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Self-assembled drug delivery systems Part 3. *In vitro*/*in vivo* studies of the self-assembled nanoparticulates of cholesteryl acyl didanosine

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ABSTRACT

Self-assembled drug delivery systems (SADDS) are defined as the self-assemblies of amphiphilic prodrugs, integrating prodrugs, molecular self-assembly and nanotechnology for drug targeting and controlled release. Cholesteryl-succinyl didanosine (CSD) and cholesteryl-adipoyl didanosine (CAD) nanoparticulate systems in water were previously prepared and optimized. In this paper, the *in vitro* and *in vivo* behavior of them was investigated. Precipitation occurred when they were mixed with acid solutions due to rapid production of hypoxanthine and subsequent disruption of supramolecular structures. They showed pH-dependent degradation and kept relatively stable in the neutral pH range. CSD is more stable than CAD due to the shorter spacer and poloxamer protection. CSD showed different degradation rates in various plasma with the descending order of rat, mouse, rabbit, dog and human. The half-life ($t_{1/2}$) of CSD is 9 days in rat plasma, and 5.9 days in rat liver homogenates. CAD has a faster degradation than CSD though the $t_{1/2}$ in rat liver homogenates is long to 23 h. CSD nanoparticulates showed no significant anti-HIV effect in MT4 cell model because of very slow degradation. CSD nanoparticulates showed the distribution $t_{1/2}$ of 7.6 min after bolus intravenous (i.v.) administration to rats, and the site-specific distribution in liver, lung and spleen with the high $t_{1/2}$ of 10 days in liver. The factors affecting achievement of successful SADDS are discussed.

1. Introduction

Self-assembled drug delivery systems (SADDS) are defined as the self-assemblies of amphiphilic prodrugs, firstly developed in our lab (Jin et al., 2006). Three technologies, prodrug, molecular self-assembly and nanotechnology are integrated into SADDS. Compared to drug carriers, such as liposomes and nanoparticles, the unique advantages of SADDS include high drug loads, no drug leakage and controlled drug release in targets because the colloidal SADDS almost or wholly consist of amphiphilic prodrugs (Jin, 2008; Jin et al., 2006). In our previous research, a long chained glyceride derivative of acyclovir was prepared and self-assembled into stable rod-like nanoparticles in water (Jin et al., 2005). After intravenous (i.v.) administration of the self-assembled nanoparticles to rabbits, targeting to the mononuclear phagocyte system (MPS) and then controlled release of parent drugs were achieved (Jin et al., 2006).

Didanosine (ddI) is one first-line anti-HIV agent, approved by FDA in 1991. Its bioavailability is reported to range from 20 to 40% depending on the formulation used. Didanosine is metabolized intracellularly to the active antiviral metabolite dideoxyadenosine triphosphate. The plasma elimination half-life is reported to be about 1–2 h (Faulds and Brogden, 1992). Didanosine was modified to various lipidic derivatives to improve the bioavailability for direct lymphatic delivery against HIV (Lalanne et al., 2007a, 2007b; Manouilov et al., 1997). Peroral bioavailability of didanosine was improved with its octanoate and benzoate prodrugs (Hasegawa and Kawaguchi, 1994). The sterically stabilized liposomes of didanosine were prepared to achieve long circulating *in vivo* (Dipali et al., 1997; Harvie et al., 1996). The mannosylated gelatin nanoparticles of didanosine were delivered to the targeted organs (Jain et al., 2008).

We perform a series of researches on SADDS currently and in future. In our previous paper, a series of cholesteryl derivatives of antiviral nucleoside analogues, involving acyclovir, didanosine and zidovudine, were prepared, and after injecting their solutions into water they self-assembled to form highly dispersed aggregates (Jin et al., 2008b). Two derivatives, cholesteryl-succinyl didanosine

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(CSD) and cholesteryl-adipoyl didanosine (CAD) were further investigated in details on the self-assembly behavior in water (Jin et al., 2008a). The morphologies of CSD self-assembled nanostructures are relevant to the amount of incorporated solvent tetrahydrofuran (THF) into bilayers. After removal of THF, only the short nanotubes of average 200 nm in size were remained, also as the most stable form. The optimal formulation of CSD self-assembled nanoparticulate systems was screened out, wherein the concentration of CSD in organic solutions and the additional amount of stabilizer poloxamer 188 were the key factors. After removing solvents and part of water, a stable concentrated nanoparticulate system was achieved, containing more than 10 mg/ml CSD, looking like milk, and totally consisting of short nanotubes. The formulation is ready for i.v. administration with the small injected volume containing an appropriate dose. However, another didanosine lipid derivative, CAD, only formed the nanoscale vesicles of average 175 nm in size, without morphological changes when further processing or storage. The relative long spacer of CAD contributed to the formation of flexible tails to further keep the vesicle form. The self-assembled nanoparticulate system of CAD could also be concentrated to a high concentration of more than 10 mg/ml CAD, maintaining stable for more than 1 month partially due to the high zeta potential of −44.5 mV.

