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Poly(ethylene oxide/propylene oxide) copolymer thermo-reversible gelling system for the enhancement of intranasal zidovudine delivery to the brain

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ABSTRACT

The purpose of this study was to investigate the olfactory transfer of zidovudine (ZDV) after intranasal (IN) administration and to assess the effect of thermoreversible gelling system on its absorption and brain uptake. The nasal formulation was prepared by dissolving ZDV in pH 5.5 phosphate buffer solution comprising of 20% polyethylene oxide/propylene oxide (Poloxamer 407, PLX) as thermoreversible gelling agent and 0.1% n-tridecyl-β-D-maltoside (TDM) as permeation enhancer. This formulation exhibited a sufficient stability and an optimum gelation profile at 27–30 °C. The in vitro permeation studies across the freshly excised rabbit nasal mucosa showed a 53% increase in the permeability of ZDV from the formulation. For in vivo evaluation, the drug concentrations in the plasma, cerebrospinal fluid (CSF) and six different regions of the brain tissues, i.e. olfactory bulb (OB), olfactory tract (OT), anterior, middle and posterior segments of cerebrum (CB), and cerebellum (CL) were determined by LC/MS method following IV and IN administration in rabbits at a dose of 1 mg/kg. The IN administration of Poloxamer 407 and TDM based formulation showed a systemic bioavailability of 29.4% while exhibiting a 4 times slower absorption process (t_{max} = 20 min) than control solution (t_{max} = 5 min). The CSF and brain ZDV levels achieved after IN administration of the gelling formulation were approximately 4.7–56 times greater than those attained after IV injection. The pharmacokinetic and brain distribution studies revealed that a polar antiviral compound, ZDV could preferentially transfer into the CSF and brain tissue via an alternative pathway, possibly olfactory route after intranasal administration.

1. Introduction

The ability of human immunodeficiency virus (HIV) to enter and harbor in the brain tissues results in numerous early and late stage abnormalities of central nervous system (CNS) (Sharer, 1992). The CNS symptoms may initiate as acute encephalitis and headache, and progress to AIDS dementia complex (Price et al., 1988). HIV can directly enter the cerebrospinal fluid (CSF) compartment and the brain via the blood–CSF and blood–brain barriers, respectively (Goswami et al., 1991). Once inside the CNS, HIV can replicate in the brain monocytes/macrophages and microglial cells. Similarly, HIV-infected macrophages are also able to cross the blood–brain barrier (BBB). The importance of adequate CNS delivery of antiviral compounds stems from the fact that improvement in cognitive function of AIDS patients has been attributed to anti-HIV nucleoside therapy. The overall low extent of CNS uptake of the nucleosides and intersubject-variations in CNS concentrations has lead to a concerted effort to increase the CNS delivery of the nucleosides by various experimental approaches (Gallo, 1994).

Zidovudine (ZDV) is structurally related to the endogenous nucleotide thymidine, differing at the 3′-OH which is replaced by an azide group (–N_3) and its antiviral activity is based on its ability to inhibit reverse transcriptase. ZDV like other dideoxynucleosides is a "prodrug" and enters mammalian cells by passive diffusion. It undergoes anabolic phosphorylation, which is the intracellular phosphorylation to the active form, ZDV-5′-triphosphate (ZDV-TP) via intracellular kinases. Orally administered ZDV is rapidly absorbed from the gastrointestinal tract with a peak plasma concentration of 1.2 μg/mL at 0.8 h. However it undergoes extensive first-pass metabolism and is converted to the inactive glucuronide, 3′-azido-3′,-deoxy-5′β-D-gluopyranuronosylthymidine (GZVD). Oral bioavailability of ZDV is 63% and elimination half-life is 1 h thus necessitating the need for frequent administration of large doses, 100–200 mg every 4 h to maintain therapeutic drug levels above 0.268 μg/mL (Thomas and Panchagnula, 2003; Klecker et al., 1987). Pharmacokinetic studies on brain uptake of ZDV conducted by Wong et al. in rabbits reported a CSF/plasma ratio of 0.18 but the thalamus/plasma ratio was only 0.07 indicating very limited transport of the com-

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pound across the blood–CSF barrier and blood–brain barrier (Wong et al., 1992). This indicates a significantly high concentration of the drug is required in systemic circulation to reach and maintain the minimum effective concentration of the drug in the brain tissue. However, significant hematological side effects such as anemia and neutropenia may develop. Important late adverse reactions include myopathy, hepatotoxicity, and carcinogenicity (Harlass, 1996).

