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Research Paper

Intranasal microemulsion for targeted nose to brain delivery in neurocysticercosis: Role of docosahexaenoic acid

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ABSTRACT

Intranasal Microemulsions (MEs) for nose to brain delivery of a novel combination of Albendazole sulfoxide (ABZ-SO) and Curcumin (CUR) for Neurocysticercosis (NCC), a brain infection are reported. MEs prepared by simple solution exhibited a globule size <20 nm, negative zeta potential and good stability. The docosahexaenoic acid (DHA) ME revealed high and rapid ex vivo permeation of drugs through sheep nasal mucosa. Intranasal DHA ME resulted in high brain concentrations and 10.76 (ABZ-SO) and 3.24 (CUR) fold enhancement in brain area-under-the-curve (AUC) compared to intravenous DHA MEs at the same dose. Direct nose to brain transport (DTP) of >95% was seen for both drugs. High drug targeting efficiency (DTE) to the brain compared to Capmul ME and drug solution ($P < 0.05$) suggested the role of DHA in aiding nose to brain delivery. Histopathology study confirmed no significant changes. High efficacy of ABZ-SO and CUR (100:10 ng/mL) DHA ME in vitro on *Taenia solium* cysts was confirmed by complete ALP inhibition and disintegration of cysts at 96 h. Considering that the brain concentration at 24 h was 1400 ng/g (ABZ-SO) and 120 ng/g (CUR), the in vitro efficacy seen at a 10 fold lower concentration of the drugs strongly supports the assumption of clinical efficacy. The intranasal DHA ME is a promising delivery system for targeted nose to brain delivery.

Abbreviations: ABZ, albendazole; ABZ-SO, Albendazole sulfoxide; ACN, acetonitrile; ANOVA, analysis of variance; AUC, area-under-the-curve; BBB, blood brain barrier; C_{max}, maximum concentration; CUR, Curcumin; CYPs, cytochrome P450; DHA, docosahexaenoic acid; DTE, drug targeting efficiency; DTP, nose to brain direct transport; FMO, flavin-containing mono-oxygenase; HPLC, high performance liquid chromatography; ICH guidelines, International Conference on Harmonisation guidelines; i.n., intranasal; i.v., intravenous; LOD, limit of detection; LOQ, limit of quantification; NCC, Neurocysticercosis; NEs, nanoemulsions; MEs, Microemulsions; PBS, phosphate buffer saline; PDI, polydispersity index; PEG 400, polyethylene glycol 400; PUFA, polyunsaturated fatty acid; RH, relative humidity; SANS, small-angle neutron scattering; $t_{1/2}$, half-life; *T. solium*, *Taenia solium*; TEM, cryogenic transmission electron microscopy; t_{max}, time to reach C_{max}.

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1. Introduction

Targeted nose to brain delivery is a proven strategy to circumvent the blood brain barrier (BBB) and hence achieve high drug concentration in the brain [1,2]. Number of drugs have been targeted to the brain for various therapeutic indications including cancer [3], schizophrenia [4], Alzheimer's [5], Parkinson's [6], migraine [7], and even infections such as encephalitis and meningitis [8]. Nevertheless an important brain infection that has been ignored for targeted drug delivery to the brain is Neurocysticercosis (NCC).

NCC, a contagious zoonotic and orphan disease, spreads rapidly through contaminated pork [9,10]. Caused by the cystic larval stage of the parasite *Taenia solium* (tapeworm), NCC manifests as adult acquired seizures and if untreated could also be fatal [11,12]. Standard therapy for NCC is a combination of albendazole (ABZ) and corticosteroids [9]. ABZ, however exhibits poor oral bioavailability with limited concentration in the brain due to the insurmountable blood brain barrier (BBB) [13]. Targeted nose to brain delivery provides a promising strategy to enhance drug concentration in the brain and thereby improved cure rates of NCC.

Microemulsions (MEs) and nanoemulsions (NEs) provide definite advantages including high loading of hydrophobic and hydrophilic drugs, feasibility of sterilization and the possibility of targeted and controlled drug delivery and are hence widely investigated for intranasal delivery [14,15]. Intranasal administration of mucoadhesive MEs of sumatriptan [16], clonazepam [17] and tacrine [5] revealed higher brain/blood ratios compared to intravenous (i.v.) suggesting effective brain targeting. A high drug targeting efficiency (%DTE) and nose to brain direct transport (% DTP) were seen with NEs of olanzapine [18], risperidone [19] and zolmitriptan ([20]. Direct nose to brain transport of nimodipine ME confirmed that a fraction of nimodipine could be transported directly into the brain following nasal delivery [21].

