages of high drug loads, no drug leakage and controlled drug release at targets.

Bolaamphiphilic molecules or bolaamphiphiles are one special type of amphiphilic molecules. In the key review about bolaamphiphiles written by Fuhrhop and Wang (2004), bolaamphiphiles are defined as the molecules containing a hydrophobic skeleton (e.g., one, two, or three alkyl chains, a steroid, or a porphyrin) and two water-soluble groups on both ends. The unique structure of bolaamphiphiles makes them to own some special functions different from traditional mono-headed amphiphiles. For example, bolaamphiphiles are inclined to form monolayers but not bilayers unless the hydrophobic skeleton is very soft and long, and the monolayer may be very strong even under harsh environments. Archaebacteria, one type of ancient bacteria with bolaamphiphilic monolayer membranes, can survive in the volcanic environment, enduring 80 °C high temperature and hot sulfuric acid (Li et al., 2009). Synthetic bolaamphiphiles may become the components of interesting structures, e.g., nanowells, ion pores, electron conductors, DNA-like nanofibers, archaebacteria-mimic membranes, nanotubes (Ambrosi et al., 2006; Fuhrhop and Wang, 2004; Iwaura et al., 2003, 2002; Kim and Thompson, 1992). Some bolaamphiphiles are also used to form carriers for delivery of gene or drugs (Fabio et al., 2003; Muzzalupo et al., 2005; Weissig and Torchilin, 2001).

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doi:10.1016/j.ijpharm.2009.11.018

A novel SADDS composed of bolaamphiphilic prodrugs was designed in this study. A long alkyl chain is the hydrophobic skeleton of bolaamphiphilic prodrug, and double zidovudine molecules are the two heads. The bolaamphiphilic prodrug can form stable self-assemblies in aqueous media with the help of stabilizers. *In vitro* and *in vivo* behaviors of the self-assemblies were explored.

2. Materials and methods

2.1. Materials

Zidovudine (AZT) was from Zhang Jiang Desano Science and Technology Co. Ltd., Shanghai, China. 1,15-Pentadecanedioic acid (purity 95.4%) was supplied by Zibo Guangtong Chemical Co., Ltd. Tween 20 (polyoxyethylene 20 sorbitan monolaurate, the mean molecular weight of 1228) was the product of Guangdong Tianma Fine Chemical Factory. Hydroxypropylmethylcellulose (HPMC, the type of HY-30T with the mean molecular weight of 30,000) was from International Specialty Products Inc. (ISP, USA). Organic solvents were of analytical grade. Other chemicals were of reagent grade. Distilled water was always used otherwise specially indicated. UV spectra, ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded respectively on a Shimadzu UV-2501PC spectrophotometer, a JNM-ECA-400 NMR spectrometer. IR spectra were recorded on a Bio-RAD FTS-65A IR spectrophotometer. ESI-MS was recorded on a Thermo LCQ Advantage mass spectrometer.

Pig liver carboxylester enzyme (PLCE, Sigma) was dissolved in the sterilized Tris–HCl buffered solutions (20 mM, pH 7.4) before use. MT4 cells and human immunodeficiency virus type-1 (HIV-1_{IIIB}) virus were from the Center of AIDS, Beijing Institute of Microbiology and Epidemiology. Plasma from Sprague–Dawley rats and rabbits was prepared in our lab. Plasma from beagle dogs and healthy human was donated by Prof. G. Dou of Beijing Institute of Transfusion Medicine.

Albino rabbits from Laboratory Animal Center of Beijing Institute of Radiation Medicine (BIRM) were used. Principles in good laboratory animal care were followed and animal experimentation was in compliance with the Guidelines for the Care and Use of Laboratory Animals in BIRM. Rabbits were sacrificed by euthanasia to remove tissues. Rabbit tissue homogenates used in the experiments of chemical stability and tissue distribution were prepared in tissue/water (1:1, w/w).

