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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

Ringer solution pH 7.4). The donor cell was filled with 3.5 mL sample solution. Samples (400 μL) were withdrawn from the receptor solution at the specified time and analyzed using the HPLC. The volume of receptor fluid withdrawn was replaced with fresh receptor fluid at each time point. Cumulative corrections were made for the drug permeated into the receptor cell after each sampling time. The steady state flux values of the drug molecules permeating across the nasal mucosa membrane were determined from the slope of graph plotted from the cumulative amount of drug permeated as a function of time.

2.5. Nasal absorption and distribution studies

In the present pharmacokinetic and distribution study, 72 male New Zealand white rabbits were divided into three groups. Each group was further subdivided into three rabbits per time point. Prior to each experiment the rabbits were weighed and kept in a restrainer. The first group of rabbits was administered IV injection of ZDV at 1 mg/kg dose in the ear marginal vein. The second group received 1 mg/kg ZDV administered IN as control solution in pH 5.5 phosphate buffer. The third group received 1 mg/kg ZDV administered IN as thermoreversible gelling formulation TDM/TR-1 with 0.1% TDM as permeation enhancer. The IN formulation (50 μL) was administered in each nostril within 10 s using a metered-dose spray device (Pfeiffer, Princeton, NJ). At predetermined time periods, i.e. 5, 10, 20, 30, 60, 120, 180 and 240 min after dosing the rabbits were euthanized with an overdose of pentobarbital (100 mg/kg). CSF samples (1 mL) were withdrawn by the cisternal puncture technique. A sagital incision was made in the occipital bone over the skin and the underlying muscle and tissue were dissected to expose the atlanto-occipital membrane. A 23-gauge needle attached to a 1 mL syringe was inserted through the atlanto-occipital membrane into the skull and 1 mL of CSF was obtained by gently pulling out the syringe plunger. Collection of CSF was terminated once blood appeared into the syringe and the contaminated blood samples were discarded. The intact brain was carefully removed from the skull by making an incision in the skin and opening the skull using a bone saw. The brain was quickly rinsed with normal saline to remove the stinted blood and wiped with Kimberly–Clark wipes and stored at −40 °C until analysis. For analysis, the brain tissues were excised in the following order: olfactory bulb (OB), olfactory tract (OT), cerebrum (CB) and cerebellum (CL). The whole brain portion and each portion of different regions were carefully weighed on a balance with a sensitivity of 0.1 mg. Thereafter, approximately 100 mg of each brain tissue was cut and analyzed for the determination of drug content in brain samples.

2.6. Analytical procedure

2.6.1. HPLC method

An HPLC analytical method was used for the analysis of drug concentration in the in vitro permeation studies. The HPLC analytical method was developed and validated by modifying the method suggested by Dunge et al. (2005). Briefly, HPLC system equipped with Waters 600E multisolvent delivery system, 717 plus autosampler, 996 photodiode array detector and Empower Data Management System was used for quantification of ZDV. The mobile phase consisting of 77% (v/v) of 0.01 M acetate buffer (pH 4.5): 23%(v/v) methanol was passed over a reversed phase Symmetry C_{18} column (5 μm; 4.6 mm × 150 mm; Waters Corporation) at a flow rate of 1 mL/min. The injection volume was set to 10 μL and detection wavelength at 265 nm.

2.6.2. Solid phase extraction

For the extraction of ZDV from the CSF, plasma and brain tissues obtained from the in vivo studies, a solid phase extraction (SPE) method was adapted and modified from that proposed by Galinsky et al. (1990). Briefly, the extraction of ZDV from the plasma, CSF and brain tissue was performed using Waters Oasis MAX flangeless cartridge 1cc/30 mg and by using D3-ZDV as internal standard. The SPE column was preconditioned with 2 mL of methanol followed by 2 mL of water. Plasma/CSF sample was loaded on the SPE cartridge and allowed to flow in a drop-wise manner. The cartridge was then washed with 0.5 mL of 90:10 water:methanol followed by vacuum drying of the cartridge for 5 min. ZDV was finally eluted from the cartridge using 2 mL of 90:10 mixture of 0.001% formic acid methanol:0.001% formic acid in water divided in five washings, 0.4 mL each. The eluted drug solvent containing ZDV and D3-ZDV was dried overnight under vacuum. The residue was reconstituted using 0.01% (v/v) formic acid in water and drug concentrations were measured using LC/MS analysis.

For the extraction of ZDV from brain tissue, 100 mg sample tissue from various parts of the rabbit brain was accurately weighed. 1 mL of acetonitrile was added to the tube and the tissue was homogenized using a VDI-12 homogenizer at 15,000 rpm. Tissue homogenate was centrifuged at 4000 rpm for 30 min at 4 °C using the Eppendorf 5804R centrifuge. The supernatant was separated into fresh clean tubes and vacuum-evaporated overnight. The dried samples were reconstituted with 0.5 mL of water. SPE columns were preconditioned as in case of plasma and CSF samples. Reconstituted sample from brain tissue was applied onto the SPE cartridge and allowed to flow drop wise to ensure maximum adsorption of drug. The cartridge was then washed with 0.5 mL of 90:10 water:methanol. Drug was eluted from the cartridge using 2 mL of 90:10 mixture of 0.001% formic acid methanol:0.001% formic acid in water divided in five washings, 0.4 mL each. The eluted drug solvent containing ZDV and D3-ZDV was dried overnight under vacuum. The residue was reconstituted using 0.01% (v/v) formic acid in water and drug concentrations were measured using LC/MS analysis.

