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Hyaluronic Acid-Based Biocompatible Supramolecular Assembly for Sustained Release of Antiretroviral Drug

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ABSTRACT

Human immunodeficiency virus (HIV) infection and its associated diseases continue to increase despite the progress in our understanding of HIV biology and the availability of a number of antiretroviral drugs. Adherence is a significant factor in the success of HIV therapy and current HIV treatment regimens require a combination of antiviral drugs to be taken at least daily for the remainder of a patient's life. A drug delivery system that allows sustained drug delivery could reduce the medical burden and costs associated with medication nonadherence. Here, we describe a novel supramolecular assembly or matrix that contains an anionic polymer hyaluronic acid, cationic polymer poly-L-lysine, and anionic oligosaccharide sulfobutylether-beta-cyclodextrin. HIV reverse transcriptase inhibitors Zidovudine and Lamivudine were successfully encapsulated into the polymer assembly in a noncovalent manner. The physicochemical properties and antiviral activity of the polymer assemblies were studied. The results of this study suggest that the supramolecular assemblies loaded with HIV drugs exert potent antiviral activity and allow sustained drug release. A novel drug delivery formulation such as the one described here could facilitate our efforts to reduce the morbidity and mortality associated with HIV infections and could be utilized in the design of therapeutic approaches for other diseases.

Introduction

The global AIDS pandemic continues to expand despite significant advances in the understanding of human immunodeficiency virus (HIV-1) pathogenesis and the development of highly effective antiviral drugs. Currently, more than 36 million people are infected with HIV-1 worldwide and approximately 2 million more people are infected each year. Effective treatment of HIV-infected individuals requires strict adherence to a multicomponent regimen of antiretroviral agents that must be taken at least daily for the remainder of a patient's life. Success of the HIV treatment regimen is heavily dependent on patient adherence. This is also true for prevention strategies that utilize antiviral chemotherapy. Nonadherence can lead to emergence of drug resistance and loss of therapeutic effectiveness. Effective prevention requires that the inhibitor be present at the right time, place, duration, and concentration to stop HIV transmission and acquisition. A drug delivery system with the capability of sustained or controlled drug release will significantly reduce the medical burden and costs associated with medication nonadherence.

In recent years, the application of supramolecular assembly and nanoparticle technology for optimizing the pharmacologic and therapeutic profiles of existing drugs has gained momentum, thus contributing to enhanced stability, prolonged circulation time, specific delivery to the target tissue, and controlled release of the active pharmaceutical ingredients (APIs).[1] Supramolecular assemblies are produced from polymeric precursors, and these structures can be formulated to encapsulate a wide variety of pharmaceuticals.[2] The use of natural or biocompatible molecules such as hyaluronic acid (HA) in the design of nanoparticles or polymeric assemblies is increasing because these molecules are biodegradable and are associated with limited, if any, adverse immune responses.

Abbreviations: API, active pharmaceutical ingredient; CD, cyclodextrin; ELISA, enzyme-linked immunosorbent assay; HA, hyaluronic acid; HIV, human immunodeficiency virus; PBMC, peripheral blood mononuclear cell; PBS, phosphate buffered saline; PL, poly-l-lysine; RLU, relative light unit; SBECD, sulfobutylether-beta-cyclodextrin.

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HA, an anionic polymer of disaccharides, composed of D-glucuronic acid and D-*N*-acetylglucosamine, is one of the major components of the extracellular matrix and plays a critical role in cell proliferation and migration.[3] Proteoglycan aggregates in the extracellular matrix of connective tissues are composed of a long chain of HA as a core surrounded by proteoglycans coupled noncovalently by linker proteins.[4] HA and the proteoglycans are both highly negatively charged and provide electrostatic interactions with the basic residues in the linker protein. A recent study suggested that HA-based hydrogels can be successfully exploited as a three-dimensional (3D) artificial extracellular matrix for various tissue engineering applications.[5] Polylysines (PLs) are generally regarded as safe by the United States Food and Drug Administration (FDA) and are widely utilized as carriers in a number of applications such as foods and pharmaceuticals. Warren et al.[6] have recently demonstrated that PL can be utilized in the design of biodegradable nanogel carriers for the delivery of the nucleoside reverse transcriptase inhibitors. Cyclodextrins (CDs) form inclusion complexes with a wide range of compounds and have been used as carriers in pharmaceutical applications to increase aqueous solubility, dissolution rate, and chemical stability of drugs, as well as to enhance drug permeability through biological membranes.[7] CD polymers have also been used in forming complex multicomponent drug delivery systems such as nanoparticles and micelles, and currently two drug delivery systems based on CD polymers have entered clinical phase II trials in cancer treatment.[8] In this regard, it is important to note that exposure of HIV to beta-CD has been shown to inactivate the virus by depletion of cholesterol.[9–11]

Based on the analogy of the controlled release of histamine and heparin from proteoglycan aggregates in living tissues during inflammation and allergic reactions,[12] Szente et al.[13] have developed a supramolecular assembly that includes the anionic polymer HA as a backbone and CD complexed with a cationic surfactant as a linker. This biodegradable polymer assembly was shown to be capable of entrapping both hydrophilic and lipophilic substances and exhibited sustained drug release *in vitro*.[13]

In the studies performed herein, we report an HA-based supramolecular assembly as a sustained release system for antiretroviral drugs. To this end, we constructed a supramolecular HA/PL/CD assembly loaded with an HIV reverse transcriptase inhibitor (Zidovudine or Lamivudine). The physicochemical properties and antiviral activity of the supramolecular assemblies were examined. Our data suggest that the drug-loaded polymer assemblies exhibit potent antiviral activity and allow sustained drug release.