2.2. Synthesis of pentadecanedioyl dizidovudine

Pentadecanedioyl dizidovudine (PDDZ, $C_{35}H_{50}N_{10}O_{10}$, Fig. 1) was synthesized by the following procedure. Pentadecanedioic acid (10 mmol) was dissolved in thionyl chloride (50 ml), refluxed for 3 h, and then dried by removing all solvents under vacuum. A brown and viscous gel of pentadecanedioyl dichloride as active intermediate was obtained. The intermediate was dissolved in methylene dichloride (6 ml), and then dropped into incubated-in-ice AZT solution in methylene dichloride/pyridine (6:11, v/v). Thirty minutes later, the reaction temperature was gradually increased to room temperature, and stopped when the residual AZT did not disappear any more. The reaction mixture was transferred to −20 °C freezer, maintained for more than 2 h followed by rapid filtration to remove pyridine chloride. The filtrate was washed with 0.1 M citric acid solution, $NaHCO_3$ solution and pure water in turn, and then dried with anhydrous Na_2SO_4. After removal of solvents, a viscous and slightly yellow gel was obtained. After purification on a silica gel column (petroleum ether/acetone, 2:1, v/v), PDDZ was obtained as gel. It should be stored in freezer. TLC: chloroform/methanol, 9:1, v/v, R_f = 0.70; UV (methanol): λ_{max} = 266 nm; δ_H (400 MHz, $CDCl_3$): 1.23–1.28 (22H, $(CH_2)_{11}$), 1.64 (4H, m, 2CH_2CH_2CO), 1.94 (6H, s, 5-CH_3), 2.37 (4H, m, 2CH_2CO), 2.48 (4H, m, double 2′-CH_2), 4.09 (2H, m, double 4′-CH),4.36 (4H, m, 2$COOCH_2$), 6.10 (2H, t, double 1′-CH), 7.25 (2H, s, double 6-CH), 9.02 (2H, s, double NH); δ_C (100 MHz, $CDCl_3$): 12.67 (CH_3), 24.82 (CH_2CH_2CO), 29.06–31.12 ($(CH2)_{11}$), 34.12 (CH_2CO), 37.68 (2′-C), 63.08 ($COOCH_2$), 77.21 (3′-C), 81.92 (1′-C), 111.17 (5-C), 135.40 (6-C), 149.96 (2-C), 163.59 (4-C), 173.16 (CO); IR (KBr): ν_{max} = 1688.9, 2104.3, 3039.8, 3189.0 cm^{-1}; ESI-MS(+): 793.47 $(M+Na)^+$ (100).

2.3. Langmuir monolayers

Surface pressure–molecular area (π–A) isotherms of PDDZ were measured using a Minitrough 2 film balance (KSV, Finland) equipped with the dual barriers and a Pt Wilhelmy plate sensing device. The area of Teflon trough was 24,300 mm^2. The subphase was the purified water of 18 MΩ cm produced with a Heal Force® Super NW Water System (Shanghai Canrex Analytic Instrument Co. Ltd., China). Experiments were performed at 25 °C. PDDZ solution in chloroform (25 μl) was deposited onto the water subphase with a Hamilton microsyringe precisely. Compression was initiated after a delay of 15 min to allow evaporation of the spreading solvent. The compression rate was 10 mm/min.

2.4. Preparation of the self-assemblies

PDDZ self-assemblies were prepared using the methanol injection method. The injection procedure was described in our previous papers (Jin et al., 2006, 2008c, 2009b). When PDDZ was used as the unique injected component, the formed self-assemblies only maintained stable within several hours and then precipitation happened due to aggregation of the self-assembled particles. It was found that some stabilizers could increase the stability of self-assemblies. The nonionic surfactant Tween 20 and the hydrophilic cellulose derivative HPMC HY-30T enhanced the stability of self-assemblies better than others. Therefore, we designed Formulation 1 containing Tween 20 and Formulation 2 containing Tween 20 and HPMC. The preparation procedures of them are described as follows. The methanol solution containing PDDZ (5 mg/ml) was injected into vortexed water by a microsyringe. After injecting 0.5 ml of PDDZ solution, 0.1 ml of 10 mg/ml Tween 20 aqueous solution was injected. A homogeneous and slightly blue-scattering transparent suspension was obtained as Formulation 1. Formulation 2 was obtained after 0.1 ml of 2 mg/ml HPMC was injected based on Formulation 1. After removing solvents and partial water from Formulation 1 or 2 under vacuum, the self-assembly suspen-

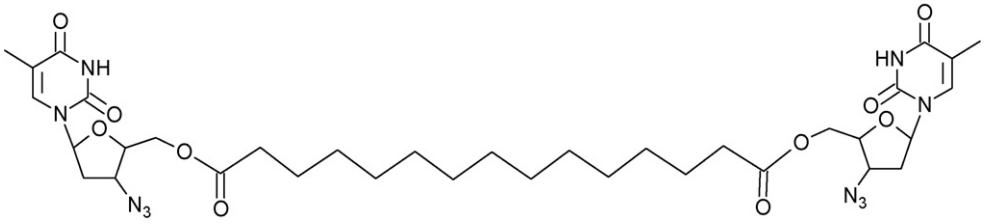

Fig. 1. Structure of pentadecanedioyl dizidovudine (PDDZ).

sion could be concentrated to a high concentration of 30 mg/ml (~40 mM) PDDZ, and keep stable for more than three months. The effect of sterilization on stability of Formulation 2 was further investigated, involving the use of 100 °C bath for 30 min, autoclave, and γ-ray irradiation of 1.5×10^4 Gy using a ^{60}Co source (BIRM, China). PDDZ was determined using HPLC after sterilization.

