Chapter 1

Introduction

The treatment of CNS disorders are challenging because of a variety of formidable obstacles for effective and persistent delivery of drugs. Even though the drugs used for the treatment of CNS disorders are potent, their clinical failure is often not due to lack of drug efficacy but mainly due to short comings in the drug delivery approach. Hence, scientists are exploring the novel approaches so that delivery of the drugs can be enhanced and /or restricted to the brain and CNS. Many advanced and effective approaches to the CNS delivery of drugs have emerged in recent years. Intranasal drug delivery is one of the focused delivery option for brain targeting as brain and nose compartments are connected to each other via olfactory/ trigeminal route via peripheral circulation. Realization of nose to brain transport and the therapeutic viability of the route can be traced from the ancient times and has been successfully investigated for rapid and effective transport in last two decades. Intranasal drug delivery delivers drug directly to the brain circumventing BBB and reduces drug delivery to the non targeted sites. This may result in reduction in dose, systemic dilution and first pass metabolism of the drug (Illum 2000). Direct nose to brain transport results into rapid and/ of higher uptake in the brain, which provides an alternative option of self medication in the management of emergencies. However, the development of nasal drug products for brain targeting is facing enormous challenges. For overcoming the obstacles, better understanding in terms of factors which are involved in the direct nose to brain transport (physicochemical factors and formulation factors) and transport mechanisms is of utmost importance. Many sophisticated and effective approaches to the CNS drug delivery have emerged in recent years. Synthesis of more lipophilic analogues, enzyme inhibitors, permeation enhancers, colloidal, bioadhesive and novel drug delivery systems like microemulsion, liposomes and nanoparticles could help in eliminating certain pharmaceutical challenges like low bioavailability, local irritation and toxicity upon long term usage. With all its inherent advantages, intranasal route has been indicated as the most promising approach for delivery of drugs to the brain/CNS.

Mucoadhesive based drug delivery systems gained importance because of longer residential time of delivery system at the site of administration and possible enhanced transport mechanism by the mucoadhesive agents. Microemulsions were

already explored as an effective carrier system for the rapid and substantial uptake of the drug to the brain (Lianli et al 2002; Vyas et al 2005; 2006a; 2006b). Prolonged residence time of the formulations can be achieved by tailoring the viscosity of the formulation by the addition of various grades and types of mucoadhesive agents. Reports in the literature reveal that carbomer derivatives and chitosan can enhance drug absorption without altering the natural physiological process of the nasal cavity/mucosa. Weekly cross link\ed polyacrylates are able to open up the tight junctions of the interstitial space in the nasal mucosa walls and allow paracellular transport (Lussen et al 1995). Chitosan have ionic interaction of positive charges of chitosan with negative charges of glycocalix in mucosa. Longer residence time is required for the absorption of high molecular weight compounds like peptides/ protein drugs. The enhanced residence time at the site of application can be achieved by tailoring viscosity of the formulation. Nasal gels were found to be effective delivery system for peptide drugs like insulin (Abdolhossein et al 2004; D'Souza et al 2005) and calcitonin (Morimoto et al 1985).

Despite enormous advances in brain research, brain and central nervous system disorders remain the world's leading cause of disability, and account for more hospitalizations and prolonged care than almost all other diseases combined. Patients suffering from fatal/and/or devastating CNS disorders such as neurogenerative disorders like Alzheimer's disease, Parkinson's disease, epilepsy, migraine, cerebrovascular diseases and HIV encephalopathy, far outnumber of those victimized from several types of systemic cancers and heart diseases. Beyond the loss of life, this broad category of disorders can have an overwhelming effect on the quality of life for the surviving patient and can lead to serious social and economic burdens on society. Cerebrovascular disease can be devastating for patients and their families. However, there is much that can be done to attenuate cerebral damage and reduce the extent of any disability. Active intervention is best seen in three phases: acute therapy, rehabilitation and secondary prevention. In developed nations, stroke is the third leading cause of death, only surpassed by heart disease and cancer. Despite the tremendous mortality and morbidity of stroke, treatment options remain limited. Many pathophysiological key mechanisms of cerebral ischemia have been identified