The LC/MS analytical system consisted of Shimadzu LC-10ADVP integrated HPLC system controlled by Analyst Software 1.4 (Applied Biosystems, Toronto, Canada). Mass spectrometer Sciex API 150EX was coupled to HPLC column via a turbo-ionspray interface. Narrow bore AQUASIL C18 (2.1 × 100 mm, 3 μm, Thermo)

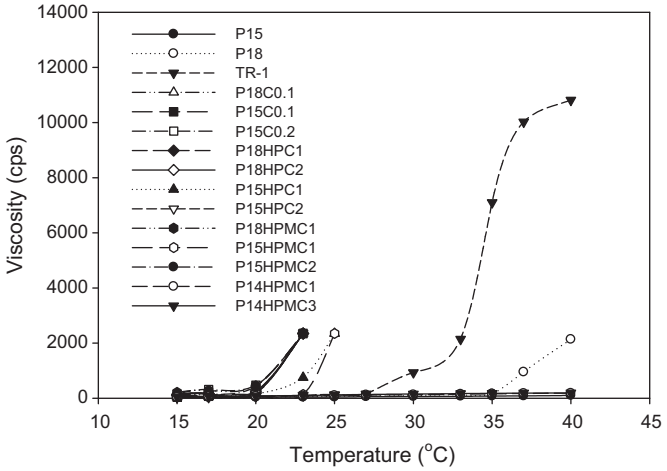

Fig. 1. Measurement of viscosity to study the effect of addition of mucoadhesive polymers to a solution of PLX in ph 5.5 buffer from 15 to 40 °C. P14 – 14% (w/w) PLX in pH 5.5 buffer, P15 – 15% (w/w) PLX in pH 5.5 buffer, P18 – 18% (w/w) PLX in pH 5.5 buffer, TR-1 – 20% (w/w) PLX in pH 5.5 buffer, P18C0.1 – P18 + 0.1% (w/w) Carbopol 934P, P15C0.1 – P15 + 0.1% (w/w) Carbopol 934P, P18HPC1 – P18 + 1% (w/w) HPC, P18HPC2 – P18 + 2% (w/w) HPC, P15HPC1 – P15 + 1% (w/w) HPC, P15HPC2 – P15 + 2% (w/w) HPC, P18HPMC1 – P18 + 1% (w/w) HPMC, P15HPMC1 – P15 + 1% (w/w) HPMC, P15HPMC2 – P15 + 2% (w/w) HPMC, P14HPMC1 – P14 1% (w/w) HPMC, P14HPMC3 – P14 + 3% (w/w) HPMC.

column was used. The mobile phase consisting of 0.01% (v/v) formic acid in 80:20 water:acetonitrile was delivered to the mass spec at a flow rate of 0.15 mL/min. The injection volume was 10 μL. The MS was operated in positive ion-scan mode under total ion chromatography (TIC) and extracted ion-chromatography (XIC). Instrumental parameters such as declustering potentials, focusing potential and entrance potential values were optimized by manual tuning with main parameters of MS set as follows; ion spray voltage 5000 V, temperature 400 °C, ion energy 1.1 V, deflector 300 V and channel electro multiplier 2200 V. Optimized MS parameters were: turbo gas 7 L/min, nebulizer gas 15, curtain gas 6, declustering potential 20.0 V, focusing potential 330 V, entrance potential 3.2 V and a temperature of 400 °C. The protonated molecules of ZDV and D3-ZDV were detected at m/z ratio of 268.2 and 271.2, respectively. Calibration curves prepared in the range of 5–1000 ng/mL for plasma and brain samples were linear with the correlation coefficients $r^2 > 0.9967$ ($n = 9$). The limit of detection for ZDV was 2 ng/mL in both plasma and brain tissue homogenate using a 3:1 signal to noise ratio. The limit of quantification was 5 ng/mL. The intraday, interday precision and accuracies were less than ±3.23% CV for six different concentrations of quality control samples. The extraction recoveries obtained were nearly complete with mean % recoveries from plasma and brain tissues at 88% and 97%, respectively.

2.7. Data analysis

Data from PK studies was analyzed using WinNonlin noncompartmental model (Pharsight Corporation, Cary, NC). C_{max} and t_{max} values were directly assessed from the time vs concentration data. The absolute bioavailability following intranasal administration was calculated using Eq. (1)

$$F\% = \frac{AUC_{IN}}{AUC_{IV}} \times \frac{Dose_{IV}}{Dose_{IN}} \times 100 \quad (1)$$

The total amount (Q_B) of ZDV and mean ZDV concentration in different regions of the brain tissue such as olfactory bulb (OB), olfactory tract (OT), cerebrum (CB) and cerebellum (CL) were calculated using Eqs. (2) and (3), respectively

$$Q_B = (C_{OB} \times F_{OB} + C_{OT} \times F_{OT} + C_{CB} \times F_{CB} + C_{CL} \times F_{CL} W_B = \sum (C_B F_B) W_B \quad (2)$$

where C represents the drug concentration in OB, OT, CB and CL, F is the percentage weight fraction of various brain tissues, W_B is the whole brain weight and Q_B is the total amount of ZDV present in the brain tissue.