2.5. Characterization of the self-assemblies

PDDZ self-assemblies were observed on a Philips CM120 80-kV transmission electron microscope (TEM) and JEM-1230 (JEOL, Japan) using the previous negatively stained method for the self-assemblies of amphiphilic prodrugs (Jin et al., 2009b). A laser light scattering instrument (Nanophox Particle Size Analysis, Sympatec, Germany) was used to analyze the particle size of self-assemblies at 25 °C. Before analysis, the suspensions were 1:10 diluted with water. The zeta potential of PDDZ self-assemblies was measured on Zetasizer 2000 (Malvern, UK) at 25 °C. The suspension was filtrated through 0.45-μm membranes, and then diluted for 100-folds with water.

2.6. HPLC determination

HPLC experiments were performed on a Shimadzu 10Avp HPLC system (Japan), consisting of LC-10Avp pump, SPD-10Avp UV detector, SCL-10Avp controller, and Shimadzu CLASS-VP 6.12 chromatographic workstation software at room temperature. The Diamonsil C18-ODS HPLC columns (5 μm, 250 mm × 4.6 mm) and the EasyGuard C18-ODS HPLC guard columns (5 μm, 8 mm × 4 mm) were purchased from Dikma Co., Ltd. (China). A manual injection valve and a 20-μl loop (7725i, Rheodyne, USA) were used. UV detector was fixed at 266 nm.

When the simple conditions were involved in degradation experiments such as aqueous media and buffered solutions, PDDZ and the degradation product AZT were simultaneously determined using the mobile phase of methanol/water (95/10, v/v) at 1.0 ml/min. The retention times (t_R) of PDDZ and AZT were 7.7 min and 3.1 min, respectively. When the solution of PLCE was applied in the degradation experiment, the same mobile phase for HPLC determination was used as above and the flow rate was altered to 0.8 ml/min. When other biological samples including plasma and tissue homogenates were used, the chromatographic peak of AZT was modified to a long t_R point due the interference of proteins. Two types of mobile phases were used for respectively determining PDDZ and AZT. The mobile phase for PDDZ determination was the same as above at 1.0 ml/min. The mobile phases for AZT determination in plasma and tissue homogenates were methanol/water (40/60, v/v) at 0.7 ml/min and methanol/water (30/70, v/v) at 1.0 ml/min, respectively.

2.7. Chemical stability of PDDZ

Chemical stability of PDDZ self-assemblies in buffered solutions was measured as follows. The aliquots of 400 μl Formulation 1 were diluted with 1.2 ml of the solutions at different pH values, involving 0.01 M HCl solutions (pH 2.0), 20 mM HAc/NaAc buffers (pH 5.0), 20 mM NaH_2PO_4/Na_2HPO_4 buffers (pH 7.4), 20 mM Tris–HCl buffers (pH 9.0), and 20 mM Na_2HPO_4/NaOH buffers (pH 12.0). The dilutions were incubated in a 37 °C bath. At predetermined time intervals, two replicates of 10 μl aliquots were removed, dissolved with 90 μl methanol, and mixed thoroughly. PDDZ and AZT were determined with HPLC as described above.

Stability experiments of PDDZ self-assemblies in the enzyme (PLCE) solution, plasma and rabbit tissue homogenates were performed like the above with a little modification. Briefly, the aliquots of 200 μl Formulation 1 or 2 were mixed with 400 μl PLCE solutions and other biological media. The samples were 1:10 diluted and deproteinized with methanol, and centrifuged at 5000 × *g* for 5 min, and then the supernatants were determined by HPLC as described above.

2.8. Anti-HIV effect on cell model

Anti-HIV effect of PDDZ self-assemblies in Formulation 2 was performed according to our previous research (Jin et al., 2009b). HIV-1_{IIIB} infected MT4 cells were used as model. PDDZ self-assemblies and AZT aqueous solutions were sterilized by film filtration with 0.22 μm filters. Drug samples were 10-fold diluted with the cultural media excluding serum. The concentrations of PDDZ in cell cultures were from 10 μM to 0.1 nM, in six levels with a 10-fold decreasing gradient and in triplicates every level. AZT aqueous solutions with the same concentration range and gradient as PDDZ were also investigated as control. After 72 h of incubation at 37 °C, the cytopathic effect (CPE) assay was performed with a light microscope and the 50% effective concentration (EC_{50}) was deduced.

