Chapter 1

Introduction

INTRODUCTION

1.1 Introduction

According to World Health Organization (WHO), neurological disorders and their prevalence are at rise, and the world-wide prevalence of neurological disorders by 2030 will be 1100 million (WHO, 2006). Central nervous system (CNS) targeting deserves much attention because of the conglomeration and high prevalence of CNS disorders. In clinical development, CNS-acting drugs have the poorest success rate (Kola and Landis, 2004). Development of more than 98% of such candidates have had to be discontinued because of poor permeability across the blood–brain barrier (BBB), and this presents a major problem to the pharmaceutical industry. (Pardridge, 2001 & Pardridge, 2002).

Perhaps, the only object which holds those advancements back is BBB, and is called for its better understanding for the better treatment of CNS disorders. Though the BBB is greeted for its neuroprotection, it symbolizes a formidable obstacle for both macro to micro and hydrophilic to lipophilic molecules in CNS targeting (Pardridge, 2001 & 2002a). In fact, until the end of 18^{th} century, medical field have not heard about the presence of BBB. It is Paul Ehrlich, a Germen bacteriologist studying staining of cells, who observed that after intravenous application of some aniline dyes, most of the animal tissues were stained with the exception of the CNS; and he explained the observation with a false theory that the brain has low affinity for vital dyes. In 1900, based on the similar observations, the existence of a barrier at the level of cerebral vessels was first postulated by Bield, Kraus and Lewandowsky (Ribatti et al., 2006; Bradbury, 1993). This hypothesis was given greater substance by the experiments of Edwin Goldman – Paul

Ehrlich's student. However, in 1900, it is Lewandowsky who coined the term "blood-brain barrier". The function of brain capillary endothelium as barrier was remained a question and the debate lasted for decades. It was Brightman, Reese and Karnovsky who solved the mystery of the blood-brain barrier in the late 1960s, with the help of electron microscopy (Brightman & Reese, 1969; Ilbay et al., 2012).

At olden days, all the drug molecules that advanced through trial-and-error drug discovery process/ rational drug discovery process were mainly of less than 500 Da in molecular weight (MW) and lipophilic in nature. Therefore, traditionally, it was believed that molecules with dual molecular characteristics viz. molecular weight (MW) <500 Da and lipid solubility transverse across the BBB.

However, few interesting findings have shown that molecules with dual molecular characteristics also do not cross the BBB at pharmacologically significant amounts (Pardridge et al., 1986; Pardridge, 2007). Subsequently, hydrogen bond and polar surface area have been acknowledged in CNS targeting. The basic criteria necessary for passive diffusion were believed to influence for CNS drug delivery. Yet, there are number of reports that disagree with these criteria. It is discredit that CNS targeting is still behind the basic criteria.

However, apart from basic criteria, expression of transporters on BBB limits the BBB permeation of molecules. With the help of positron emission tomography, the in vivo activity of human BBB transport systems has been evaluated. Multiple transporters are expressed in the brain microvessel endothelial cells (BMEC) that line cerebral capillaries of the BBB, and the BMEC has been equipped with three different specialized

mechanisms of solute transfer (Begley, 2003; Kaur et al., 2008; Ohtsuki and Terasaki, 2007). Briefly, blood-to-brain influx transport system that supplies nutrients, including glucose, amino acids and nucleotides, to the brain. Accordingly, xenobiotics recognized by influx transporters are expected to have high permeability across the BBB. Brain-to-blood efflux transport system that functions to eliminate metabolites and neurotoxic compounds from brain interstitial fluid; and molecules recognized by this efflux transporter are expelled from the brain parenchyma. Drug efflux pump that prevents entry of xenobiotics into the brain by pumping them out.

Therefore, It is obvious that unless the molecules do not imitate the structure of substrate of the endogenous receptor expressed at BBB, it will not be transported into the brain. Otherwise it should not be recognized by or should not be the substrate of brain-to-blood efflux transport system (Ohtsuki, S. and Terasaki, 2007).

1.2 Rational of the Study

Amidst the presence of effective efflux transporters, BBB transportation of endogenous substrates and even therapeutics that mimic endogenous substrates occurs with no restrictions. Transportation of drugs that are not recognized by receptor on their own accord could be achieved through chimeric peptide technology. Herein, a non-transportable drug is conjugated to a BBB transport vector/ ligand which has its receptor expression in the BBB, and undergoes transcytosis (Kaur et al., 2008; Pardridge, 1997; Wu and Pardridge, 1999). The transport vectors could be conjugated either directly to the drug compounds or to the surface of particulate colloidal carriers *viz*. nanoparticles,

liposomes, etc. through covalent or noncovalent linkage (Kaur et al., 2008; Pardridge, 2002b).

Folic acid (pteroylglutamic acid), also known as Vitamin B_9, and its double-reduced form tetrahydrofolate are cofactors of several enzymes and a notable endogenous substrate. . Further, its receptor has wildly been expressed in the cells of brain and CP. Folate is relatively easy to ligate to any therapeutics, and retains its ability to bind to its receptor with normal affinity when attached via its γ-carboxylate, and thereby enter receptor-bearing cell by endocytosis. Gelatin, as a protein-based product, possesses several functional groups which are available for covalent modifications of drug or ligand binding.

Among the CNS disorders, the prevalence of epilepsy is as high as other abreast CNS disorders. The epilepsies are common and have frequently devastating influences, affecting approximately 2.5 million people in the United States alone and about 4 % of individuals (50 million) over their lifetime worldwide. More than 40 distinct forms of epilepsy have been identified. People with epilepsy are well known to be at increased risk of sudden death.

Phenytoin is an excellent anticonvulsant and because of its putative mechanisms of action, it masters three different conditions like Grandmal, Partial & status epileptics. (Aaron et al., 1999). However, it is also a good candidate of P-glycoprotein (P-gp), Mrp1, Mrp2, and BCRP (Van vliet et al., 2005, Van vliet et al., 2006, Prasad et al., 2011). Recent studies have suggested that overexpression of P-glycoprotein in the hippocampal region affects brain uptake of phenytoin in epileptic rats and causes a decrease of local

PHT levels in the rat brain (Van Vliet et al., 2007). Even, it has been observed that expression of multidrug resistance–associated proteins MRP1 and MRP2 and breast cancer–resistance protein (BCRP) was upregulated shortly after status epilepticus, during the Latent Period, and in Chronic Epileptic Rats, and affects distribution of phenytoin (PHT) in the brain (Van vliet et al., 2005).

Gelatin is a natural, inexpensive, low immunogenic, non-toxic, and good biodegradable macromolecule which is registered as excipient for pharmaceutical formulations. As a protein-based product, gelatin possesses several functional groups which are available for covalent modifications for drug or ligand binding and useful in targeted drug delivery (Balthasar et al., 2005). Furthermore, the high physiological tolerance of gelatin, its biocompatibility, and its biodegradability are well established for years. The US Food and Drug Administration (FDA) classified gelatin as a "Generally Recognized as Safe" excipient. Gelatin has been used for decades in parenteral formulations and as an approved plasma expander (Schwick and Heide, 1969 & Zwiorek et al., 2008).

In this context, the objective of the study is to attach the folic acid molecules covalently on gelatin, and to formulate phenytoin sodium loaded gelatin folate nanoparticles for CNS drug delivery.