$$C_{mean} = \frac{Q_B}{W_B} = \sum (C_B F_B) \quad (3)$$

Chow et al. proposed comparing the ratio of brain to plasma concentration of drug at different time points or AUCs attained after intravenous and intranasal administration, respectively. Following nasal administration, if the drug is absorbed via the systemic pathway, the brain to plasma concentration ratio should be equal to or less than that obtained after intravenous administration. Therefore, drug targeting efficiency (DTE) can be calculated using Eq. (4) and represents a time average partitioning ratio (Chow et al., 1999).

$$DTE\% = \frac{AUC_{brain}}{AUC_{plasma}} \times 100 \quad (4)$$

Zhang et al. (2004) introduced the term direct transport percentage (DTP) that can be calculated using the Eq. (5)

$$\frac{B_{IV}}{P_{IN}} = \frac{B_x}{P_{IN}} \quad (5)$$

$$DTP\% = \frac{B_{IN} - B_x}{B_{IN}} \times 100\% \quad (6)$$

where, P_{IV}, B_{IV}, P_{IN} and B_{IN} denote the AUC of drug in plasma and brain tissues obtained after intravenous and intranasal administration, respectively. B_x represents the brain AUC fraction contributed by systemic circulation through the blood–brain barrier after nasal dosing. Since ZDV has demonstrated linear pharmacokinetics (Blum et al., 1988; Unadkat et al., 1990), the drug amount is proportional to its AUC. Thus, as per Zhang et al. (2004), it can be assumed that the brain AUC fraction contributed by systemic circulation through BBB represented by B_x divided by plasma AUC from nasal route is equal to that of intravenous route. Then DTP represents the percentage of drug directly transported to the brain via olfactory pathway.

2.8. Statistical analysis

Student's t-test and ANOVA were used appropriately for statistical analysis. Probability value $p < 0.05$ was considered as statistically significant.

3. Results and discussion

3.1. Characterization of thermoreversible gelling formulations

Fig. 1 illustrates the effect of concentration of the mucoadhesive agent on the temperature dependant rheological behavior of PLX in pH 5.5 buffer solutions. A solution of 15% (w/w) PLX in pH 5.5 buffer does not form a gel up to a temperature of 40 °C whereas at 18% (w/w), the solution starts gelling at approximately 35 °C. Increasing the concentration of PLX to 20% (w/w) causes gelation in the range of 27–30 °C. This temperature and concentration dependant gel formation has been reported due to the tendency of PLX to undergo a decrease in the critical micelle concentration with an increase in temperature and concentration. The process is known as hard sphere micellization and has been explained by Mortensen and Pedersen (Mortensen and Pedersen, 1993). Addition of 0.2% (w/w) of Carbopol 934P to a solution containing 18% (w/w) of PLX increased its viscosity from 27 to 140 cps at 15 °C. This formulation tends to form a gel at 22–23 °C thus making it unacceptable for sprayability reasons. Therefore the concentration of Carbopol was reduced to 0.1% (w/w) effectively reducing the initial viscosity to 59 cps. However, this did not increase the gelling temperature of the formulation. Further, to study the effect of gelling temperature as a function of PLX concentration in the solution containing 0.1% (w/w) Carbopol, the concentration of PLX was reduced from 18 to 15% (w/w). Viscosity of the formulation at 15 °C was reduced from 59 to 39 cps but the gelation temperature was close to 22–23 °C. Further reduction in PLX below 15% (w/w) led to complete loss of gelation properties up to 40 °C. Similarly, the addition of 1% (w/w) HPC to 18% (w/w) PLX in buffer led to an increase in viscosity up to 210 cps at 15 °C making it non-sprayable even at that temperature. A reduction in PLX to 15% (w/w) caused a reduction in viscosity but the formulation formed a gel at 25 °C prohibiting its application. Similar issues observed with the use of HPMC led to the elimination of any other mucoadhesive agents and it was decided to further pursue a 20% (w/w) solution of PLX in water (TR-1) as the base for the final formulation.

Fig. 2 represents the effect of addition of ZDV and permeation enhancer TDM on the rheological behavior of TR-1. The addition of ZDV at saturation solubility and TDM at 0.1% (w/w) concentration had no effect on the viscosity and gelation temperature of TR-1. This also indicates that ZDV and TDM do not interfere with the micelle formation of PLX thus leaving gelation unaffected.

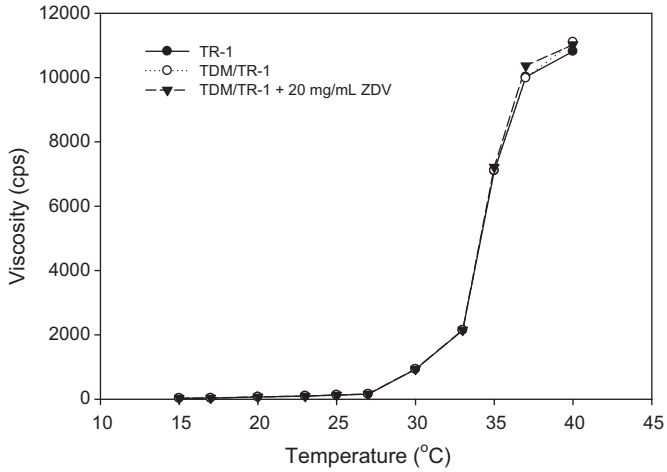

Fig. 2. Effect of addition of TDM and ZDV on the viscosity and gelation temperature of TR-1 formulation.