2.9. Pharmacokinetics and tissue distribution in animals

Pharmacokinetics and tissue distribution of PDDZ self-assemblies were studied after bolus i.v. administration of Formulation 2 to rabbits. The suspensions containing 20 mg/ml PDDZ were sterilized by 0.22 μm filters, and then injected into rabbits through ear vein. A dose of 30 mg/kg PDDZ was applied to rabbits. About 0.5 ml of rabbit blood sample was collected from the ear veins, and then put into heparinized centrifuge tubes at 1, 2, 3, 5, 8, 10, 15, 20, 30, 40, 50, 60 min. Plasma was isolated by centrifugation at 3000 rpm for 10 min, and then plasma of 50 μl was mixed with 100 μl methanol followed by vortex, centrifuged at 5000 × *g* for 5 min and HPLC determination. In the tissue distribution experiments, rabbits were sacrificed after 15, 30, 60 min of administration, and the tissues were removed, weighted and disrupted to homogenates followed by the same measurement procedure as plasma samples from the mixing process.

3. Results and discussion

3.1. Langmuir monolayer of PDDZ

Langmuir monolayer can be used to reveal the two-dimensional behavior of amphiphiles in nanoscale level. Bolaamphiphiles show the special behavior at the air/water interface different from mono-headed amphiphiles due to the unique structure (Meister and Blume, 2007). In this study, PDDZ as a symmetric bolaamphiphile showed an interesting compression behavior at the air/water interface (Fig. 2). When a little amount of PDDZ molecules were spread, i.e. PDDZ concentration of spreading solution was low (1 mM), the π–A isotherm initially showed a rapid lift followed by a maximal point or an inflexion appearing at 7 mN/m (Fig. 2A). This special abrupt change should be related to the unique structure of PDDZ. After the inflexion, a slowly ascending curve of a horseshoe conformation appeared. If a mono-headed amphiphile is applied, the horseshoe-like conformation of isotherm means the partially squeezing out of molecules from the monolayers (Jin et al., 2008b). After the plateau stage, the curve went into another rapidly ascending phase. However, when a lot of PDDZ molecules were spread, i.e. PDDZ concentration in the spreading solution was up to 2 mM, the isotherm showed a different profile from the above (Fig. 2B). At this time, the behavior of PDDZ monolayer was similar to traditional amphiphiles. According to the isotherm conformation, PDDZ could own a moderately soft lipid chain improving the bolaamphiphiles

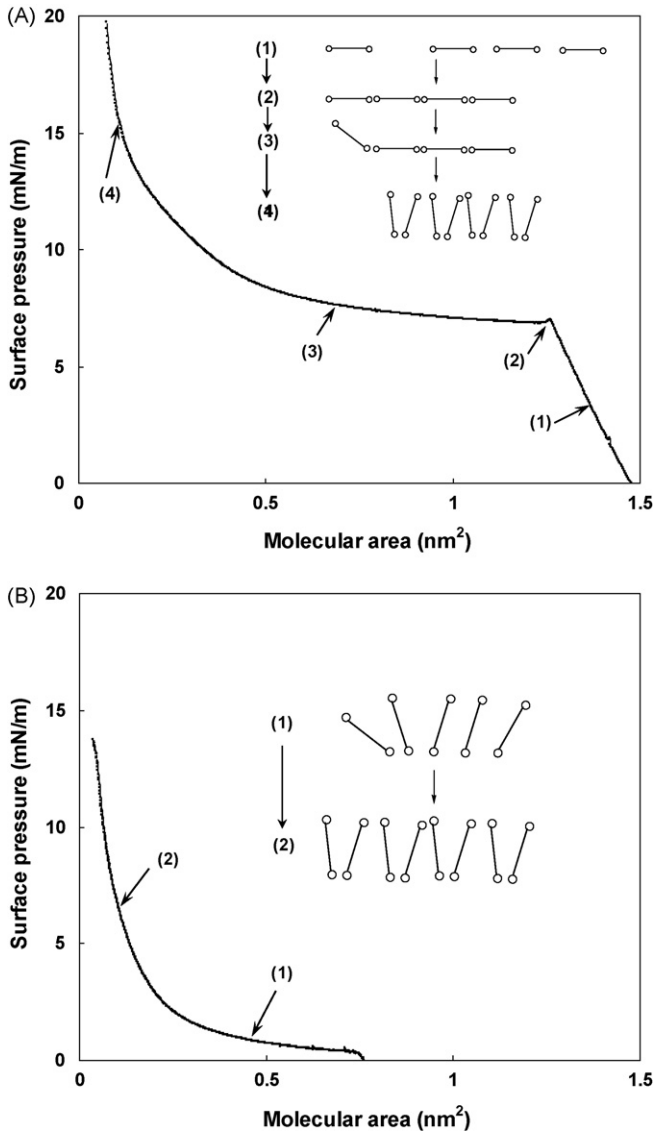

Fig. 2. Langmuir monolayer behavior of PDDZ at the air/water interface. PDDZ concentration in spreading solutions was 1 mM (Graph A), and 2 mM (Graph B).

to form stable monolayers and self-assemblies in aqueous media (Jin et al., 2008a,b, 2005).