in recent years, but drug treatment targeting one or a few of these mechanisms has failed to improve clinical outcome after stroke. The most plausible reason for this failure is the multiplicity of mechanisms involved in causing neuronal damage during ischemia. Drugs targeting a multimodal mode of action could potentially overcome this dilemma, and have recently been shown to provide a remarkable benefit in preclinical studies. There are very few treatments for stroke and the development of new therapeutics is imperative. At present the only FDA approved treatment is to provide tissue plasminogen activator to reopen occluded blood vessels, however, due to a narrow time window, this treatment is only appropriate for a very small number of patients. Antiplatelet, antihypertensive, and lipid-lowering therapies are approved for secondary stroke prevention.Epilepsy is one of the most common of the serious neurological disorder characterized by recurrent unprovoked seizures (CEP 1993; Blume et al 2001). Beyond symptoms of the underlying diseases that can cause certain epilepsies, people with epilepsy are at risk for death from four main problems: status epilepticus (most often associated with anticonvulsant noncompliance), suicide associated with depression, trauma from seizures, and sudden unexpected death in epilepsy (SUDEP). About 50 million people worldwide have epilepsy, with almost 90% of these people being in developing countries.

Clobazam(7-Chloro-1,5-dihydro-1-methyl-5-phenyl-1,5-benzodiazepine-*2,4 (3H) -dione)* is benzodiazepine derivative used in the treatment of CNS disorders like epilepsy, anxiety and schizophrenia. Clobazam and its active metabolite, N-desmethylclobazam (norclobazam), work by enhancing GABA-activated chloride currents at $GABA_A$-receptor-coupled Cl^- channels. Clobazam is rapidly and extensively absorbed following oral administration, with the bioavailability close to 87%.While the half life of clobazam has been reported to be about 48 hours, N-desmethyl clobazam possess a longer half life about 72 hours (Brogden et el 1980). Morever N-desmethyl clobazam is accumulated during long term treatment achieving concentration levels upto10 times greater than clobazam and therefore it may be an important factor in both therapeutic and toxic responses. It is mainly excreted in urine (87-91%) and accumulation is expected in impaired renal functions (Guberman et al 1990 and Bun et al 1990). The higher oral bioavailability and longer half life of

clobazam can be additive in terms of tolerance, drug dependence and withdrawal symptoms associated with the long term use of benzodiazepines.

Clopidogrel bisulphate (Thieno[3,2-c]pyridine-5(4H)-acetic acid, α-(2-chloro phenyl) 6,7-dihydro-,methyl ester,(S)-,sulfate (1:1)) is a thioenopyridine-derived antiplatelet drug which has been used for the management of chronic ischemia. It inhibits ADP receptor/$P2Y_{12}$, thereby it inhibits ADP induced platelet aggregation which is important in stroke development, both in the pathogenesis of atherosclerosis and in the occurrence of acute cerebral artery occlusions. Inhibition of platelet aggregation occurs 24-48 h following the oral intake of clopidogrel and reaches its maximum level in 3 to 5 days. Restoration of platelet functions occurs slowly within 7-14 days, after withdrawal of the drug (Di Minno et al 1985, Dunn et al 1984). Clopidogrel was shown to have neuroprotective effects on hippocampal regeion of brain against hypoxia (Huber et al 2005). The neuroprotection of hippocampus of brain by clopidogrel during ischemic events needs the selective delivery of drug to the brain with minimal systemic exposure. The systemic exposure of clopidogrel found to show prolonged bleeding time which would be lethel/ unwanted effect during the stoke prevention therapy.

Insulin like growth factor-1 is an investigation peptide drug and shown to effective in the protection of brain in ischemic events. Insulin-like growth factor-I (IGF-I) is a 70 amino acid neurotrophic factor with a molecular weight of 7649 Da and structural homology to proinsulin (Rinderknecht and Humbel 1978). The major source of IGF-I in the body is the liver, although it is expressed in many other tissues, including the CNS. Circulating IGF-I plays a prominent role in normal growth and development by mediating the indirect effects of growth hormone, with which it has a complex relationship. Despite of the high-affinity receptor for IGF-I is found on brain capillary endothelial cells, blood-borne IGF-I has difficulty in crossing the blood–brain barrier except in specific hypothalamic and anterior thalamic nuclei (Rein-hardt and Bondy, 1994). IGF-I may be more efficiently transported across the blood–CSF barrier (Pulford and Ishii 2001). But the IGF-I binding capacity present in both blood and CSF may hinder significant transport from the bloodstream into CNS parenchyma by this route. Apart from poor permeability through BBB, systemic administration of IGF-1 not only carries the inherent risk of causing acute hypoglycemia, but also