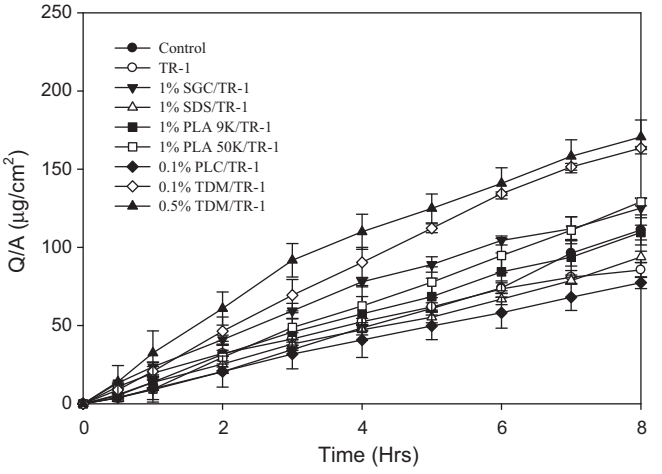

Fig. 3. Transmucosal permeation of ZDV in TR-1 using various permeation enhancers and pH 5.5 phosphate buffer as control, across the rabbits nasal mucosa using side-by-side diffusion cells at 37 °C.

3.2. In-vitro permeation studies

As shown in Table 1, TR-1 formulation showed 1.5-fold increase in the permeability coefficient value (P) (3.86×10^6–5.88×10^6 cm/s) of ZDV as compared to an aqueous solution in pH 5.5 buffer. This increase in transmucosal permeability can be explained by the presence of PLX which is a non-ionic surfactant and can also act as a permeation enhancer. The greater permeability of ZDV from TR-1 as compared to the aqueous solution shows that the entrapment of ZDV in a hydrogel network does not inhibit the diffusivity of the molecule. Thus it may be concluded that the absorption of ZDV is not limited by the rate of drug release from the gel but is governed by the rate of drug diffusion through the biological membrane.

A wide range of chemicals is known to modify the membrane transport of drugs. Surfactants, bile salts and their derivatives, phospholipids, cyclodextrins, cationic polymers, lipids and other miscellaneous systems are widely used as chemical enhancers in IN delivery. The permeability of ZDV from TR-1 was further evaluated across the rabbits nasal mucosa in the presence of various permeation enhancers such as sodium glycocholate (SGC), sodium dodecyl sulfate (SDS), poly-L-arginine (PAG) of molecular weight 9KD and 50KD, hydrogenated L-α phosphatidylcholine (PLC) and n-tridecyl-β-D-maltoside (TDM).

As seen in Fig. 3 and 1, the inclusion of 1% SGC, 1% PAG 9KD, 1% PAG 50KD and 0.1% TDM to TR-1 formulations resulted in a statistically significant ($p < 0.05$) increase in the permeability of ZDV across the biological membrane whereas the addition of 1% SDS and 0.1% PLC showed an insignificant effect on the permeability of the antiviral agent. The addition of 1% SGC to TR-1 resulted in a 1.5-fold increase in permeability as compared to TR-1 alone. Similarly, the addition of 1% PAG 9 K and 1% PAG 50 K to TR-1 led to a 1.3–1.6-fold increase in permeability of ZDV, respectively. Maximum increase in permeability of ZDV from TR-1 was obtained with the addition of 0.1% (w/v) concentration of an alkylglycoside derivative, n-tridecyl-β-D-maltoside as a permeation enhancer. A solution of 0.1% (w/v) TDM in TR-1 (TDM/TR-1) led to a two-fold increase in permeability of ZDV from 5.88×10^{-6} cm/s in TR-1 to 11.8×10^{-6} cm/s in TDM/TR-1. However, there was no statistically significant increase in the permeation of ZDV when evaluated with 0.5% TDM in TR-1 as compared with the permeation of 0.1% TDM in TR-1, suggesting a saturation effect on the permeation of the drug at 0.1% TDM level. Hence, further evaluation was carried out on the formulation containing TR-1 with 0.1% TDM. This formulation will henceforth be referred to as TDM/TR-1. TDM is a non-ionic surfactant consisting of alkyl chains attached to a sugar moiety and belong to the group known as alkylglycoside. Arnold et al. demonstrated a 24-fold increase in the nasal bioavailability of insulin using 0.125% TDM as compared to an aqueous solution. The mechanism is believed to be due to a combination of fluidization of cell membranes and increasing drug movement into the cell (Arnold et al., 2004).

3.3. In-vivo absorption, CSF and brain distribution studies of ZDV

This in vivo study was undertaken to investigate whether a relatively polar compound, ZDV, is capable of transporting through the olfactory pathway to the CSF and brain tissues following intranasal administration of selected formulation in rabbits. In order to evaluate the effect of IN administration of ZDV on the nasal systemic absorption and distribution of the drug into the CSF and brain tissues, we conducted simultaneous determination of drug concentrations in CSF, plasma and 6 different regions of the brain, i.e., the olfactory bulb (OB), olfactory tract (OT), cerebellum (CL) and the anterior (CB1), middle (CB2), and posterior cerebrum (CB3) tissues

Table 1
Transmucosal permeation of ZDV from various formulations across rabbits nasal mucosa at 37 °C ($n = 3$).