Recent developments in intranasal (IN) drug delivery have highlighted the possibilities of exploiting the nasal route for direct transport of drug molecules to the brain tissues. Drug substances, in particular, relatively polar drug compounds such as methotrexate and hexarelin have demonstrated a high direct brain targeting efficiency through the olfactory pathway after intranasal administration in animal models (Wang et al., 2003; Yu and Kim, 2009; Zhang et al., 2004) whereas lipophilic compounds such as diazepam that can cross the blood–brain barrier very easily have demonstrated a very minimal advantage, if any, when administered through the nasal route as compared to its systemic administration (Kaur and Kim, 2008). ZDV itself is a moderately polar compound and hence it is possible ZDV may be able to transport through the olfactory pathway directly into the CSF and brain tissue. It is also possible to achieve higher drug concentrations in the CNS following intranasal administration as compared to IV administration of the drug which proves to be an advantage considering the hematological side effects discussed earlier. However, mucociliary clearance in the nasal cavity tends to clear the formulation from the site of absorption in approximately 10–15 min after administration. This reduces the time available for absorption and direct transport of the drug to the brain via the olfactory route. Most recently, there has been renewed interest in the use of viscous gelling and bioadhesive polymers to prolong contact time in the various mucosal routes of drug administration. Because of rapid mucociliary clearance, the ability to retain drug formulation on the mucosal layer in the nasal cavity for an extended period of time has great appeal for the improvement of systemic bioavailability and drug distribution to the brain tissue via olfactory region. Several literature reports mention somewhat successful and optimized delivery of drugs by the application of mucoadhesive polymers such as chitosan (Yu et al., 2004), Carbopol (Tas et al., 2006), methylcellulose, hydroxypropyl methylcellulose, sodium carboxymethylcellulose, and polyacrylic acid (Andrews et al., 2009). However, in this case, the addition of these polymers to the nasal formulations significantly increased the viscosity of the formulations and thus it impairs the accuracy and reproducibility of dosing volume in the intranasal spray of the formulation.

Commercially available mucoadhesive poly(ethylene oxide-b-propylene oxide-b-ethylene oxide) polymer (Poloxamer 407 – PLX), which is available in various molecular weights, exhibits phase transitions from sol to gel at low temperature gel–sol boundary and from gel to sol at high temperature gel–sol boundary. The polymer exists in gel form only between two critical temperatures (Jeong et al., 2002). Temperature of gel formation can be regulated by adjusting the concentration of PLX in solution. PLX is also known for its concentration dependant mucoadhesive properties as reported by Juhaz et al. (1991).

As part of development studies of ZDV intranasal delivery system for use in the treatment of HIV mediated CNS disorders, the objective of the present study was to investigate the plasma pharmacokinetics and brain distribution profiles of a relatively hydrophilic antiviral compound, ZDV after intravenous and intranasal administration of a Poloxamer 407 based thermoreversible gelling system in rabbits and to assess whether there is a direct nose-to-brain transport pathway for the antiviral agent.

2. Materials

2.1. Chemicals

ZDV was purchased from Sigma (St. Louis, MO, USA). D3-zidovudine (D3-ZDV) was purchased from Toronto Research Chemicals (North York, Ontario, Canada). Methanol, acetonitrile, formic acid, sodium glycocholate, sodium dodecyl sulfate, poly-L-arginine, sodium acetate and acetic acid were of high profile liquid chromatography or analytical grade, purchased from Sigma (St. Louis, MO, USA) and used as such. Phosphatidylchonine and n-tridecyl-(-D-maltoside (TDM) was purchased from Anatrace (Maumee, OH). Poly (ethylene oxide/propylene oxide) copolymer (poloxamer 407, PLX) was a kind gift from BASF.

2.2. Animals

New Zealand white rabbit's (2–2.5 kg) obtained from Millbrook Farms, Amherst, MA were used for pharmacokinetic studies. All experiments were conducted according to protocol for animal use approved by the Institutional Animal Care and Use Committee (IACUC) at St. John's University. Animals were housed in individual cages with free access to food and water in a room with automatically controlled illumination (12-h light–dark cycle), temperature and relative humidity.

2.3. Thermoreversible gel formulation development and characterization

Thermoreversible gels were prepared using the cold method as suggested by Schmolka (Schmolka, 1972). Briefly, PLX, TDM and ZDV were solubilized in pH 5.5 phosphate buffer prepared in distilled water at 4 °C. The liquid was left at 4 °C until a clear solution was obtained. The formulation prepared using a concentration of 20% (w/w) PLX was termed as TR-1. Other mucoadhesive agents such as carbopol 934P, HPC and HPMC at a concentration of 0.1–3% (w/w) were added to TR-1 with continuous agitation to ensure complete solubilization.

Rheological measurements were performed on the thermoreversible gelling formulations using a thermostatically controlled Brookfield Programmable Rheometer fitted with CP-52 spindle. The cone/plate geometry was used due to the pseudoplastic behavior of PLX solution. The cone had a 1.2 cm radius and an angle of 3°. The shear stress was controlled to maintain a shear rate of 5 s^{-1}. This value was chosen for precise determination of the gelling temperature. The temperature was increased in a stepwise manner from 15 to 40 °C to precisely determine solution/gel transition point. The gelling point was determined graphically as the inflection point on the curve of the apparent viscosity as a function of temperature (°C). Each preparation was tested thrice to control the reproducibility of measurement.

2.4. In-vitro permeation studies

In-vitro permeation studies of 0.5% ZDV in the thermoreversible gelling formulation (TR-1) were conducted using side-by-side diffusion cells at 37 °C ± 0.5 °C. Permeation enhancers such as 1% sodium glycocholate (SGC), 1% sodium dodecyl sulfate (SDS), 1% poly-L-arginine 9KD (PAG 9KD), 1% poly-L-arginine 50KD (PAG 50KD), 0.1% phosphatidylcholine (PLC) and 0.1–0.5% n-tridecyl-(-D-maltoside (TDM) were individually evaluated with the gel formulation to screen for the most effective permeation enhancer and its optimum concentration. The freshly excised rabbit nasal mucosa was mounted with the centre area over the cell opening, and the mucosal epithelia facing the donor cell. The receptor cell was then filled with the receptor fluid (oxygenated Krebs Glucose