The mechanism of PDDZ monolayer behavior is analyzed based on the unique molecular structure. The mimicking illustrations are inserted in Fig. 2 along with the isotherms. If a little PDDZ is used for spreading as shown in Fig. 2A, the molecules should horizontally lie on the surface due to the dual heads. As compression proceeding, the bolaamphiphiles could directly interact with each other by a head-to-foot mode so that the surface pressure quickly increases. Continually, when all of PDDZ molecules at the interface contact each other, there is no distance to be compressed. Some bolaamphiphiles could be forced to stand up or tilt with one head on the surface, like squeezing out (Jin et al., 2008b). Therefore, an abrupt short descending phase appears in the curve. After almost all the molecules stand up, the surface pressure rapidly increases again. Additionally, a method could be used to demonstrate the horizontally lying state of PDDZ molecules at the interface at the inflexion. The molecular area at the inflexion is 1.25 nm^2 based on the experiment. PDDZ molecule is three-dimensionally optimized by the software ChemSketch and mimicked as a rectangle with the width of 0.55 nm and the length of 2.7 nm (Advanced Chemistry Development Inc., 2006), and the theoretical molecular area of long side is 1.485 nm^2, close to the above experimental value. If enough PDDZ molecules have been spread as shown in Fig. 2B, the initial state of molecules is to tilt on the surface so that the behavior is ordinary. Some of other bolaamphiphiles also show the similar monolayer behavior as PDDZ (Gao and Liu, 2006).

3.2. Preparation of PDDZ self-assemblies

PDDZ self-assemblies are nanoscale and spherical vesicles based on the TEM graphs (Fig. 3). The driving force of self-assembly is regarded as the hydrophobic interaction between the long lipid chains of PDDZ, and the formed monolayers may further bend into closed vesicles (Fig. 4). Methanol is selected as the solvent in the injection method, but not the other solvents especially the generally used THF in the previous researches, although PDDZ is very soluble in THF and soluble in methanol. The lipid derivatives of nucleosides are very soluble in THF because hydrogen bonding is formed between the derivatives (Cook et al., 2007; Jin et al., 2008c). If THF was used as solvent and PDDZ solutions were injected into water, hydrophobic interaction could not immediately be constructed between the lipid chains of PDDZ due to the strong hydrogen bonding between the heads of PDDZ. However, methanol has hydrogen bonding interaction with AZT moieties of PDDZ. When the PDDZ solution in methanol contacts water, water

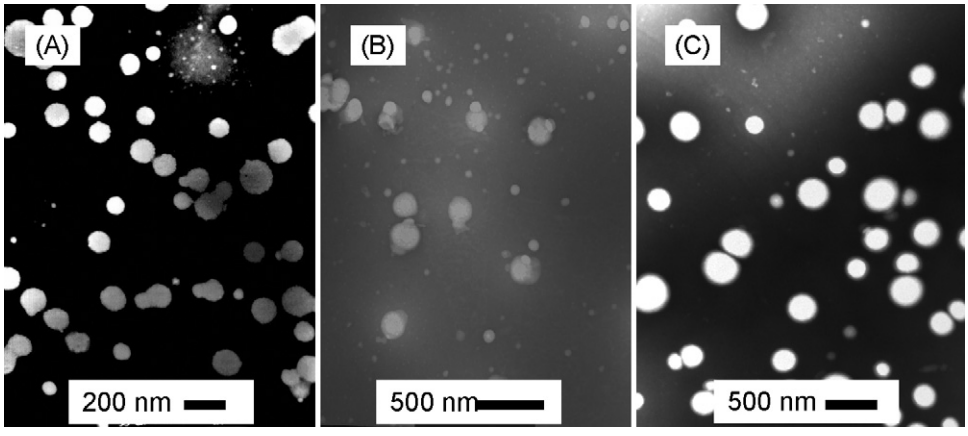

Fig. 3. TEM graphs of PDDZ self-assemblies. Graphs A–C are from the suspension without additives, Formulation 1 containing Tween 20, and Formulation 2 containing Tween 20/HPMC, respectively.

