Evaluation of Polycaprolactone Matrices for Sustained Vaginal Delivery of Nevirapine in the Prevention of Heterosexual HIV Transmission

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ABSTRACT: Nevirapine (NVP) was loaded in polycaprolactone (PCL) matrices to produce vaginal inserts with the aim of preventing HIV transmission. NVP dispersions in PCL were prepared, at 10% (w/w) theoretical loading, measured with respect to the PCL content of the matrices, in the form of (1) NVP only, (2) a physical mixture of NVP with polyethylene glycol (PEG) 6000 or (c) a solid dispersion (SD) with PEG produced by co-dissolution in ethanol. Characterisation of SD by differential scanning calorimetry and attenuated total reflectance–Fourier transform infrared spectroscopy suggested transformation of the crystalline structure of NVP to an amorphous form which consequently increased the dissolution rate of drug. A low-loading efficiency of 13% was obtained for NVP-loaded matrices and less than 20% for matrices prepared using physical mixtures of drug and PEG. The loading efficiency was improved significantly to around 40% when a 1:4 NVP–PEG SD was used for matrix production. After 30 days, 40% of the drug content was released from NVP-loaded matrices, 55% from matrices containing 1:4 NVP–PEG physical mixtures and 60% from matrices loaded with 1:4 NVP–PEG SDs. The *in vitro* anti-viral activity of released NVP was assessed using a luciferase reporter gene assay following the infection of HeLa cells with pseudo-typed HIV-1. NVP released from PCL matrices in simulated vaginal fluid retained over 75% anti-HIV activity compared with the non-formulated NVP control. In conclusion, 1:4 NVP–PEG SDs when loaded in PCL matrices increase drug loading efficiency and improve release behaviour. © 2014 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 103:2107–2115, 2014
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INTRODUCTION

The global AIDS epidemic remains one of the world's most serious health challenges with 35.3 million (32.2–38.8 million) people living with HIV worldwide at the end of 2012.[1] However, the health burden varies considerably between countries and regions. Sub-Saharan Africa, for example, remains the most severely affected region, with nearly one in 20 adults infected with HIV and accounting for around 70% of the global incidence. Most HIV infections occur through heterosexual intercourse and young women between the ages of 15 and 24 are at least three times more likely to be HIV-positive than men.[2] The scale of the AIDS epidemic, the absence of a vaccine and the major gender imbalance in infection rate has heightened awareness of the need for more effective preventative measures that reduce virus transmission and are controlled by women independently of a sexual partner. As a consequence, research on vaginal delivery of microbicides that kill or inactivate viruses at the mucosal barrier has been receiving increasing attention in recent years.[3,4] Early work employed non-specific microbicides, including anionic polymers such as cellulose acetate phthalate, and carbohydrate-binding polymers such as carrageenan that bind to viral envelope proteins (gp120 and gp141 in HIV) and inhibit virus entry into host target cells ($CD4^+$-T cells, macrophages and dendritic cells) in the genital sub-mucosal region. However, the ability of these "first generation" microbicides to prevent HIV transmission has not been clinically proven.[5] Several antiretroviral drugs, including the nucleoside reverse transcriptase inhibitor (NRTI), tenofovir and the non-nucleotide reverse transcriptase inhibitor (NNRTI), dapivirine, are currently being evaluated as "specific" vaginal micro-bicides.[6] Tenofovir, formulated in a 1% hydroxyethylcellulose vaginal gel, was reported to be modestly effective in a Phase IIb trial (CAPRISA-004) when applied in a pre-exposure prophylaxis study.[7] However, tenofovir gel was found to perform no better than placebo in the subsequent, larger-scale Phase IIb VOICE trial.[8] The ineffectiveness of the formulation was ascribed to poor adherence of the trial participants to the dosing regimen.[9] The negative trial outcomes tend to highlight the major disadvantages of semi-solid vaginal formulations; they are inherently "messy" to apply, they tend to leak, and concerns exist over ineffective coverage of the epithelium. In addition, such formulations often need to be applied prior to sexual intercourse to reduce the risk of infection and this can entail use of an application device. As a consequence, intra-vaginal ring (IVR) devices are being investigated intensively for the delivery of anti-virals[10–12] to improve user compliance and to maintain therapeutic concentrations of the drug in vaginal fluid over long time periods. Conventional silicone elastomer or thermoplastic poly(ethylene vinyl acetate) IVRs are normally confined to the delivery of low molecular weight; hydrophobic compounds such as dapivirine and IVR manufacture involves the curing of silicone elastomer

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at 80°C or hot-melt extrusion of poly(ethylene vinyl acetate) at 110°C, which may degrade heat-sensitive bioactives.

We have previously shown the potential utility of microporous polycaprolactone (PCL) matrices for vaginal delivery of hydrophilic antibacterial (ciprofloxacin) and hydrophobic anti-fungal agents (miconazole) in the treatment of gonorrhea and candida.[13] More recently, we incorporated the hydrophilic NRTI, tenofovir, in PCL matrices and achieved gradual release over 30 days in simulated vaginal fluid (SVF) with over 70% retention of anti-viral activity against pseudo-typed HIV-1 viruses.[14] In this study, we describe the development of PCL matrices incorporating a hydrophobic NNRTI, nevirapine (NVP), as part of an on-going evaluation of the materials for prevention and treatment of sexually transmitted infections. NVP is an attractive option as an anti-retroviral drug because it is inexpensive and is widely used in resource-poor areas for treating both HIV and preventing mother-to-child transmission. However, low-water solubility of NVP (0.7046 mg/L[15]) can hinder drug dissolution and thus release into vaginal fluid from an IVR. In addition, it has been pointed out in our previous study[13] that the use of a drug with high solubility in organic solvents, such as NVP, could result in a low loading efficiency in the matrices because of elution into methanol during the solvent extraction stage of matrix production. Polyethylene glycol (PEG) 6000 exhibits limited solubility in methanol[16] but can act as a hydrophilic carrier for NVP in a solid dispersion (SD) to improve dissolution rate of drug in aqueous environment. Thus, physical mixtures and SDs of PEG6000 and NVP were incorporated in the matrices with the aim of improving drug loading and release properties.

