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Development of risperidone liposomes for brain targeting through intranasal route

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ABSTRACT

The present paper is aimed at development of functionalized risperidone liposomes for brain targeting through nasal route for effective therapeutic management of schizophrenia. The risperidone liposomes were prepared by thin film hydration method. Various parameters such as lipid ratio and lipid to drug ratio were optimized by using Design-Expert® Software to obtain high entrapment with minimum vesicle size. The surface of the optimized liposomes was modified by coating stearylamine and MPEG-DSPE for enhanced penetration to the brain. The formulations were evaluated for vesicle size, zeta potential, and entrapment efficiency. The morphology was studied by Transmission Electron Microscopy (TEM). *In vivo* efficacy was assessed by performing pharmacokinetic study in Wistar albino rats following intranasal administration of the formulations in comparison to intravenous bolus administration of pure drug. The mean vesicle size of optimized liposomes ranged from 90 to 100 nm with low polydispersity index (<0.5). The entrapment efficiency of optimized liposomes was between 50 and 60%, functionalized liposomes showed maximum entrapment. The TEM images showed predominantly spherical vesicles with smooth bilayered surface. All formulations showed prolonged diffusion controlled drug release. The *in vivo* results showed that liposomal formulations provided enhanced brain exposure. Among the formulations studied, PEGylated liposomes (LP-16) had shown greater uptake of risperidone into the brain than plasma. High brain targeting efficiency index for LP-16 indicating preferential transport of the drug to brain. The study demonstrated successful formulation of surface modified risperidone liposomes for nasal delivery with brain targeting potential.

1. Introduction

Psychotic disorders included severe mental diseases with disordered thinking, loss of connections with reality due to delusions and/or hallucinations. Schizophrenia is one of the well-established and common psychotic disorders [1]. Majority of the schizophrenia cases require psychosocial treatment along with pharmacotherapy [2]. The anti-schizophrenic drugs generally have poor bioavailability apart from their adverse drug reactions [3]. The lack of drug availability at the site of action is mainly attributed to the blood–brain barrier (BBB), which restrict the penetration of drugs into the central nervous system (CNS) [4]. Hence, much efforts are being made to develop novel drug delivery systems such as solid-lipid nanoparticles, nanoemulsions, liposomes and polymeric nanoparticles to transport the drug to the brain [4,5].

Liposomes have desirable biological properties, including the biocompatibility and biodegradability [6]. Liposomes, due to their sub-cellular size allow relatively higher intracellular uptake than other particulate systems. Apart from other routes of drug administration, nano-sized drug loaded systems can be delivered through nasal route. Upon nasal instillation of the formulation, drug is directly transported to the brain by circumventing BBB and provide rapid onset of action due to presence of high vascularisation [7]. The drugs can reach the central nervous system through extracellular or intracellular transport along olfactory nerves and also by trigeminal pathway [8]. Liposomes through intranasal administration provide benefit in terms of improved penetration into the brain [9]. Liposomes are increasingly being explored for the intranasal delivery of drugs and vaccines for enhanced systemic availability and targeting. Some examples of the drugs tried

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for administration *via* intranasal liposomes are insulin [10], desmopressin [11], calcitonin [12], rivastigmine [13], and acyclovir [14].

The aim of the present study was to formulate and evaluate nano-sized liposomes of risperidone for brain-targeting though the nasal route. Risperidone is a second generation atypical-antipsychotic drug with combined 5-HT_{2A} and dopamine D2-receptor antagonism property, generally used to ameliorate the negative symptoms of schizophrenia because of its additional α_{2B} adrenergic receptor antagonism [15]. Among the antipsychotic drugs risperidone is widely used to treat schizophrenia [16]. Risperidone is a highly potent drug, structurally classified under the benzioxazoles, extensively metabolised in the liver and excreted by the kidney [17]. It is marketed as oral solutions, tablets and depot injections. The depot intramuscular preparation given once every two weeks, slowly releases risperidone and maintains constant plasma levels [18]. This type of formulations help in improving compliances [19]. Nevertheless, these formulations suffer from the similar drawback as other antipsychotics by delivering a low amount of the drug to the brain and possess extensive first-pass metabolism. Risperidone nanoemulsions [20,21], solid-lipid nanoparticles [22] and polymeric nanoparticles [23] have been reported by other authors. Risperidone was used as model drug for development of implantable drug delivery device using polypyrrole-film electrically stimulated controlled release system [24,25]. However, this delivery system is therapeutically insignificant in schizophrenia. In the present study risperidone liposomes were attempted for nasal administration as risperidone being low molecular weight drug and highly lipophilic, is well suited for nasal delivery [26–28]. Liposomes were evaluated for various formulation parameters and the optimized formulations were studied for their pharmacokinetics behaviour in rats.

2. Materials and methods

2.1. Materials

Risperidone was a gift sample from Aurobindo Pharma Ltd., Mumbai. Soya phosphatidylcholine (SPC), cholesterol, octadecylamine/stearylamine (SA), sephadex-G25 and Triton®-X 100 were procured from Sigma Aldrich, USA. distearylphosphatidylethanolamine-mPEG-2000 (DSPE-mPEG-2000; MPEG-DSPE) was purchased from Genzyme Pharmaceuticals, Switzerland. All other reagents and chemicals used were of laboratory/analytical or HPLC grade.

