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Self-assembled drug delivery systems 2. Cholesteryl derivatives of antiviral nucleoside analogues: Synthesis, properties and the vesicle formation

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Abstract

Self-assembled drug delivery systems (SADDS) are defined as the self-aggregates of amphiphilic prodrugs. Prodrug, molecular self-assembly and nanotechnology are involved in SADDS manufacturing. But the knowledge of the self-assembly of amphiphilic prodrugs and the formation rules of SADDS is very limited. In this paper, five cholesteryl derivatives of antiviral nucleoside analogues were synthesized, involving antiviral acyclovir, didanosine and zidovudine, and the different acyl linkers, succinyl, adipoyl and phosphoryl. The derivatives are typical amphiphiles with nucleosides as polar heads and long-chained lipids as hydrophobic tails. The derivatives showed the similar soluble behavior, and the solubility highly depended on the types of solvents. Two forces, hydrogen bonding and hydrophobic interaction in alcohol solutions could improve the derivatives dissolving. However, the molecular self-assembly of derivatives could prefer to happen in the noncompetitive solvents including chloroform and tetrahydrofuran (THF) based on the intermolecular hydrogen bonding between nucleobase moieties, which could greatly increase their solubility. The derivatives formed nanosized vesicles based on hydrophobic interaction after injecting their THF solutions into water. The volume ratios of polar heads and hydrophobic tails of amphiphiles could determine the vesicle size, and the amphiphiles with large ratios would prefer to form small vesicles. The self-assembled vesicles would likely become SADDS.

Keywords: Antiviral; Cholesteryl; Molecular self-assembly; Nucleosides; Prodrugs; Vesicles

1. Introduction

In our previous researches, the long-chained lipid derivatives of acyclovir were prepared and one of them (the glyceride derivative) formed self-assembled nanoparticles (SAN) in water due to its amphiphilic property (Jin et al., 2005). After i.v. administration to rabbits, the SAN showed the significant mononuclear phagocyte system (MPS, mainly including liver, lung and spleen) specific distribution and drug sustained-release effect in the targeted sites (Jin et al., 2006a). The novel drug delivery systems have many unique properties different from traditional drug carriers such as liposomes and nanoparticles, which are defined as self-assembled drug delivery systems (SADDS). The combination of prodrug, molecular self-assembly and nanotechnology in SADDS could take them many unique advantages over traditional carriers. They can deliver themselves in vivo, and show high drug loading, very low drug leakage in preservation and circulation, drug targeting and controlled-release.

The knowledge about SADDS is very limited up to now although the preliminary researches have been done (Jin et al., 2005, 2006a,b; Jin, 2007). It was known that a polar drug head such as acyclovir and a long-chained glyceride-type lipid other than double-chained lipids were necessary to prepare an appropriate amphiphilic prodrug that would then

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form nanosized self-aggregates. The physicochemical properties including molecular self-assembly of amphiphilic prodrugs must be known before manufacturing SADDS, and the obtained enough information can help to prepare ideal SADDS. In the previous case of the glyceride derivative of acyclovir, the bilayers were first formed based on the hydrophobic interaction between lipid chains, and then overlapped layer-by-layer to form cuboid-shaped nanoparticles in water (Jin et al., 2005). However, a lot of researches on different drugs, lipids and their various combinations are necessary to obtain the deep and extensive knowledge of SADDS.

Nucleoside analogues could become important active agents such as antivirals (Simons et al., 2005), anticancer agents (Lech-Maranda et al., 2006) and oligonucleotide antisense agents (Stahel and Zangemeister-Wittke, 2003). Due to a relatively strongly polar group (nucleobase) and one or more reactive groups (hydroxyl or amino) in molecules, nucleoside analogues are appropriate model drugs to study their amphiphilic prodrugs and SADDS. And after the research, both the general self-assembling rules and the potential pharmacotherapeutic agents would be obtained. Five cholesteryl derivatives of antiviral nucleoside analogues containing representative nucleobase groups were synthesized and investigated on their physicochemical properties including solubility and molecular self-assembly in the paper. The obtained useful information benefits to well understand the intermolecular interaction and molecular self-assembly of amphiphilic antiviral nucleosides, and prepare optimal SADDS in future.

2. Materials and methods

2.1. Materials

Acyclovir (ACV) was purchased from Zhejiang Jiayuan Pharmaceutical Co. Ltd., China. Zidovudine (AZT) and didanosine (ddI) were from Zhang Jiang Desano Science and Technology Co. Ltd., Shanghai, China. Organic solvents were of analytical grade. Other chemicals were of reagent grade. Water was distilled. 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, and FAB-MS and ESI-MS were respectively recorded on a Micromass ZabSpec high-resolution mass spectrometer and a Thermo LCQ Advantage mass spectrometer.