Formulation	Concentration (mg/mL)	J_{ss} (μg/cm²/h)	$P \times 10^6$ (cm/s)	T_{lag} (h)	R^2
Control	1.00	13.90 ± 1.07	3.86 ± 0.29	0.35 ± 0.10	0.9904
TR-1	0.50	10.58 ± 0.37	5.88 ± 2.08	0.34 ± 0.10	0.9804
1% SGC/TR-1	0.50	15.50 ± 0.89	8.61 ± 0.05	0.68 ± 0.15	0.9827
1% SDS/TR-1	0.50	11.28 ± 0.32	6.26 ± 0.18	0.23 ± 0.02	0.9921
1% PAG (9KD)/TR-1	0.50	13.57 ± 1.19	7.54 ± 0.66	0.11 ± 0.01	0.9916
1% PAG (50KD)/TR-1	0.50	16.40 ± 0.79	9.11 ± 0.43	0.09 ± 0.10	0.9902
0.1% PLC/TR-1	0.50	9.73 ± 0.64	5.40 ± 0.36	0.45 ± 0.23	0.9868
0.1% TDM/TR-1	0.50	21.30 ± 0.54	11.8 ± 0.03	0.39 ± 0.02	0.9913
0.5% TDM/TR-1	0.50	21.40 ± 1.29	11.9 ± 0.17	0.33 ± 0.07	0.9710

Table 2
Bioavailability and pharmacokinetic parameters of ZDV after IV administration and IN administration of TDM/TR-1 at a dose of 1 mg/kg in rabbits. Data represented as mean ± SEM. (n = 3 rabbits/time point).

Formulation	C_{max} (ng/mL or ng/g)	t_{max} (min)	AUC_{0-4h} (h × ng/mL or g)
Plasma-IV	5575.13 ± 413.00	0.00	3728.86 ± 112.94
Plasma-IN-Control	449.33 ± 46.18	5.00	302.33 ± 8.88
Plasma-IN-TDM/TR-1	901.67 ± 46.61	20.00	1094.50 ± 31.92
CSF-IV	915.67 ± 52.65	5.00	495.53 ± 46.79
CSF-IN-Control	352.33 ± 27.59	5.00	87.68 ± 4.69
CSF-IN-TDM/TR-1	1377.00 ± 102.14	20.00	2319.64 ± 61.64
Brain-IV	113.08 ± 19.82	5.00	27.16 ± 3.46
Brain-IN-Control	154.79 ± 1.42	5.00	34.63 ± 0.78
Brain-IN-TDM/TR-1	939.44 ± 68.13	20.00	1521.69 ± 59.62

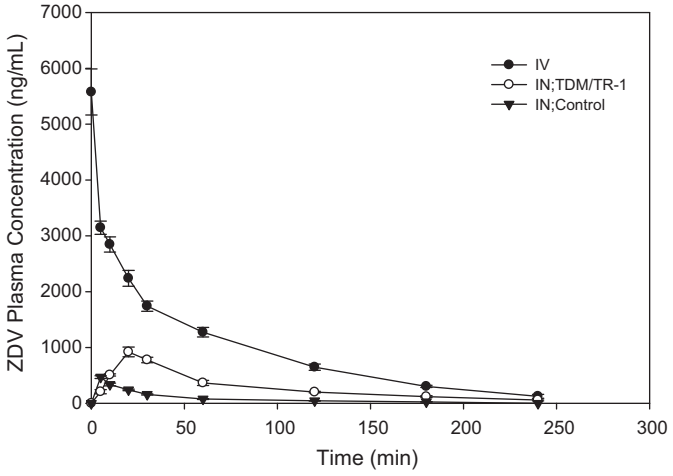

Fig. 4. Mean ZDV concentration–time profile in plasma after IV injection and IN administration of selected ZDV formulation at 1 mg/kg dose in rabbits. Data represented as mean ± SEM. (n = 3 at each time point).

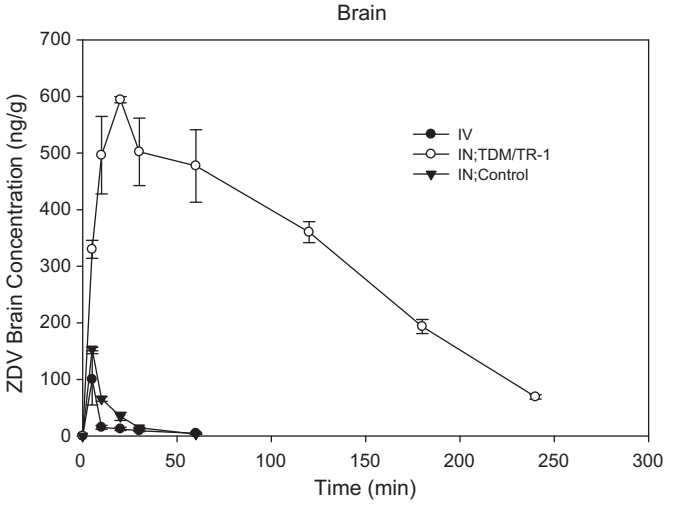

Fig. 6. Mean ZDV concentration-time profile in the brain after IV injection and IN administration of selected ZDV formulations at 1 mg/kg dose in rabbits. Data represented as mean ± SEM. (n = 3 at each time point).

after IV and IN administration in rabbits. For PK and distribution studies, three rabbits were sacrificed at each time point (8 points per formulation) thereby accounting for a total of 24 rabbits per test formulation. The mean ZDV concentration–time profiles determined in the plasma, CSF and brain tissue after IV injection and IN administration of TDM/TR-1 at a dose of 1 mg/kg in rabbits are presented in Figs. 4–6. The corresponding PK parameters obtained from the simultaneous PK studies are listed in Table 2.