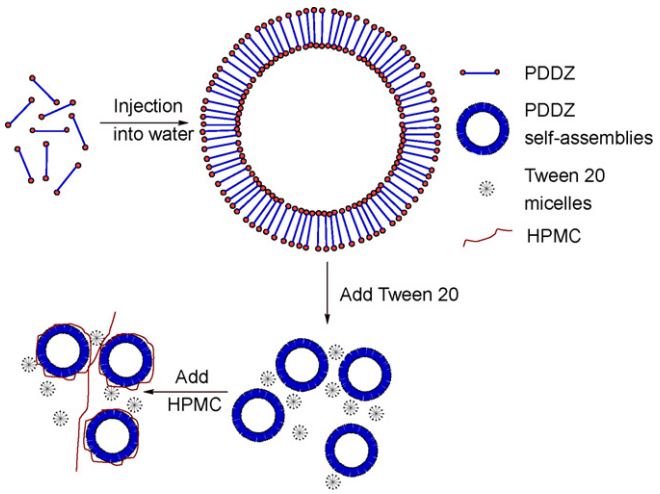

Fig. 4. Illustration of PDDZ self-assembly.

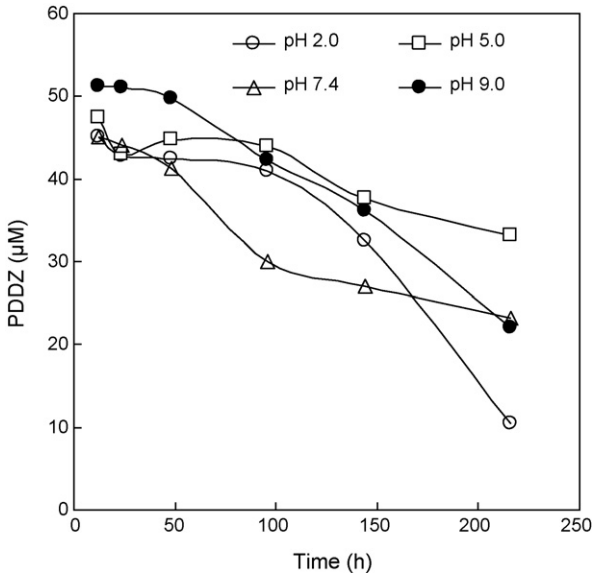

Fig. 5. Stability of PDDZ in Formulation 1 in buffered solutions at 37 °C ($n = 3$).

molecules would replace methanol rapidly, and the hydrophobic interaction between PDDZ molecules would immediately been formed. Moreover, PDDZ self-assemblies are still instable and easily aggregated mainly because of the low surface charge (see Section 3.4). Stabilizers are needed to ensure the stability.

Tween 20 is a hydrophilic nonionic surfactant, the critical micelle concentration (CMC) of which is 6×10^{-5} M in water (Krielgaard et al., 1998). In this study, the concentration of additional Tween 20 after removal of organic solvents was about 0.5 mg/ml, i.e. 4×10^{-4} M, exceeding the CMC, so that micelles were present in Formulation 1 to prevent the self-assemblies from aggregating (Fig. 4). If the concentration of Tween 20 in suspensions is lower than the CMC, the self-assemblies are also relatively instable. Additionally, some of Tween 20 molecules could insert into the vesicles although the morphology of assemblies has no change (Lasch, 1995). Furthermore, HPMC in Formulation 2 can prevent the rapid degradation of prodrugs (see Section 3.3.2).

3.3. Degradation of PDDZ

3.3.1. Degradation in buffered and enzyme solutions

The degradation of PDDZ in Formulation 1 was rapid in the pH 12.0 buffered solution, and almost all PDDZ was degraded within 20 min under 37 °C. However, the degradation in the pH range from 2.0 to 9.0 was slow (Fig. 5). Based on the pseudo-first degradation kinetics, the degradation half-life ($t_{1/2}$) of PDDZ was 108, 433, 198, and 173 h at pH 2.0, 5.0, 7.4, and 9.0, respectively. However, PDDZ in Formulation 1 was rapidly degraded by carboxylesterase (PLCE) under 37 °C, even faster than in pH 12.0 solutions. At room temperature, the prodrug was completely degraded to AZT by enzyme within 1.5 h.

3.3.2. Degradation in plasma and screening of formulations

PDDZ in Formulation 1 was degraded to AZT in rat or rabbit plasma very rapidly, even though in diluted plasma. PDDZ was completely degraded within 20 min in 20% rat plasma though slower in 20% rabbit plasma. Tween 20 micelles can prevent the self-assemblies from aggregating, but not change the activity of enzyme (Savelli et al., 2000). The self-assemblies would be i.v. administered to animals in the *in vivo* study, and the rapid degradation of prodrug in plasma could not benefit to tissue targeting. Therefore, it is necessary to find a method to improve the chemical stability of prodrugs.

