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Application of quality by design approach for intranasal delivery of rivastigmine loaded solid lipid nanoparticles: Effect on formulation and characterization parameters

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ABSTRACT

In the present investigation, Quality by Design (QbD) approach was applied on the development and optimization of solid lipid nanoparticle (SLN) formulation of hydrophilic drug rivastigmine (RHT). RHT SLN were formulated by homogenization and ultrasonication method using Compritol 888 ATO, tween-80 and poloxamer-188 as lipid, surfactant and stabilizer respectively. The effect of independent variables (X1 – drug: lipid ratio, X2 – surfactant concentration and X3 – homogenization time) on quality attributes of SLN i.e. dependent variables (Y1 – size, Y2 – PDI and Y3 – %entrapment efficiency (%EE)) were investigated using 3^3 factorial design. Multiple linear regression analysis and ANOVA were employed to indentify and estimate the main effect, 2FI, quadratic and cubic effect. Optimized RHT SLN formula was derived from an overlay plot on which further effect of probe sonication was evaluated. Final RHT SLN showed narrow size distribution (PDI- 0.132 ± 0.016) with particle size of 82.5 ± 4.07 nm and %EE of 66.84 ± 2.49. DSC and XRD study showed incorporation of RHT into imperfect crystal lattice of Compritol 888 ATO. In comparison to RHT solution, RHT SLN showed higher *in-vitro* and *ex-vivo* diffusion. The diffusion followed Higuchi model indicating drug diffusion from the lipid matrix due to erosion. Histopathology study showed intact nasal mucosa with RHT SLN indicating safety of RHT SLN for intranasal administration.

1. Introduction

Alzheimer's disease is a neurodegenerative disorder characterized by deficiency of acetyl choline in the brain resulting in loss of neurons, synapses, memory dysfunction and pathological changes like formation of abnormal protein aggregates known as senile plaques and neurofibrillary tangles (Lazenby, 2010; Serpell, 2000; Yates and McLoughlin, 2008; Williams et al., 2003). Rivastigmine hydrogen tartrate (RHT), a USFDA approved reversible cholinesterase inhibitor is a candidate of choice, used in the treatment of Alzheimer's disease to treat mild to moderate dementia due to its favorable effect on patient's cognitive and behavioral symptoms. RHT is a non-competitive dual inhibitor, which inhibits the metabolism of both acetyl cholinesterase and butyryl cholinesterase and helps in enhancing acetyl choline level to moderate Alzheimer's disease by increasing central cholinergic function (Scarpini et al., 2003; Grossberg, 2003).

RHT, a phenyl carbamate derivative undergoes extensive first-pass metabolism in the liver resulting in reduced absolute

Abbreviations: BBB, blood brain barrier; CPP, critical process parameters; Compritol, Compritol 888 ATO; CQA, critical quality attribute; CV, coefficient of variation; D: L, drug: lipid; DOE, design of experiment; DSC, differential scanning calorimetry; %EE, % entrapment efficiency; EMEA, European Medicines Agency; GMS, glycerol monostearate; HLB, hydrophilic lipophilic balance; HPLC, high performance liquid chromatography; HSH, high speed homogenizer; HT, homogenization time; MHRA, medicines and healthcare products regulatory agency; PA, Precirol ATO 5; PBS, phosphate buffer saline; PDI, polydispersity index; Pol-188, Poloxamer 188; QbD, quality by design; RES, reticulo endothelial system; RHT, rivastigmine hydrogen tartrate; SLN, solid lipid nanoparticle; TEM, transmission electron microscopy; TPGS, alpha-tocopherol polyethylene glycol 1000 succinate; USFDA, United States food and drug administration; UV–Vis, UV–visible; XRD, X-ray diffraction.

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bioavailability of only 36% after 3 mg dose, leading to restricted entry into brain and lesser concentration at the target site (Fazil et al., 2012). Owing to its hydrophilic nature, oral delivery of RHT necessitates frequent oral dosing, resulting into accumulation of severe cholinergic side effects in the systemic circulation (Wilson et al., 2008; Yang et al., 2013).

Brain targeting of hydrophilic drugs like RHT is even more challenging, because the therapeutic molecules must be transported not only across the brain cell membrane, but also across the blood brain barrier (BBB). BBB an obstructive gatekeeper being lipophilic in nature, hinders the permeability of hydrophilic drugs and molecules above 500 Da, thereby making it difficult to treat many severe and life threatening neurological disorders including Alzheimer's disease (Kaur and Bhandari, 2013; Eyal et al., 2009; Pardridge, 2012).

Various strategies capable of delivering neurotherapeutics across BBB, includes invasive techniques requiring disruption of barrier integrity and noninvasive techniques. Noninvasive intranasal delivery has come to the forefront as an alternative to invasive delivery due to presence of direct connection between olfactory and trigeminal region in the upper nasal mucosa. This connection helps in delivering therapeutics into brain by circumventing BBB and minimizing systemic exposure thereby providing rapid absorption and enhancing drug influx at BBB (Alam et al., 2010; Illum, 2000).