2.2. Analysis of risperidone

The amount of risperidone in plasma and brain homogenate was analysed by validated reversed-phase high performance liquid chromatography (RP-HPLC) method. The HPLC LC–2010 CHT (Shimadzu, Kyoto, Japan) equipped with low pressure quaternary gradient pump along with dual wavelength UV-detector was used. The samples (20 μL and 80 μL each for analytical and bioanalytical respectively) were injected into Phenomenex Luna C_{18} column (250 mm × 4.6 mm, 5 μm) maintained at 25 °C. The mixture of acetonitrile and 20 mM ammonium acetate buffer, pH 4.2 (30:70 ratio) was used as mobile phase at 1.0 mL/min flow rate. The eluted risperidone was analysed at 276 nm. For bioanalysis, the plasma and brain samples were maintained at temperature of 4 ± 0.5 °C before processing. The risperidone was extracted from plasma/brain homogenate (brain homogenate was prepared with phosphate buffer saline pH 7.4, 10% w/w of brain) by liquid/liquid extraction method prior to the injection of samples to RP-HPLC column. Aliquot of (450 μL) blank plasma or brain homogenate was mixed with 100 μL sodium carbonate solution (1 M) and 25 μL of IS (quetiapine, 5 μg/mL). Extraction was accomplished by adding 2.5 mL of Methyl tert-butyl ether (TBME) followed by vortex mixing for 20 min. The mixture was then centrifuged for 10 min at 10,000 rpm at 4 °C. The organic supernatant was transferred to a clean glass vial and evaporated using nitrogen gas, TurboVap® LV (15 psi) at 60 °C for 10 min. The residue was then reconstituted with 150 μL mobile phase mixture (MP) and 80 μL was injected to HPLC. The retention time was found to be 7.28 min for Risperidone and 14.1 for IS. The main validation parameters as per USFDA guidelines are listed in Table 1.

2.3. Preparation of risperidone loaded liposomes

Risperidone loaded conventional liposomes consisting of soyaphosphatidylcholine (SPC) and cholesterol were prepared by lipid film hydration method as per the literature with slight modification [29]. The ratios of lipids used were as shown in Table 2. Accurately weighed amount of drug, SPC and cholesterol were added to a 250 mL round bottom flask (RBF) and then dissolved in 10 mL of chloroform. The RBF was attached to the rotavapour (Buchi, Switzerland) and maintained at 40 °C. Rotation speed of RBF was adjusted to 40 rpm and vacuum was applied for 20 min to aid the evaporation of chloroform and to form a thin layer of lipids on the inner wall of the RBF. Vacuum was released and RBF was kept in a vacuum desiccator for 24 h to completely remove the residual traces of chloroform. Phosphate buffer pH 6.4 (10 mL) was added as the hydration media and lipid film were removed by hand shaken method in water bath at 40 °C. A milky white uniform multi-lamellar liposomal suspension was obtained which were further reduced in size by probe sonication (in an ice bath) at amplitude 60%, time 5 min and 2 pulse/s. Uniform suspension of liposomes obtained was transferred to sterilized vials and stored at 4 °C until use.

Similarly, cationic liposomes containing SPC/cholesterol/stearylamine and PEGylated liposomes consists of SPC/cholesterol/MPEG-DSPE were prepared as given in Table 3 wherein stearylamine and MPEG-DSPE were dissolved along with the SPC and cholesterol in chloroform.

2.4. Optimization and validation of the experimental design

The liposome formulations contained different variables such as lipid ratio and lipid to drug ratio. The liposomal formulation was optimized by using Design-Expert® Software Version 9-Stat-Ease, Inc. Central Composite Design (CCD) can be used to derive two or more factors (X1, X2) and three level (−1, 0, +1) design can be developed by inclusion of a central point. The present study consists of 2 variables/factors (X1:Lipid (SPC:cholesterol) ratio and X2:Lipid drug ratio) and 3 levels for each of them (2:1, 4:1, 8:1 and 5:1, 7.5:1, 10:1). The central points were 4:1 (lipid ratio) and 7.5:1 (lipid drug ratio), with experimental trials being performed at all 13 possible combinations, as the batch containing central point LP-5 was prepared five times to compute the results (Table 2). The response variables were R1: % Entrapment Efficiency, R2: Zeta Potential, *R*3: Size, R4: Release at 4 h.

2.5. Entrapment efficiency

The entrapment efficiency of risperidone loaded liposomes was determined by separating the free drug from the liposomes. Unentrapped drug was separated from entrapped drug by ultracentrifugation of the risperidone loaded liposomal formulation at 60,000 rpm for one hour (Optima MAX-XP, Beckman Coulter Inc., USA). Pellet of the entrapped

Table 1
Validation parameters of the HPLC method used for the quantification of risperidone ($n = 3$).

Validation parameters	Analytical samples	Plasma	Brain
Calibration range (μg/mL)	0.5–20	0.01–0.6	0.01–0.6
coefficient of determination (R^2)	0.998	0.991	0.993
Accuracy (% mean ± SD)	99.8 ± 0.25	99.5 ± 0.37	99.2 ± 0.35
Precision (% RSD)	0.84	0.92	0.87
LOD (μg/mL)	0.01	0.005	0.005
LOQ (μg/mL)	0.5	0.01	0.01
Recovery (%)	–	89.5 ± 0.78	88.7 ± 1.2