MATERIALS AND METHODS

Materials

Polycaprolactone (MW 115,000 Da, Capa 650) was obtained from Solvay Interox (Warrington, UK). NVP (MW 267 g/mol) was supplied by Huahai Pharmaceutical Company (Zhejiang, China). PEG6000 was obtained from BDH Laboratory Supplies (Leicestershire, UK). Sodium chloride, potassium hydroxide, calcium chloride, bovine serum albumin, glucose, glycerol, urea, lactic acid and acetic acid were purchased from Sigma–Aldrich (New South Wales, Australia). All solvents [acetone, methanol, ethanol and dichloromethane (DCM)] were of analytical grade and obtained from Sigma–Aldrich. SVF (pH 4.2) was prepared following the method of Owen and Katz[17] and consisted of 3.51 g NaCl, 1.40 g KOH, 0.222 g $Ca(OH)_2$, 0.018 g bovine serum albumin, 2.00 g lactic acid, 1.00 g acetic acid, 0.16 g glycerol, 0.4 g urea and 5.0 g glucose in 1 L of distilled water.

Preparation of SDs of NVP

Solid dispersions of NVP–PEG6000 in weight ratios of 1:1; 1:2 and 1:4 (labelled as SD1:1, SD1:2 and SD1:4, respectively) were prepared by co-dissolution in ethanol. The appropriate amount of PEG6000 (1, 2 or 4 g) was added to a solution of NVP (1 g) in ethanol (50 mL), the solvent was removed under reduced pressure at 40°C and the NVP–PEG dispersion was dried under vacuum at room temperature for 5 h. The samples were ground using a mortar and pestle and 0.05–0.25 mm particle size fractions were obtained by sieving. Physical mixtures of NVP and PEG6000 were prepared by grinding process followed by manually mixing appropriate amounts of the 0.05–0.25 mm particle size fractions of each powder to obtain NVP–PEG6000 ratios of 1:1, 1:2 and 1:4 (labelled as PM1:1, PM1:2 and PM1:4, respectively).

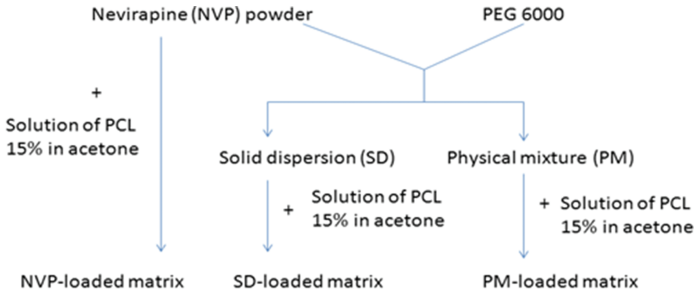

Figure 1. A diagram of matrix production.

Preparation of NVP-loaded PCL Matrices

A PCL solution (15%, w/v) was prepared by dissolving the polymer in acetone at 50°C. NVP powder or NVP in the form of a SD or a physical mixture with PEG6000 was then dispersed in the PCL solution to produce theoretical drug loadings equivalent to 10% (w/w) of the PCL content. The resulting suspensions were poured into a polypropylene syringe body (3 mL) and rapidly cooled in ethanol at −80°C for 2 h to allow crystallisation of PCL. Following the hardening process, the matrices were removed from the moulds and immersed in methanol for 12 h to extract the residual acetone. Solvents (acetone and methanol) remaining in the matrices were removed by evaporation under ambient conditions for 24 h. A schematic diagram illustrating matrix production is presented in Figure 1.

Thermal Analysis

Thermal analysis of NVP and PEG6000 in isolation, as physical mixtures, or as SDs, was performed using differential scanning calorimetry (DSC) (Mettler Toledo DSC1 Star System; Mettler-Toledo Ltd., Victoria, Australia). Samples (5–10 mg) were heated in hermetically sealed aluminium pans at a heating rate of 10°C/min from −80°C to 300°C under a nitrogen atmosphere. Thermal analysis of PCL matrices loaded with NVP or physical mixtures of NVP and PEG6000 or SDs of NVP and PEG6000, respectively, was also carried out under the same conditions.

Attenuated Total Reflectance–Fourier Transform Infrared Spectroscopy

A Nicolet Fourier Transform Infrared Spectroscopy (FTIR) Spectrometer (Thermo Scientific, Brisbane, Australia) with Diamond Attenuated Total Reflectance (ATR) was employed for investigations of NVP and PEG6000 in isolation, as physical mixtures and as SDs. Spectra were obtained at a resolution of 4 cm^{-1} in the 4000—5000 cm^{-1} range.

Determination of the NVP Content of PCL Matrices by HPLC Analysis

Sections (∼100 mg) were cut from matrix samples, weighed and dissolved in DCM (2 mL). Precipitation of PCL was induced by adding 5 mL of methanol, followed by vortex mixing (Vibrax VXR; IKA, Werke Staufen, Germany) at 1000 rpm overnight to allow DCM to evaporate and the drug content to partition into the methanol phase. The residue was washed twice with

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