Nanotechnology based delivery systems hold great potential for delivery to brain when given intranasally. These systems are efficiently taken up by nasal mucosa thereby increasing their specificity, bioavailability, duration of therapeutic effect, besides offering higher drug loading and protection against enzymatic and/or chemical degradation (Kumar et al., 2014a; Mittal et al., 2014). Nanoparticulate carriers (lipid emulsions, polymeric nanoparticles, liposomes and micelles) within size range of 10–400 nm, allow easy access across BBB by efficiently encapsulating drug molecules and increasing their diffusion through biological membranes (Bagwe et al., 2001; Chang et al., 2009). However, brain delivery with nanoparticulate system like polymeric nanoparticles shows carrier's interaction with reticulo endothelial system (RES), resulting in rapid clearance from blood circulation (Costantino et al., 2009). In this regard, solid lipid nanoparticles (SLN) are considered as an attractive colloidal carrier system for brain targeting, since they showed higher ability to escape the RES, thereby prolonging the residence time (Brioschi et al., 2009).

SLN are defined as lipidic nanocarriers generally spherical in shape with an average diameter between 10–1000 nm containing biocompatible solid lipid core matrix (mono-di and tri glycerides, fatty acids, steroids and waxes) stabilized by various classes of emulsifiers. They are preferred over other colloidal carriers because of their lipophilic nature and other versatile properties like high drug payload, controlled release, drug targeting and feasibility to incorporate both hydrophilic and lipophilic drugs, make SLN an efficacious carrier system for a wide range of therapeutics facing challenges in the area of brain targeted delivery system (Patel et al., 2011; Kaur and Bhandari, 2013). The mechanism behind SLN uptake by the brain is believed to be an interaction between plasma proteins adsorbed onto SLN surface and endothelial cells of BBB thereby facilitating adhesion and subsequently activating endocytotic process (Kreuter, 2001; Brioschi et al., 2009).

In the past few years, regulatory agencies like USFDA, EMEA and MHRA etc. are emphasizing on the concept of quality by design (QbD) to develop a better quality product by understanding critical process and product parameters based on risk management (ICH, 2009; FDA, 2006). Design of experiment (DOE), a part of QbD has a very significant role in evaluating the effect of large number of critical process parameters (CPP) on critical quality attributes (CQA) of the product (Wechsler, 2008). DOE helps in the development of quality product by minimizing the number of experiments which are often costly and time consuming.

The present investigation was aimed at applying QbD approach for the development of hydrophilic drug loaded SLN for intranasal delivery. 3^3 factorial design was applied to optimize process parameters affecting quality attributes of SLN. RHT SLN were formulated and they were characterized for physicochemical, morphological, *in-vitro, ex-vivo* diffusion and histopathological parameters.

2. Material and methods

2.1. Materials

RHT was received as a gift sample from Cadila Pharmaceuticals Ltd. (Ahmedabad, India). Apifil, Compritol 888 ATO (Compritol) and Precirol ATO 5 (PA) were gift samples received from Gattefosse Pvt. Ltd. (Mumbai, India). Stearic acid, Tween 80, Poloxamer-188 (Pol-188), D-alpha-Tocopherol polyethylene glycol 1000 succinate (TPGS) and Glycerol monostearate (GMS) were purchased from Sigma-Aldrich (Bangalore, India). All other chemicals and reagents were of analytical reagent grade and were used without further purification.

2.2. High-performance liquid chromatography analysis of RHT

RHT was analyzed using high-performance liquid chromatography system (HPLC) LC-2010C HT (Shimadzu, Japan) which consisted of UV/VIS detector and Labsolutions chromatographic software. A reverse phase C18 column (250 x 4.6 mm, 5 μ, kinetex, Phenomenex, USA) was used at room temperature. Mixture of acetonitrile and potassium dihydrogen orthophosphate buffer pH 6.0 (20:80 v/v) was used as a mobile phase at a flow rate of 1.0 ml/min. Injection volume was 10 μl and elute was analyzed at 215 nm. The R^2 value of 0.9996 was found to be linear in the concentration range of 1–100 μg/ml.

2.3. Solubility study of RHT in different solid lipids

It was not possible to determine the solubility of RHT by equilibrium method since different lipids taken into study were solid in nature. Hence an alternative method was followed, wherein drug and lipids were mixed in two different drug: lipid ratios (D: L) *viz*., 1:2 and 1:3 individually as shown in Table 1. Each test tube containing mixtures of drug and lipids were melted 5 °C above the melting point of lipid using water bath and mixed using a cyclo mixer (CM 101, REMI, Mumbai, India). Process of heating and mixing were continued for five minutes and test tubes were observed visually for miscibility and clarity (Das et al., 2011).

2.4. Formulation of RHT SLN

SLN were formulated by homogenization and ultra sonication method (Mehnert and Mäder, 2012). Formulation procedure was divided into two parts in which one part contained lipid and drug while other part contained aqueous solution of surfactant and stabilizer. Drug and lipid mixture was melted 5 °C above the melting point of lipid. Aqueous part was heated at the same temperature. When both parts attain equilibrium, the aqueous phase was incorporated into lipid phase and emulsified using high speed homogenizer (HSH, Kinematica AG, Polytron PT 1600 E, Switzerland). Temperature was maintained constant throughout the emulsification process. Primary emulsification was followed by ultrasonication using a probe sonicator (SONICS, VibraCell, VC 505, USA) and temperature was kept constant (Das et al., 2011). Resulting lipidic dispersion was cooled down at room temperature for 15 min to obtain RHT SLN.