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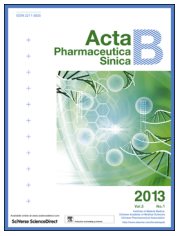

ORIGINAL ARTICLE

Tween 80 containing lipid nanoemulsions for delivery of indinavir to brain

Kandadi Prabhakar, Syed Muzammil Afzal, Goparaboina Surender, Veerabrahma Kishan*

Nanotechnology Laboratory, Department of Pharmaceutics, University College of Pharmaceutical Sciences, Kakatiya University, Warangal 506009, India

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KEY WORDS

Tween 80;
Indinavir;
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Brain delivery;
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Fluorescent DiD oil

Abstract Indinavir is a protease inhibitor used in the treatment of HIV infection. However, it has limited efficacy in eradicating the virus in the brain due to efflux by P-glycoprotein (P-gp) expressed at the blood–brain barrier (BBB). The objective of this work was to develop an o/w lipid nanoemulsion (LNE) of indinavir using Tween 80 as co-emulsifier to improve its brain specific delivery. LNEs were prepared with different compositions and were characterized for globule size, polydispersity index, zeta potential and *in vitro* drug release. Five formulations were then evaluated for drug content, entrapment efficiency and stability after which brain uptake studies were carried out using fluorescent labeled LNEs and pharmacokinetic (PK) and tissue distribution studies were conducted after intravenous administration in mice. Brain uptake of indinavir was shown to be improved for a 1% Tween 80 containing formulation (F5) compared to a formulation containing 0.3% cholesterol (F2). In PK studies, the brain level of indinavir subsequent to administration of F5 was significantly ($P<0.05$) higher than produced by administration of a drug solution (2.44-fold) or a control nanoemulsion (F1) (1.48-fold) or formulation F2 (1.6-fold). The increased brain specific accumulation of indinavir from F5 is probably due to enhanced low density lipoprotein-mediated endocytosis and P-gp inhibition by Tween 80 at the BBB. These results suggest Tween 80 containing LNEs could provide a simple but effective means of delivering indinavir to brain.

*Corresponding author. Tel.: +91 870 2446259, +91 9440973245; fax: +91 8702453508.
E-mail address: vbkishan@yahoo.com (Veerabrahma Kishan).

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1. Introduction

Infection with the human immunodeficiency virus (HIV) often results in progression to the acquired immune deficiency syndrome (AIDS). The HIV retrovirus is found in the brain soon after infection and leads to a variety of central nervous system adverse effects[1]. HIV in the periphery is significantly reduced by highly active antiretroviral therapy (HAART) comprised of multiple small molecule therapeutics. However, certain tissues including brain, macrophages and testis remain reservoirs for HIV infection even with HAART[1,2]. This is because the drugs used in HAART have only limited ability to cross the blood–brain barrier (BBB) into brain parenchyma.

Among the drugs used to treat HIV infection, indinavir is an anti-retroviral protease inhibitor used as a part of the HAART regimen in patients with AIDS. The sub-therapeutic concentration of indinavir in brain leads to failure of treatment and results in the development of drug-resistant viral strains in brain despite the presence of adequate plasma concentrations[3]. The reason for the sub-therapeutic concentration in brain is due to efflux by P-glycoprotein (P-gp) expressed at the BBB[4–7]. Increasing the brain concentration of indinavir by improving its permeation of the BBB is therefore a key to reducing the viral burden in brain during antiretroviral therapy.

Different strategies can be used to improve levels of indinavir in brain, including co-administration of P-gp inhibitors[8] and use of colloidal carriers[9]. Among the various colloidal carriers, lipid nanoemulsions (LNEs) have been used as drug carriers for poorly aqueous soluble drugs such as indinavir to improve their oral bioavailability[10], sustained release[11] and targeting[12,13]. The LNEs of indinavir coupled with holo-transferrin[14] or pegylation[15] or by incorporation of lipoaminoacids as ligands[16] have been reported to provide brain specific/targeted delivery of indinavir. Furthermore, administration of a suspension of a lipid-associated indinavir complex reduced the HIV viral RNA load in macaques[17].

Tween 80 (Polysorbate 80, polyoxyethylene sorbitan monooleate) is a hydrophilic nonionic surfactant widely used in emulsification and dispersion of substances in medicinal and food products[18,19]. Not only it is useful as an emulsifying agent in emulsions[20] and multiple emulsions[21,22], and as a co-emulsifying agent for reducing the globule size in lipid emulsions[23], but also has the advantage in relation to indinavir of being an inhibitor of intestinal P-gp. Thus it has been used to increase the permeability of numerous drugs in rats *in vivo*[24], in Caco-2 cells *in vitro*[25], in tissues such as rat intestinal membrane *ex vivo*[26] and in the inverted rat intestinal sac[27]. In addition, it has been used in conjunction with nanoparticles to improve brain specific delivery of several drugs including tacrine[28], doxorubicin[29], the hexapeptide dalargin[30], loperamide[31] and tubocurarine[32], by increasing LDL-mediated endocytosis.

The objective of this work was to develop indinavir LNEs using lecithin as emulsifier and Tween 80 as co-emulsifier. The effect of Tween 80 containing LNEs on brain delivery of indinavir was investigated in mice by determining plasma and tissue levels of drug after intravenous administration. The therapeutic availability and targeting potential were calculated to evaluate the efficacy of Tween 80 containing formulations in comparison to drug solution and control LNEs.

2. Materials and methods

2.1. Materials

Indinavir was generously provided by Matrix Laboratories (Hyderabad, India). Egg phosphatidyl choline and EPC-80 were gifts from Lipoid (Germany). Refined soyabean oil was obtained from Fluka (Mumbai, India). Tween 80, cholesterol, glycerol, methanol and acetonitrile were products of Merck (Mumbai, India). DiD oil (1,1′-dioctadecyl-3,3,3′,3′-tetramethyl-indocarbocyanine perchlorate), α-tocopherol, triethylamine and phosphoric acid were purchased from Sigma (Mumbai, India). Sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium chloride and oleic acid were from S.D. Fine Chemicals (Mumbai, India). Centrisart tubes and dialysis membranes were products of Sartorius (Germany) and HiMedia (Mumbai, India), respectively. All other chemicals were of reagent grade and used as received.

2.2. Preparation of LNEs

LNEs were prepared with the compositions shown in Table 1 using a hot homogenization and ultrasonication process. The formula consisted of 10% *w/v* soybean oil as the oil core, 1.2% *w/v* EPC-80 as a phospholipid emulsifier, oleic acid (0.3% *w/v*) as a negative charge inducer, α-tocopherol (0.25% *w/v*) as antioxidant and glycerol (2.25% *w/v*) to maintain isotonicity for intravenous

Table 1 Composition of lipid nanoemulsions of indinavir.

Formulation ingredient (% *w/v*)	No cholesterol	Cholesterol (0.3%)		Tween 80			
	F1	F2	*F2	F3	F4	F5	*F5
Indinavir	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Soya bean oil	10	10	10	10	10	10	10
Egg lecithin	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Cholesterol	0	0.3	0.3	0.3	0.3	0.3	0.3
α-Tocopherol acetate	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Oleic acid	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Tween 80	0	0	0	0.2	0.6	1.0	1.0
Glycerol	2.25	2.25	2.25	2.25	2.25	2.25	2.25
Did oil (Dil C_{18})	–	–	0.1	–	–	–	0.1
Doubly distilled water (mL)	10	10	10	10	10	10	10

*Fluorescent dye (DiD oil) containing LNEs.