Materials and Methods

Materials

HIV reverse transcriptase inhibitors Zidovudine and Lamivudine were purchased from Sigma-Aldrich (St. Louis, MO). HA was obtained from Pentapharm (Basel, Switzerland; Lot No. H12990001/295-03). Sulfobutylether-beta-cyclodextrin-Na salt, USP pharmaceutical grade (Dexolve7), is a product of CycloLab (Budapest, Hungary). Poly-L-lysine was obtained from JNC Corporation (Tokyo, Japan; Lot No. 21450203). Luviquat-Mono-CP was purchased from Fluka/Sigma-Aldrich (Lot No. 4168893/1).

A variety of media that included Dulbecco's modified Eagle's medium, RPMI 1640 medium, fetal bovine serum, and penicillin-streptomycin were purchased from ThermoFisher Scientific (Waltham, MA). Phytohemagglutinin-M (PHA-M) and interleukin-2 (IL-2) were obtained from Sigma-Aldrich. The CellTiter 96® AQueous One Solution Cell Proliferation Assay Kit and Luciferase Assay System were purchased from Promega (Madison, WI).

Cells and Viruses

TZM-bl cells are HeLa-derived HIV-1 reporter cells that express the HIV-1 receptor CD4, the co-receptors CXCR4/CCR5, and the HIV-1 long terminal repeat (LTR)-driven firefly luciferase.[14] JLTRG cells are T-lymphocyte-derived HIV-1 reporter cells that express the HIV-1 receptor CD4, the co-receptors CXCR4/CCR5, and the HIV-1 LTR-driven enhanced green fluorescent protein (EGFP).[15] The TZM-bl and JLTRG cell lines were obtained from the NIH AIDS Reagent Program (USA). The T-lymphocyte-derived Jurkat cell line was obtained from the American Type Culture Collection. The TZM-bl cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal bovine serum and 100 U/mL of penicillin-streptomycin at 37°C in a 5% CO_2 humidified incubator. The JLTRG and Jurkat cells were maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum and 100 U/mL of penicillin-streptomycin at 37°C in a 5% CO_2 humidified incubator. Human peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Hypaque centrifugation from the whole blood obtained from the UCLA CFAR Virology Core Laboratory and cultured in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum and 100 U/mL of penicillin-streptomycin as described.[16]

The HIV-1_{IIIB} (CXCR4-tropic) and HIV-1_{BaL} (CCR5-tropic) stocks were purified from the Jurkat-IIIB and PM1-BaL cell lines that have been chronically infected with HIV-1_{IIIB} and HIV-1_{BaL}, respectively. The culture supernatant containing virus was filtered using a 0.45-μm filter and the cleared supernatant was subjected to centrifugation for 2 h at 100,000 × *g*. The virus pellets were resuspended in a small volume of phosphate buffered saline (PBS) to achieve a 100-fold concentration. The purified virus stock was aliquoted in small volumes and stored at −80°C. The concentration of the virus stock was determined by assaying the capsid protein p24 by enzyme-linked immunosorbent assay (ELISA) and for infectivity using the TZM-bl cell line.

Preparation of Supramolecular Assemblies

For Zidovudine/HA/PL/SBECD (sulfobutylether-beta-cyclodextrin) assembly, 8.3 g of SBECD was dissolved in 10 mL of deionized water. To this solution, 0.09 g of poly-L-lysine was added and the resulting solution was stirred at room temperature together with 1.02 g of Zidovudine. The system became slightly opalescent suspension during stirring. Finally, 0.2 g of HA was added and the reaction mixture was stirred for 4 h at room temperature resulting in a slightly opalescent gel. The gel was chilled to −70°C and water was removed by freeze-drying. This procedure resulted in a yield of 9.3 g of white amorphous powder with a Zidovudine content of 10.9% by weight. The same procedure was used for the Lamivudine/HA/PL/SBECD assembly. In other assemblies, the cationic surfactant hexadecyl(2-hydroxyethyl)dimethylammonium dihydrogen phosphate (also known as Luviquat-Mono-CP) was used instead of poly-L-lysine under the same reaction conditions.

Size-Exclusion High-Performance Liquid Chromatography

High-performance liquid chromatography (HPLC) measurements were carried out using a Hewlett-Packard 1050 System equipped with ERC-7515B refractive index detector and Hewlett-Packard 1050 variable wavelength (VW) detector. TSK-GEL G3000SW, silica gel–based column (TosoHaas) (300 × 7.5 mm), and TSK-GEL SW (75 × 7.5 mm) guard column were applied. The column temperature was set to 30°C. As an eluent, a water-to-methanol (65:35) mixture containing 1% NaCl was used with a flow rate of 1.0 mL/min. The refractive index detector temperature was