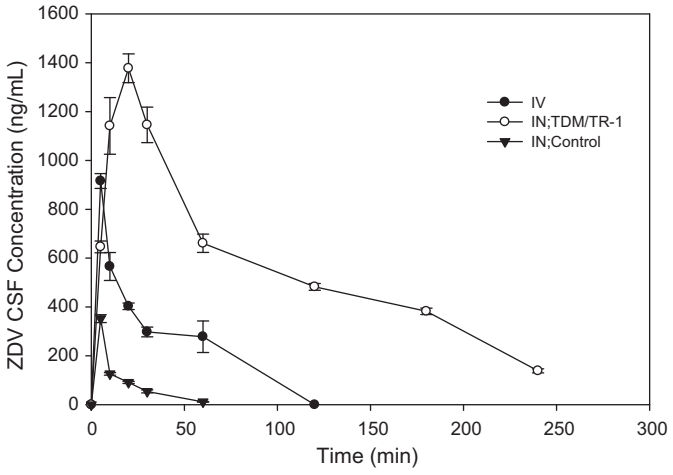

Fig. 5. Mean ZDV concentrations–time profile in the CSF after IV and IN administration of selected ZDV formulations at 1 mg/kg dose in rabbits. Data represented as mean ± SEM. (n = 3 at each time point).

IN administration of ZDV (1 mg/kg) from control solution in pH 5.5 buffer shows rapid absorption and distribution into systemic circulation, CSF and brain tissue as seen from the t_{max} value of 5 min whereas, the IN administration of TDM/TR-1 produced a 4-fold delay in attaining t_{max} (20 min). The C_{max} level attained in the plasma was highest after IV administration of ZDV (5575.13 ng/mL), followed by IN administration of TDM/TR-1 (901.67 ng/mL), and control solution (449.33 ng/mL). Contrary to this, IN administration of TDM/TR-1 shows a 1.5–8.3 times higher C_{max} and 4.6–56 times higher AUC_{0-4h} in the CSF and brain tissue respectively as compared to IV administration of the drug, even though the systemic bioavailability via this formulation was only 29%. This higher concentration and bioavailability observed in the CSF and brain tissue following intranasal administration of TDM/TR-1 indicates the possibility of an alternate route of transport for the drug from the nasal cavity into the brain, possibly by the olfactory pathway. However, the IN administration of control solution of ZDV in pH 5.5 phosphate buffer produced a significantly lower AUC_{0-4h} in the plasma (302.33 h × ng/mL) and CSF (87.68 h × ng/mL) and there was no statistically significant difference observed in the AUC_{0-4h} in the brain tissue (34.63 h × ng/mL) as compared to IV administration of drug. The ratio of AUC_{0-4h} for CSF/plasma and brain tissue/plasma following IV administration of drug would indicate the fraction of drug capable of passing from the systemic circulation to the CSF and brain tissue through the blood–CSF barrier and blood–brain barrier, respectively. In the absence of any alternative pathway, the IN administration of drug should produce a fraction equal to or less than that obtained after IV administration of the drug. IV administration of ZDV indicates a ratio of 0.13 and 0.007 for the

amount of drug available in the CSF and brain tissue as compared to the amount in the plasma following IV administration of ZDV. In contrast, IN administration of the control solution in pH 5.5 buffer shows a significantly higher ratio of 0.29 and 0.11 in the CSF and brain tissue respectively as compared to that obtained following IV administration of drug. The highest ratios were observed following IN administration of TDM/TR-1, 2.11 in the CSF and 1.39 in the brain tissue. Thus, a higher ratio of drug concentration in the CSF and brain tissue following IN administration of the control solution and TDM/TR-1 further indicates the possibility of an alternate route of transport for the drug from the nose to the brain.