NE components often play a crucial role in enabling delivery across the BBB. MEs comprising long chain triglycerides exhibit prolonged circulation half-life and hence greater brain accumulation [22]. Flax-seed oil, enabled significantly high and comparable brain targeting of saquinavir following intravenous and oral administration in comparison with safflower oil although both comprised polyunsaturated fatty acid (PUFA). Nevertheless flaxseed oil is naturally more PUFA enriched than safflower oil. The slow metabolism and elimination of PUFA oils also favoured sustained drug concentration in the brain [22]. PUFA, specifically docosahexaenoic acid [DHA, 22:6(n-3)] has natural transporters across the BBB and furthermore DHA enabled enhanced permeation across sheep nasal mucosa ex vivo. DHA being an important nutrient for brain health could provide additional advantage [23]. We therefore designed MEs containing DHA in the oil phase for intranasal delivery. Although ABZ is the drug of choice for NCC we selected ABZ-SO the active metabolite of ABZ, as the active.

ABZ a prodrug is metabolized to the active metabolite ABZ-SO by cytochrome P450 (CYPs) and the flavin-containing monooxygenase (FMO) system predominantly in the liver and intestine [11,24]. The limited presence of CYPs in the nasal mucosa precluded the intranasal administration of ABZ dictating the use of the metabolite ABZ-SO. The long term therapy associated with NCC and the related side effects of the co-administered corticosteroids prompted us to replace the same with a safe and natural anti-inflammatory agent Curcumin (CUR) [25].

Our study therefore presents a novel combination of ABZ-SO and CUR in a ME formulation comprising a PUFA rich oil (DHA rich oil) with the objective of targeted and sustained brain delivery following intranasal administration.

The enhancement in brain concentrations following nasal administration was compared with the same formulations administered intravenously. The specific objective of study was to evaluate the role of the DHA rich oil in the ME on enhanced brain targeting.

An in vitro efficacy study of the MEs on *Taenia solium* (*T. solium*) cysts was also undertaken as an indicator of possible efficacy in vivo. The decrease in cyst size and alkaline phosphatase (ALP) levels was retained as parameters of in vitro efficacy study.

2. Materials and methods

2.1. Materials

Albendazole sulphoxide and curcumin were obtained as a gift sample from SeQuent Scientific Limited (India) (assay 99.9%) and Konark herbals and Healthcare, India (assay 99.9%) respectively. DHA rich oil [INCHROMEGA DHA 500 TG SR, Croda Chemicals (India) Private Limited] and Capmul MCM (Abitec Corporation Ltd., India) were obtained as gift samples. Tween 80, ethanol, propylene glycol, N,N-dimethylacetamide, methanol, and acetonitrile were procured from Merck India Pvt. Ltd. RPMI 1640 medium supplemented with L-glutamine (2 mM), HEPES buffer (25 mM; Gibco), penicillin (10,000 U/mL), streptomycin (10 mg/mL), amphotericin B (0.25 mg/mL) and 10% foetal calf serum; all were purchased from Gibco-Invitrogen. The in vitro assays were carried out in cell culture 12 well plates (Corning, USA). All other chemicals were of analytical reagent grade or HPLC grade.

2.2. Solubility study

The solubility of drugs in the ME components was determined by adding excess of drug (ABZ-SO/CUR) to 1 mL each of selected oils, surfactants and co-surfactants in Eppendorf tubes. Solubility in combination with oil was also evaluated. The Eppendorf tubes were maintained at 37 ± 1 °C in a shaker water bath for 48 h to attain equilibrium. The equilibrated samples were centrifuged at 10,000*g* for 15 min and the supernatant was analyzed by UV spectrophotometry at λ_{max} 290 nm (ABZ-SO) and 425 nm (CUR).

2.3. Pseudoternary phase diagram

Selection of appropriate components for the formation of o/w ME was based on pseudoternary phase diagrams. The surfactant and cosurfactant selected were tween 80 and ethanol respectively. The oils employed in the present study were DHA, Capmul MCM and their combination. Pseudoternary phase diagrams of oil, surfactant, cosurfactant (CoS), and water were constructed using the oil titration method to obtain the components and their concentration ranges that can result in large existence area of ME. The surfactant was blended with CoS (S_{mix}) in fixed weight ratios (1:1, 2:1 and 3:1). Water and S_{mix} were mixed at room temperature (25 °C) in the ratios 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9 (w/w). Oil was added dropwise to each water–S_{mix} mixture and vortexed. After equilibrium, the samples were visually checked using optical polarizer and determined as being clear ME or emulsions, or gels, and the phase diagrams plotted.

2.4. Drug loaded microemulsions

The ME composition comprised 60% tween 80:ethanol (3:1) and 30% water by weight. The oil concentration was maintained at 10% and comprised either DHA rich oil:Capmul MCM (1:1) or Capmul MCM. MEs comprising DHA rich oil:Capmul MCM (1:1) in the oil phase are referred to as DHA ME while those with Capmul MCM as oil are referred to as Capmul ME. ABZ-SO (1 mg/mL) and CUR

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