results in unwanted side effects due to the complexities of the relationship between IGF-1, growth hormone and somatostatin. Subcutaneous administration may be an alternative to deliver IGF-1 to motor neuron axons and terminals via the blood stream. However for the effective delivery of IGF-1 to pathology occurring within the brain in cases of cerebral ischemia, Alzheimer's diseases and central manifestations of amyotropic lateral sclertosis and multiple sclerosis, an different strategy is required for the brain delivery of this peptide. Previous studies showed that that intranasally delivered IGF-I from solution can bypass the blood– brain barrier via olfactory and trigeminal-associated extracellular pathways to rapidly elicit biological effects at multiple sites within the brain and spinal cord. Since the solution may get cleared rapidly from the site of administration by natural clearance mechanism, a better delivery approach for increasing more contact time which will facilitate the transport of high molecular weight peptide would be required. Because of the hydrophilic nature and high molecular weight of the insulin like growth factor-1, mucoadhesive nasal gel would be a preferred delivery system for the intranasal delivery of IGF-1.

In the light of above facts, an alternative drug delivery system is needed which can selectively target the candidate drugs to the various regions of brain. Due to preferential transport of drugs to the brain, intranasal delivery approach may be expected to reduce the wide distribution of drug to the non targeted sites such as systemic/ peripheral circulation. The delivery system must be meticulasly designed to provide rapid transport of the drug across nasal mucosa and longer residence time in nasal cavity. Since mucoadhesive based drug delivery systems provide longer residence time at the site of application, systems like mucosdhesive microemulsions and mucoadhesive gel would expected to have enhanced nose to brain transport.

RESEARCH ENVISAGED

Hence the aim of this investigation was envisaged to deliver clobazam, clopidogrel bisulphate and insulin like growth factor via nasal route for the effective treatment of CNS disorders like epilepsy and ischemic stroke. It was hypothesized that mucoadhesive drug delivery system loaded with these drugs will result into selective and effective nose to brain transport and will restrict its distribution to the

desired sites in the brain. It will help in rapid drug delivery to the brain, reduce side effects, maximize therapeutic index and reduce dose and dosing frequency.

The proposed plan of research includes

I. Review of literature with reference to CNS disorders, epilepsy and stroke, intranasal delivery for brain targeting, intranasal delivery of peptides, delivery system based approaches for intranasal delivery of drugs, micrioemulsion, mucoadhesive agents, nasal gel, radiolabeling, analytical profile and physico-chemical properties of the selected therapeutic agents.

II. Preparation of solutions containing selected drugs, preparation and optimization of microemulsions with the help of pseudo ternary diagrams and titration method.

III. Characterization of drug loaded microemulsions for their globule size, zeta potential, % transmittance, drug content, pH, viscosity, nasal mucosa tissue compatability and evaluation of stability of the formulations under normal and accelerated conditions.

IV. Preparation and characterization of mucoadhesive nasal gel of insulin like growth factor-1.

V. *In vitro* diffusion studies of drug solutions/ microemulsions/ mucoadhesive microemulsions across nasal mucosa of sheep using franz diffusion cell.

VI. Radiolabeling of the selected formulations and optimization of radiolabeled complex for it's suitability for *in vivo* studies.

VII. Biodistribution studies and gamma scintigraphy imaging in animals to ascertain nose to brain transport of drug.

VIII. Pharmacodynamic studies of the drugs on suitable animal models (Pentylene tetrazole induced convulsions in mice model for epilepsy and transient global ischemic model for stroke).