2.2. Synthesis of the cholesteryl derivatives of antiviral nucleoside analogues

2.2.1. Cholesteryl-succinyl acyclovir (1, CSA, $C_{39}H_{59}N_5O_6$)

Succinyl acyclovir (SACV) was synthesized according to the literature (Colla et al., 1983). SACV (1 eq.) and cholesterol (3 eq.) were dissolved in *N*,*N*-dimethyl formamide/tetrahydrofuran (DMF/THF, 1:1, v/v), and 4-dimethylaminopyridine (DMAP, 0.2 eq.) and dicyclohexylcarbodiimide (DCC, 1.5 eq.) as catalysts were added. The solution was sealed and agitated at room temperature for about 30 h. Most solvents were removed under vacuum, and the remained solution was poured into the saturated $NaHCO_3$ solution. The white suspension was filtered. The residual was dried, recrystallized from 2-propanol and isolated by silica gel column chromatography. Yield 55%; TLC: chloroform/methanol, 8.5:1.5 (v/v), $R_f = 0.65$; UV (methanol): $\lambda_{max} = 252.8$ nm; δ_H (400 MHz; $CDCl_3$) 0.66–2.06 (43H cholesteryl), 2.31, 2.62 (m, 4H, $OCCH_2CH_2CO$), 3.75 (s, 2H, OCH_2CH_2OCO), 4.00 (t, 2H, $J = 11.7$ Hz, OCH_2CH_2CO), 4.25 (m, 1H, OCH cholesteryl), 5.35 (d, 1H, $J = 5.6$ Hz, $CCHCH_2$ cholesteryl), 5.46 (s, 2H, NCH_2O), 6.72 (s, 2H, NH_2), 7.76 (s, 1H, NCHN guanine), 11.70 (s, 1H, OCNHC guanine); δ_C (100 MHz; $CDCl_3$) 11.8–27.7, 28.9–56.6 (24C cholesteryl), 28.0, 28.2 ($OCCH_2CH_2CO$), 63.4 (CH_2CH_2O), 67.1 (CH_2CH_2O), 72.7 (NCH_2O), 74.4 (OCH cholesteryl), 116.5 (C-5 guanine), 122.7 (CH cholesteryl), 137.9 (CH-8 guanine), 139.5 (C cholesteryl), 151.8 (C-6 guanine), 153.8 (C-2 guanine), 156.8 (C-4 guanine), 171.7, 172.4 ($OCCH_2CH_2CO$); FAB-MS: 695.2 ($M+H^+$).

2.2.2. Cholesteryl-succinyl didanosine (2, CSD, $C_{41}H_{60}N_4O_6$)

The synthesis of cholesteryl hemisuccinate (CHS) was referred to as the literature with a little modification (Deng et al., 2001). Cholesterol (1 eq.), succinic anhydride (3 eq.) and DMAP (0.1 eq.) were dissolved in dichloromethane (DCM), and refluxed at 55 °C for 3 days. The solvent was removed under vacuum. The residual was dissolved in ethanol and then poured into the ice solution containing 15% NaCl. The obtained suspension was adjusted to pH 2.0 by adding 1 M HCl solution. The suspension was filtered and washed to neutral by water. The dried solid residual was recrystallized from ethanol/ethyl acetate (10:1, v/v) with the CHS yield near 100%. CHS (2 eq.), ddI (1 eq.), DCC (1.2 eq.) and DMAP (1 eq.) were dissolved in DMF/THF (1:1, v/v), sealed and agitated at 50 °C for 2 days. Most solvents were removed under vacuum, and the remained solution was poured into the saturated $NaHCO_3$ ice solution. The white suspension was filtered and the residual was dried. CSD was recrystallized from methanol and isolated by silica gel column chromatography. Yield 65%; TLC: chloroform/methanol/ammonia, 85:15:5 (v/v/v), $R_f = 0.70$; UV (methanol): $\lambda_{max} = 249.6$ nm; δ_H (400 MHz; $CDCl_3$) 0.66–2.17 (43H cholesteryl), 2.31, 2.65 (m, 4H, $OCCH_2CH_2CO$), 4.36 (m, 1H, OCH cholesteryl), 5.35 (d, 1H, $J = 3.9$ Hz, $CCHCH_2$ cholesteryl), 6.29 (t, 1H, $J = 4.5$ Hz, NCH), 8.11 (s, 1H, C(8)H hypoxanthine) 8.14 (s, 1H, NC(2)HN hypoxanthine), 12.71 (s, 1H, NH); δ_C (100 MHz; $CDCl_3$) 11.8–27.7, 29.0–56.7 (24C cholesteryl), 28.0, 28.2 ($OCCH_2CH_2CO$), 65.0 (OCH_2), 79.5 (OCH cholesteryl), 85.7 (NCH), 122.7, 138.4, 139.6, 148.2, 160.0 (5C hypoxanthine), 125.3, 144.5 (2C cholesteryl), 171.6, 172.3 ($OCCH_2CH_2CO$); FAB-MS: 705.5 (M^+).

2.2.3. Cholesteryl-adipoyl didanosine (3, CAD, $C_{43}H_{64}N_4O_6$)

Cholesterol hemiadipate (CHA) was synthesized like CHS except for using DCM as solvent. The immediate product had the yield near 100%. CAD was also synthesized like CSD. Yield 60%; TLC: chloroform/methanol/ammonia,