Formulation 2 was designed based on Formulation 1 to achieve good chemical stability. The degradation $t_{1/2}$ of PDDZ in Formulation 2 in rabbit, dog and human plasma were 0.2, 31 and 1.9 h, respectively. The protection of HPMC molecules could result from their adsorption on the self-assemblies (Fig. 4). It is reported that HPMC adsorbs onto liposomes through hydrophobic interactions (Rubalcava et al., 2000). The adsorption could hinder the attack of enzyme, resulting in the slow degradation of PDDZ.

3.4. Characteristics of PDDZ self-assemblies

The mean particle size of PDDZ self-assemblies in Formulations 1 and 2 was 152.3 and 156.2 nm, respectively. The surface charge of self-assemblies in Formulation 2 was low to −20.7 mV of zeta potential so that the self-assemblies were instable if no stabilizers. It is known that low zeta potential (less than 30 mV) leads to particles instable due to very high surface area if no other stabilizing mechanism is present (Heurtault et al., 2003; Wang et al., 2006).

The relatively low temperature, such as 37 °C, did not influence the stability of self-assemblies. However, a high temperature of 100 °C or higher, involving boiling water incubation and autoclave, led to the aggregation and fusion of self-assemblies based on the TEM investigations although the prodrug kept stable temporarily. The high temperature could make HPMC desorption and the increasing of Tween CMC. Another sterilization method, γ-ray radiation, greatly improved the degradation of prodrugs and resulted in the production of nanotubes according to the TEM observation. Some unsymmetrical bolaamphiphilic molecules also show the morphological changes (Shimizu et al., 2005; Sirieix et al., 2000). Therefore, the film filtration was finally selected to sterilize the self-assemblies.

3.5. In vitro antiviral activity of PDDZ self-assemblies

The anti-HIV EC_{50} of PPDZ self-assemblies on MT4 cell model was 5 nM, equal to the EC_{50} of AZT in the control experiment. Therefore, PDDZ is a strong anti-HIV agent. Because only AZT has anti-HIV action, PDDZ could be rapidly degraded to AZT in the culture medium and/or MT4 cellular plasma. Generally, the degradation rate of prodrugs determines the therapeutic effect of SADDS (Jin et al., 2009a, 2006). In the previous research, the self-assemblies of cholesteryl-phosphonyl zidovudine (CPNZ) have the potent anti-HIV activity because of the production of a highly active intermediate phosphonyl zidovudine (Jin et al., 2009b). The simi-

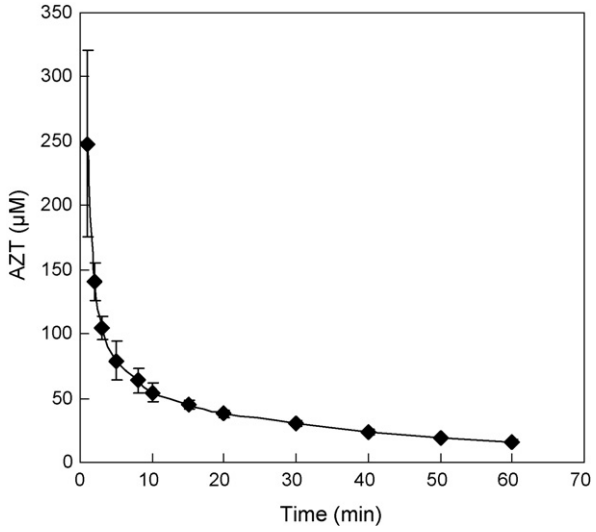

Fig. 6. Time profile of AZT concentrations in plasma after bolus i.v. administration of PDDZ self-assemblies to rabbits ($n = 3$).

lar bolaamphiphilic prodrugs will be designed to achieve a high antiviral level in future.

3.6. Pharmacokinetics and tissue distribution

It was difficult to detect PDDZ in plasma and tissue samples due to rapid degradation in spite of use of Formulation 2, so that only AZT was determined in the *in vivo* study. The produced AZT was rapidly eliminated from circulation after i.v. administration of the self-assemblies to rabbits (Fig. 6), in agreement with the pharmacokinetics of other colloidal systems due to immune response (Torchilin, 2006). Other SADDS previously prepared also showed the rapid elimination (Jin et al., 2009a,b, 2006). Based on the pharmacokinetic profile, the 0–5 min range was considered as the distribution phase with the $t_{1/2\alpha}$ of 2.3 min, and no AZT was detected in circulation after 60 min. The majority of PDDZ may rapidly distribute to targeted tissues. AZT distribution in liver, spleen, brain and testis of rabbits was explored. The relatively high concentrations of AZT was found in liver, spleen and testis though little drug was in brain (Fig. 7). The concentration of AZT in liver reached a high level of 102 μg/g after 15 min of administration, while 85 μg/g in spleen and 32 μg/g in testis.