The higher C_{max} and $AUC_{0-4\ h}$ value obtained following IN administration of TDM/TR-1 as compared to control solution can be explained by the in vitro permeation data as presented in Table 1. The permeability coefficient value obtained using TDM/TR-1 is almost three times higher than the value obtained using the control solution in pH 5.5 buffer. ZDV is a polar compound; hence it may not be able to efficiently penetrate across the nasal mucosa without the aid of a permeation enhancer. The control solution of ZDV in pH 5.5 phosphate buffer does not contain any permeation enhancer whereas the thermoreversible gelling formulation TDM/TR-1 has been formulated with an alkylglycoside derivative, 0.1% n-tridecyl-β-D-maltoside, a non-ionic surfactant consisting of alkyl chains attached to a sugar moiety and is a very effective permeation enhancer. The thermoreversible gelling property of a 20% (w/w) solution of PLX in pH 5.5 buffer at 27–30 °C led to the gelation of TDM/TR-1 in the nasal cavity of the rabbits. This increase in viscosity is accompanied by mucoadhesion, causing a longer retention of the formulation in the nasal cavity. This prolonged retention is evident from the delay in t_{max} as observed in case of TDM/TR-1 (20 min) as compared to that of the control solution (5 min). Also, the higher C_{max} and AUC_{0-4h} obtained with IN administration of TDM/TR-1 as compared to that obtained following IN administration of control solution justifies that the delay in t_{max} value was in fact due to a greater retention time of the formulation in the nasal cavity and not due to the slower absorption of ZDV from the gel. Also, as seen in Fig. 6, 60 min after IV administration of ZDV (1 mg/kg) and IN administration of control solution (1 mg/kg), drug concentration in the brain tissue was below the detection limit (5 ng/mL) of the developed LC/MS method, whereas, during the same time period, ZDV concentrations available in the brain tissue after IN administration of TDM/TR-1 (1 mg/kg) was greater than the minimum inhibitory concentration of 268 ng/g. The concentration of IN administered ZDV increased to 939 ng/g at the t_{max} point of 20 min then declined relatively slowly and was detectable up to the last point of measurement at the end of 4 h (Fig. 6). This explains the 56 times greater ($p < 0.05$) AUC_{0-4h} obtained in the brain tissue following IN administration of TDM/TR-1 (1521 h × ng/mL) as compared to that obtained after IV (27.16 h × ng/mL) administration of ZDV. The extended drug absorption profiles following IN administration of TDM/TR-1 may be due to the possible accumulation of drug molecules at the site of administration after IN delivery of TDM/TR-1 and thus facilitating the direct olfactory epithelial transport of ZDV over the extended period of time. Hence it can be safely concluded that the combination of TDM as a permeation enhancer and the supposed mucoadhesive property of PLX produced the largest increase in bioavailability of ZDV effectively due to an increase in drug permeability and a longer residence time at the site of absorption.

Another notable observation from Table 2 was that the amount of ZDV available in the CSF after IV and IN administration of each formulation appeared to be significantly higher than the amount available in the brain tissue. This observation was similar to Galinsky et al.'s (1990) findings with IV administration of ZDV. ZDV is a hydrophilic molecule and cannot cross the BBB via passive diffusion. Also, there is no specific nucleoside transport process available at the blood–brain barrier for the active transport of ZDV in the brain tissue. Hence, the only available pathway for absorption is through the choroid plexus in the CSF with subsequent diffusion to the ependymal lining of the ventricle and finally, diffusion into the brain parenchyma. In addition, the diffusion of the drug molecules from the CSF into the brain tissue will be against the flow of CSF and the rapid turnover rates of CSF would decrease the penetration of drug into the brain. Thus, the polar nature of ZDV, the absence of specific transporters at the BBB and the flow of CSF each restrict redistribution of drug into the brain tissue thereby causing a gradient in concentration across the CSF and brain tissue.

Table 3
Parameters for the evaluation of brain targeting efficiencies after IV and IN administrations of selected formulations at a dose of 1 mg/kg in rabbits.

Formulation	DTE	DTP
IV	0.73	
IN-control	11.51	99.27
IN-TDM/TR-1	139.15	99.48

Higher AUC and C_{max} values in the CSF and brain tissue following IN administration of TDM/TR-1 can be explained by the direct nose–brain pathway available as a conduit for direct transport of agents into the CNS as has been demonstrated by many substances such as viruses, metals, dyes, small molecular drugs, proteins and hormones (Mathison et al., 1998). Some lipophilic drugs, such as diazepam (Kaur and Kim, 2008) which can easily pass through the BBB do not show any expected increased uptake in CSF and brain tissues after nasal administration. The rapid and full absorption of diazepam across the nasal mucosa into systemic circulation assists the transport of drug across the blood–brain barrier. This systemic drug transport made any transport via the olfactory pathway into the CSF and brain tissue insignificant. Contrastingly, compounds that exhibit low BBB permeability, such as ZDV, tend to exhibit direct nose to brain transport into the CNS via the olfactory pathways. Sakane et al. reported the preferential uptake of cephalexin into the CSF after nasal administration as compared to IV and intraduodenal administration in rats. Drug concentration in the CSF was 166-fold higher 15 min after nasal administration as compared with the other two routes. This was also the case with methotrexate as reported by Wang et al. (2003). In their study, the AUC_{CSF}/AUC_{plasma} ratio of IN dosing was more than 13 times as high as IV injection. Illum illustrated that the direct pathway from nose-to-brain may only be significant for compounds that are poorly absorbed from the nasal cavity to the systemic circulation or have low BBB transport properties (Illum, 2000).

As seen in Fig. 7, the drug distribution patterns in the OB, OT, CB1, CB2, CB3 and CL tissues of rabbits after IV and IN administration at a dose of 1 mg/kg clearly indicate that ZDV was homogenously distributed into various regions of the brain tissue after IV and IN administration. These drug levels appeared quite similar with no significant region-to-region difference observed after IV and IN administration. ZDV is a small and polar molecule with a molecular weight of 267.2 and log P of 0.1. Hence the distribution of ZDV throughout the brain tissue might be unobstructed and it was homogenously distributed.