Self-assembled nanoparticulates could preferentially distribute in the MPS due to their colloidal characteristic, as shown in our previous study (Jin et al., 2006). The MPS-targeted distribution benefits to anti-HIV therapy because macrophages are the main reservoirs of HIV (Aquaro et al., 2002). In this study, the *in vitro* and *in vivo* studies of the self-assembled nanoparticulates of CSD and CAD were performed. Chemical stability of them was investigated. Anti-HIV action on cell model was detected. Site-specific distribution and sustained release were achieved after i.v. administration of CSD nanoparticulates. The key factors determining successful SADDS are discussed, and the prospect of SADDS is described at the end of the paper.

2. Materials and methods

2.1. Materials

Two cholesteryl acyl derivatives of didanosine, i.e. cholesteryl-succinyl didanosine ($C_{41}H_{60}N_4O_6$) and cholesteryl-adipoyl didanosine ($C_{43}H_{64}N_4O_6$) were prepared according to the previous research (Jin et al., 2008b). Analytical reagents were used otherwise specially indicated. Chromatographic reagents were used in high-performance liquid chromatography (HPLC). Distilled water was always used. A polyoxyethylene–polyoxypropylene copolymer, poloxamer 188 (P188) was supplied by Shenyang Jiqi Pharmaceutical Co. Ltd., China. The pig liver carboxylester enzyme (PLCE, Sigma) was dissolved in sterilized Tris–HCl buffer 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. The plasma from BALB/c mice, Sprague-Dawley rats and albino rabbits was prepared in our lab. The plasma from beagle dogs and healthy human was donated by Prof. G. Dou of Beijing Institute of Transfusion Medicine.

Sprague-Dawley rats 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. The rats were sacrificed by euthanasia to remove tissues. The rat tissue homogenates used in the experiments of chemical stability and tissue distribution were prepared in tissue/water (1:1, w/w).

2.2. Preparation of nanoparticulates

The self-assembled nanoparticulates of CSD and CAD were prepared according to our previous researches (Jin et al., 2008a, 2008b). In brief, a CAD solution (5 mg/ml) in THF was slowly injected into the vortexed water containing 5% (v/v) THF by a microsyringe. The injecting processes were repeated for several times until to obtain a homogeneous and slightly opalescent suspension. When preparing CSD self-assembled nanoparticulates, the injection solution containing 5 mg/ml CSD and 1 mg/ml P188 in the THF/ethanol (4:1, v/v) mixture solvent was injected as above. We usually mixed CSD stock solution in THF and P188 stock solution in ethanol before injection. After removing solvents and partial water, a stable concentrated nanoparticulate system was obtained, containing more than 10 mg/ml CSD or CAD. Both kinds of nanoparticulate systems can keep stable for more than 1 month at room temperature.

2.3. HPLC determination of didanosine and its derivatives

HPLC experiments were performed on a Shimadzu 10Avp HPLC system (Japan) at room temperature, consisting of LC-10Avp pump, SPD-10Avp UV detector, SCL-10Avp controller, and Shimadzu CLASS-VP 6.02 chromatographic workstation software. Diamonsil™ C18-ODS HPLC columns (5 μm, 250 mm × 4.6 mm) and EasyGuard™ C18-ODS HPLC guard columns (5 μm, 8 mm × 4 mm) were purchased from Dikma (China). A manual injection valve and a 20-μl loop (7725i, Rheodyne, USA) were used. UV detector was fixed at 249 nm.

Didanosine and its cholesteryl derivatives were separately determined with different mobile phases due to their significant differences of polarity, and the samples from different resources, including as the primitive nanoparticulate systems and the samples in various environments for the stability measurement, were also determined using different mobile phases. The details of HPLC are referred to in Table 1.

2.4. Degradation of didanosine derivatives

2.4.1. Degradation in buffer solutions

Aliquots of 800 μl of CSD or CAD self-assembled nanoparticulate systems, were diluted with 2.4 ml of various buffers including 20 mM phosphate buffers (pH 5.0 and 7.4) and 20 mM Tris–HCl buffers (pH 9.0 and 12.0), and the dilutions were incubated in a 37 °C bath. At predetermined time intervals, aliquots of 20 μl were removed, dissolved with 180 μl methanol, and assayed with HPLC. The chemical stability of derivatives in acid solutions (pH 1.0 and 2.0, HCl solutions) was also detected as above.

2.4.2. Degradation in enzyme solutions, plasma and tissue homogenates

The effects of PLCE solution, animal and human plasma, and rat tissue homogenates on the chemical stability of derivatives at 37 °C were investigated as above. CSD or CAD self-assembled nanoparticulate systems of 200 μl were mixed with the enzyme solutions (20 U/ml) of 800 μl, and 300 μl of nanoparticulate systems were mixed with 600 μl of plasma or tissue homogenates. For the determination of CSD, aliquots of 20 μl were withdrawn and deproteinized with isopropanol of 80 μl, followed by vortex for 2 min and centrifugation at 5000 × *g* for 10 min. The deproteinizing reagent for CAD determination was methanol with the same dilution fold as above. The extracting reagent of didanosine was methanol/5% $ZnSO_4$ (1:3, v/v) followed by centrifugation as above. The supernatants were determined with HPLC.