REFERENCES

1. Abdolhossein, Rouholamini Najafabadi, Payam Moslemi, Hosnieh Intranasal Bioavailability of Insulin from Carbopol-Based Gel Spray in Rabbits. *Drug Delivery* 2004;11(5): 295 – 300.
2. Blume W, Lüders H, Mizrahi E, Tassinari C, van Emde Boas W, Engel J. "Glossary of descriptive terminology for ictal semiology: report of the ILAE task force on classification and terminology". *Epilepsia* 2001; 42 (9): 1212–8.
3. Brogden RN, Heel RC, Speight TM and Avery GS. Clobazam: A review of it's pharmacological properties and therapeutic use in antianxiety. *Drugs* 1980; 20: 161 -178.
4. Bun HS, Monjanel M, Noel F, Durand and Cano JP. Effect of age and antiepileptic drugs on plasma levels and kinetics of clobazam and N-desmethyl clobazam. *Pharmacol Toxicol* 1990; 67: 136-140.
5. Commission on Epidemiology and Prognosis, International League against Epilepsy. "Guidelines for epidemiologic studies on epilepsy. Commission on Epidemiology and Prognosis, International League against Epilepsy". *Epilepsia* 1993; 34 (4): 592– 596.
6. D'Souza, Mutalik S, Venkatesh M, Vidyasagar S, Udupa N. Nasal Insulin Gel as an Alternate to Parenteral Insulin: Formulation,Preclinical, and Clinical Studies. *AAPS PharmSciTech* 2005; 6 (2) Article 27: E184-E189.
7. Di Minno G, Cerbone AM, Matioli PL, Turco S, Iovine C and Mancini M. Functional thrombasthenic state in normal platelets following the administration of ticlopidine. *J. Clin. Invest.*1985; 75, 328-338.
8. Dunn FW, Soria J, Soria C, Thomaidis A, Lee H and Caen JP. In vivo effect of ticlopidine on fibrinogen-platelet cofactor activity and binding of fibrinogen to platelets. *Agents Actions* 1984; 15, 97-104.
9. Guberman A, Outure MC, Blaschuk K, Sherwin A. Add on trial of clobazam in intractable adult epilepsy with plasma level correlation. *Can J Neuol Sci* 1990; 17: 167 -171.
10. Huber R, Reepe MW. Improved posthypoxic recovery invitro on treatment with drugs used for secondary stroke prevention. *Neuropharmacology* 2005; 48: 558-565.

11. Illum L. Transport of drugs from the nasal cavity to the central nervous system. Eur. J.Pharam Sci. 2000; 11:1-18.
12. LianLi, Indranil Nandi and Kim KH. Development of an ethyl laurate-based microemulsion for rapid-onset intranasal delivery of diazepam. *Int J Pharmaceutics* 2002; 237(1-2): 77-85.
13. Luessen HL, Verhoef JC, Lehr CM, Borchard G, deBoer AG, and Junginger HE, Bioadhesive polymers for peroral peptide drug delivery. II. Carbomer and polycarbophil are potent inhibitors of intestinal proteolytic enzyme trypsin. *Pharm Res* 1995; 12: 1293-1298.
14. Pulford BE and Ishii DN Uptake of circulating insulin-like growth factors (IGFs) into cerebrospinal fluid appears to be independent of the IGF receptors as well as IGF-binding proteins. *Endocrinology* 2001; 142: 213–220.
15. Reinhardt RR and Bondy CA. Insulin-like growth factors cross the blood-brain barrier. *Endocrinology* 1994; 135:1753–1761.
16. Rinderknecht E, Humbel RE. The amino acid sequence of human insulin-like growth factor I and its structural homology with proinsulin. *J Biol Chem* 1978; 253:2769 –2776.
17. Vyas TK, Babbar AK, Sharma RK, Misra AN. Intranasal mucoadhesive microemulsions of zolmitriptan: Preliminary studies on brain-targeting *J Drug Target* 2005; 13(5) 317-324.
18. Vyas TK, Babber AK, Sharma RK, Singh S. and Misra AN. Intranasal mucoadhesive microemulsions of clonazepam: Preliminary studies on brain targeting. *J Pham Sci* 2006; 95: 570-580.
19. Vyas TK, Babber AK, Sharma RK, Singh S. and Misra AN. Preliminary brain targeting studies on intranasal mucoadhesive microemulsions of sumatriptan. *AAPS Pharm SciTech* 2006; 7, E1-E9.