3.4. Evaluation of brain drug targeting efficiency

The drug targeting efficiency values (DTE%), defined as $[AUC_{brain}/AUC_{plasma}] \times 100$, determined after IV and IN administration are tabulated in Table 3. In this study, drug targeting efficiency (DTE%) for ZDV after IV administration is 0.7%, after IN administration of control solution of ZDV is 11.51% and after IN administration TDM/TR-1 formulation is 139%. IN administration of control solution of ZDV and TDM/TR-1 provided 16.44 and 231.7 times greater

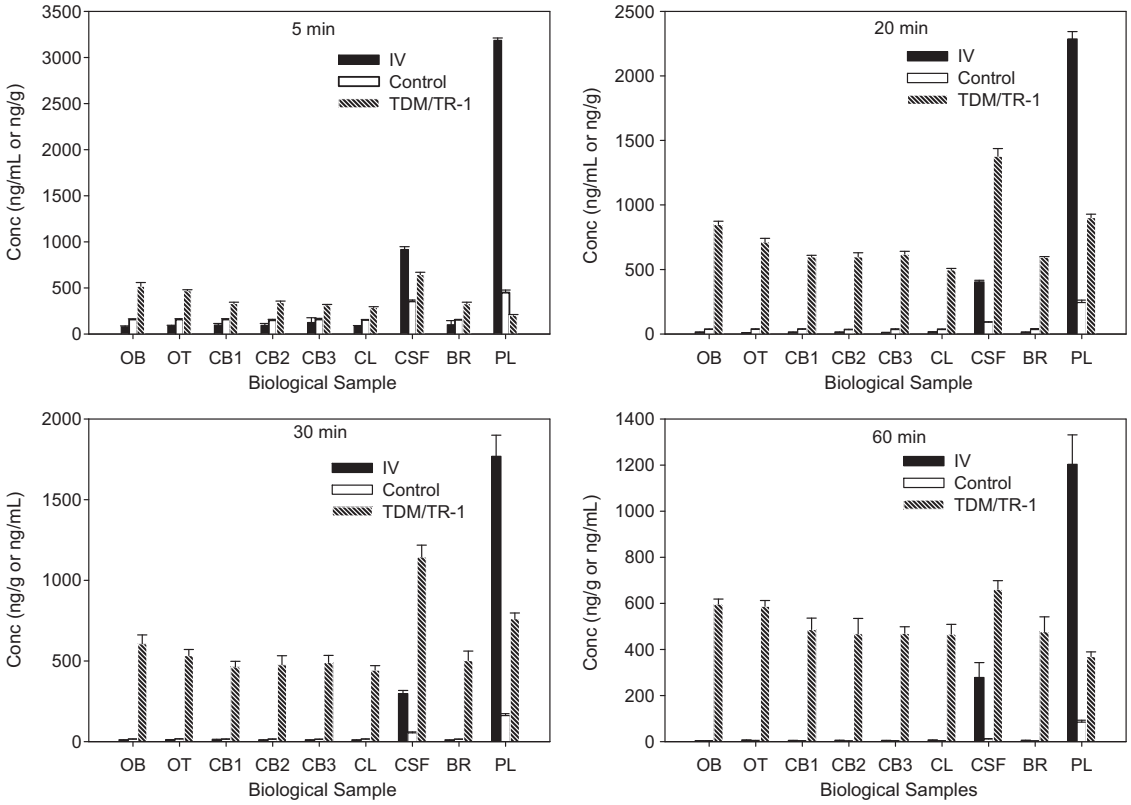

Fig. 7. ZDV concentrations (ng/g or ng/mL) in various biological samples at 5–60 min after IV injection and IN administration of selected ZDV formulations at a dose of 1 mg/kg in rabbits. Data represented as mean ± SEM. (n = 3 rabbits/time point).

($p < 0.05$) ZDV brain targeting efficiency, respectively, as compared to the IV administration. Similar results have been reported by Kumar et al. (2008) when evaluating the drug targeting efficiency of risperidone administered intranasally in the form of a nanoemulsion. The higher DTE value obtained after IN administration of ZDV supports the existence of an alternative pathway of entry to the brain as explained by Chow et al. (1999). In order to more clearly demonstrate the direct nose-to-brain transport of ZDV after nasal administration, the brain drug direct transport percentage (DTP) values were calculated as per Eqs. (5) and (6). According to this method, to calculate the amount of drug reaching the brain via the olfactory route, the amount of drug entering the brain after nasal application via systemic circulation should be subtracted from the total amount of drug existing in the brain. As shown in Table 3, following IN administration, approximately 99% of ZDV content in the brain was directly transported from the nasal cavity via the olfactory pathway. These results can be supported from the fact that when ZDV was administered via the IV route, only 0.7% (Table 3, $DTE\% = AUC_{brain}/AUC_{plasma} \times 100$) of the systemically administered drug reached the brain tissue. These results also strongly suggest the existence of direct pathway for the transport of ZDV from the nasal cavity to the brain tissue via the olfactory pathway.

4. Conclusions

The in vivo absorption and brain distribution studies in rabbits revealed that a polar antiviral agent, zidovudine could preferentially transfer into the cerebrospinal fluid and brain tissues from the nasal cavity possibly via olfactory pathway. Intranasal administration of ZDV solution formulated with a thermo-reversible gelling system prepared with Poloxamer 407 in aqueous buffer solution containing n-tridecyl-β-D-maltoside as permeation enhancer may provide a promising and durable therapeutic option for the treatment of CNS disorders caused by human immunodeficiency virus.

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