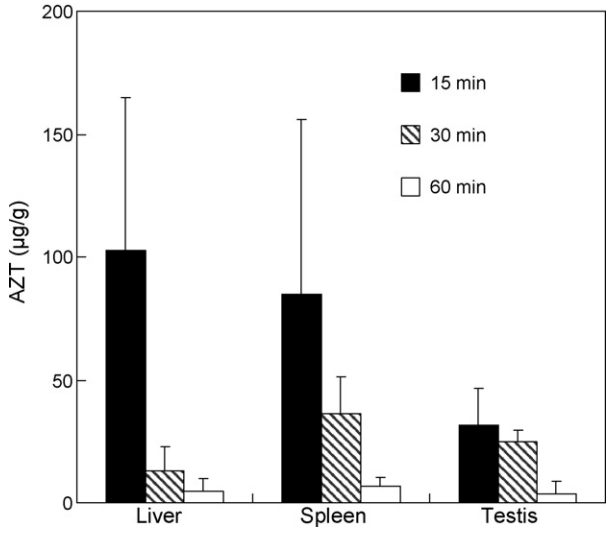

Fig. 7. Tissue distribution of AZT after bolus i.v. administration of PDDZ self-assemblies to rabbits ($n = 3$).

Macrophages in liver, spleen and testis belong to the mononuclear phagocyte system (MPS) that plays a decisive role as a reservoir for HIV (Aquaro et al., 2002). Despite treatment with highly active antiretroviral drugs and the suppression of plasma HIV-1 RNA to undetectable levels for a long time, HIV-1 transcription persists in peripheral-blood mononuclear cells (Furtado et al., 1999). Testis was reported as the reservoir of HIV, and infected cells appeared to be testicular macrophages located within the interstitial tissue (Roulet et al., 2006). In HIV-1-infected men who are receiving potent antiretroviral therapy and who have no detectable levels of viral RNA in plasma, the virus may be present in seminal cells and therefore may be capable of being transmitted sexually (Zhang et al., 1998). Anti-HIV agents-loaded carriers were prepared for targeting of the MPS, such as liposomes (Garg et al., 2006). Therefore, the macrophage targeting effect of PDDZ self-assemblies and rapid release of AZT at targets would benefit to anti-HIV therapy.

3.7. Design of bolaamphiphilic prodrug self-assemblies

Novel bolaamphiphilic prodrug self-assemblies were created in this study based on the SADDS theory. Unlike traditional mono-headed amphiphilic prodrugs, the design of bolaamphiphilic prodrugs needs to regard special factors because of the unique structure. In detail, it is important to consider the volume of drug heads and the length and flexibility of lipid chains in the design of previous amphiphilic prodrugs. But for bolaamphiphilic prodrugs, the relationship between two factors may be very complicated (Yan et al., 2007). The use of computer-aided design should be a good method. Furthermore, the meaning of bolaamphiphilic SADDS is not limited to one simple type of prodrug self-assemblies. Two different drug molecules could be conjugated together into one prodrug molecule using this design. Though the 'co-drug' concept has been well known, especially for the application of co-drug in the design of antiviral and antitumor drugs, all of them cannot be designed as bolaamphiphilic prodrugs (Cappellacci et al., 2008; Guenther and Nair, 2002; Lau et al., 2008; Lazrek et al., 2007; Romeo et al., 2008). The bolaamphiphilic prodrug self-assemblies containing two different drugs would benefit to combination therapy with the advantages of nanoscale and controlled release. We are now performing the related research.

4. Conclusion

A symmetric bolaamphiphilic prodrug containing dual zidovudine, PDDZ, was prepared in this study. The stable and concentrated vesicular self-assemblies were prepared through injecting PDDZ solution into water followed by addition of stabilizers and removal of solvents. Nanoscale size, stability, anti-HIV activity and macrophage targeting effect demonstrate that PDDZ self-assemblies are a promising SADDS. Moreover, an asymmetric bolaamphiphile containing different drug heads would benefit to combination therapy of some serious diseases such as AIDS and cancer.

Acknowledgements

This work is supported by the National Natural Science Foundation of China (No. 30672542) and the National Key Technologies R&D Program for New Drugs (No. 2009ZX09301-002). We also thank Prof. Minghua Liu of Chinese Academy of Science for the suggestive communication.

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