CHAPTER 1

INTRODUCTION

1. INTRODUCTION

Nearly 12.5 million new cancer cases are diagnosed worldwide each year. Every year in the US, 20,000 new primary and nearly 200,000 secondary (metastatic) brain tumor cases are reported. Worldwide numbers are more distressing (Ningaraj, 2007). A brain tumor is a mass of cells that have grown and multiplied uncontrollably. Primary brain tumors originate in brain and may be benign with definite boundaries or malignant that spread to other parts of body. Metastatic (or secondary) brain tumors come from cancer cells in another part of the body. The diseased cells spread to the brain by moving through the bloodstream. Gliomas (astrocytoma) are the most common primary brain tumors. Forty to sixty percent of the primary brain tumors are gliomas. They originate from the star shaped glial cells (astrocytes) that support nerve cells. The symptoms of glioma are headaches, seizures or convulsions, difficulties in thinking or speaking, behavioral changes, loss of balance and vision changes. The treatment modalities of brain tumor include surgery, radiation therapy, immunotherapy and chemotherapy. (www.fda.gov-. A primer of brain tumors-a patient's reference manual). Currently used chemotherapeutic agents suffer from major drawbacks of extremely severe toxicities like cardiotoxicity (carmustine), peripheral neuropathy (vincristine) and CNS depression (procarbazine). These limitations can partially be alleviated by use of a drug known for less severe side effects.

However, drug delivery to brain is challenged by a variety of formidable obstacles like blood brain barrier (BBB), brain cerebrospinal fluid barrier and brain tumor barrier. The BBB comprising of the endothelial cells forming tight junctions separates brain from the systemic circulation, thereby restricting delivery of therapeutics to brain. (Begley, D.J., 1996, Partridge, 1999, Schlossauer, B 2002) Further, the multidrug resistance proteins like P-glycoprotein abundant at the luminal membrane of BBB; remove the drugs before they penetrate brain parenchyma. (Terasaki T. 1999, Akira Tsuji, 1997)

Interestingly, the BBB is provided with active transport mechanisms like carrier mediated transport, adsorption mediated transport and receptor mediated transport for nutrient

supply to the brain. Receptor mediated transport (RMT) employs the interaction of ligand with the receptors located at the luminal membrane, for the transport of nutrients across the BBB. Receptors localized at the BBB include the transferrin receptor, the insulin receptor, and the transporters for low-density lipoprotein, leptin and insulin-like growth factors (Angela R. J., Eric V. S., 2007). Transferrin receptor, a transmembrane glycoprotein, over-expressed on the BBB at the luminal end facilitates the delivery of iron to the brain through endocytosis of the iron binding protein transferrin (Jefferies W. A., 1984, Moos T, 2000). This receptor mediated transport mechanism can be useful for delivery of therapeutics to the brain.

Amongst the various strategies proposed for improving drug delivery to brain, the research on exploitation of nanoparticles as vectors is gaining impetus (Misra et. al, 2003). Nanoparticles are used as transport vectors for delivery of many drugs to brain. Nanoparticles alter the characteristics and tissue distribution pattern of drug and allow the passage of the inaccessible drugs to the brain. Polymeric nanoparticles are interesting colloidal systems that allow the enhancement of therapeutic efficacy and reduction of toxicity of large variety of drugs. Nanoparticles of biodegradable polymers are safe and also provide prolonged release of the drug (Misra et. al, 2003, Kreuter J. 2001, Christophe J. O., 2005).

Surface engineering of nanoparticles with ligand like transferrin offers promising tool for brain delivery of otherwise inaccessible drugs. Several researchers across the globe have successfully targeted inaccessible drugs across BBB by incorporation into the nanocarrier and surface modifying the nanoparticles with transferrin ligand. Hydrophilic drug like azidothymidine was successfully delivered to brain in the form of albumin nanoparticles surface engineered with transferrin (Mishra V. et. al, 2006).

An alternative CNS drug delivery strategy that has received relatively little attention is the intranasal route. Intranasal route of drug delivery is non invasive and delivers the drug rapidly and directly to CNS by circumventing the BBB. The administration of

nanoparticles through the intranasal route may not require the any ligand attachment or any surface modifier for delivery to brain. (Misra et. al, 2005)

Etoposide is a relative safe chemotherapeutic agent with low toxicities, but delivery of etoposide to brain is limited due to its physicochemical nature and in spite of more useful therapeutic application, it does not find role in brain tumor chemotherapy. Etoposide reported to be substrate of multidrug resistance proteins (P-glycoprotein) is effluxed at BBB and hence inadequately available in brain for treatment of brain tumors (Abe T., 1994, Koike K. 1996). Hence there is need for selective brain targeting of etoposide for use in effective treatment of brain tumor.

Temozolomide, currently used in therapy of brain tumors, is a prodrug and converts into its active metabolite monomethyl triazeno imidazole carboxamide (MTIC) at physiologic pH for producing a therapeutic response. Temozolomide and its metabolite are reported to have short half life of 1.5-2hrs and hence require high dosing with increased dosing frequency. (www.fda.gov, Darkes et. al., 2002) Temozolomide is currently available in capsule dosage form and is 30% of the serum concentration is reported to reach brain. Hence enhancing the delivery of Temozolomide to the brain may help in effective treatment of brain tumors.

Hence, the aim of this investigation was envisaged to deliver anticancer agents for the effective treatment of brain tumor. It was hypothesized that after incorporating the selected drugs into polymeric nanoparticles and surface modification with ligand will help in brain specific drug delivery. The surface modified nanoparticulate drug delivery system will lead to enhanced delivery of the drug to the brain by prevention of the clearance by reticuloendothelial system and probable inhibition of the efflux mechanism of brain. Intranasal drug delivery will help in delivering drug directly to the brain, bypassing the blood brain barrier. Hence, it will not require attachment of any ligand or other surface modifiers for drug delivery to the brain.

The proposed plan of research includes:

1. Review of literature regarding brain targeting approaches, intranasal drug delivery, ligand for receptor mediated uptake and its conjugation, analytical profiles of the selected drugs, optimization techniques, invitro characterization of the nanoparticles, invitro cell culture studies for cytotoxicity and intracellular uptake of nanoparticles, invivo models for evaluation of brain targeting, suitable methods for analysis of drug for biodistribution studies.

2. Preparation and optimization of nanoparticles of selected drugs using suitable statistical design, surface modification with ligand for selective brain delivery and characterization of nanoparticles for particle size, drug entrapment efficiency, invitro release, surface morphology.

3. Stability studies of the prepared nanoparticles in accordance with ICH guidelines.

4. Invitro cell line studies to evaluate the delivery system cytotoxicity studies, intracellular uptake studies and fluorescent microscopy of nanoparticles loaded with fluorescent dye.

5. Radiolabeling and optimization of anticancer agents, prepared nanoparticles.

6. Pharmacokinetics and Biodistribution studies of nanoparticles after intravenous and intranasal administration in mice.

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