CHAPTER 1

INTRODUCTION

1 INTRODUCTION

Pain is escalating health problem globally affecting 19% of the population. Many diseases like cancer, multiple sclerosis, herpes zoster infection, accidental surgeries and diabetes patients suffer from different types of pain. A survey by World Health Organization stated that more than 50% of patients still suffer severe and intolerable pain after surgery and trauma (http://www.painrelifehumanright.com). To live everyday with any type of pain is extremely adverse experience that challenges every fiber of an individual's being. The pain disorder brings very high direct and indirect costs to patients and society in terms of suffering and lost productivity. Pain is able to alter a patient's quality of life by interfering with mood, sleep and emotional well-being (Francesca F et al., 2007).

Pathophysiological nociceptive pain occurs when the tissue is inflamed or injured. This pain may appear as spontaneous pain (pain in the absence of any intentional stimulation) and/or as hyperalgesia and/or allodynia. Hyperalgesia is a higher pain intensity that is felt upon noxious stimulation and allodynia is the occurrence of pain that is elicited by stimuli that are normally below the pain threshold. While nociceptive pain is elicited by noxious stimulation of the sensory endings in the tissue, neuropathic pain results from injury or disease of neurons in the peripheral or central nervous system. (Schaible HG and Richter F, 2004) This pain does not primarily signal noxious tissue stimulation and, therefore, feels abnormal. It often has a burning or electrical character and can be persistent or occur in short episodes. Pathological nociceptive input often causes central sensitisation. Central sensitisation amplifies the processing of nociceptive input and is thus an important mechanism that is involved in clinically relevant pain states (Farquhar-Smith PW, 2007).

Opioid analgesics are increasingly being prescribed for the treatment of multiple and diverse acute and chronic painful conditions. Their use for acute pain or terminal pain is well accepted. Their role in the long-term treatment of chronic non-cancer pain is, however, controversial for many reasons. One of the primary reasons is the well-known phenomenon of psychological addiction that can occur with the use of these medications. Abuse and diversion of these medications is a growing problem as the availability of these medications increases and this public health issue confounds their clinical utility. (Benyamin R et al., 2008) Also, the extent of their efficacy in the treatment of pain when utilized on a chronic basis has not been definitively proven. Lastly, the role of opioids in the treatment of pain is also influenced by the fact that these potent analgesics are associated with a significant number of side effects and complications. Common side effects of opioid administration include sedation, dizziness, nausea, vomiting, constipation, physical dependence, tolerance,

and respiratory depression. Physical dependence and addiction are clinical concerns that may prevent proper prescribing and in turn inadequate pain management.

Non-opiods based drugs are normally used for postoperative and non–cancer pain. Patients are not frequently receiving the appropriate drug therapy with inadequate drug dosing and are frequently dissatisfied with treatment (Colombo B et al., 2006). Analgesics very effective for acute inflammatory traumatic pain, are not very helpful in treating the majority of chronic pain conditions (Nitu A et al., 2003). Most current therapies have come about from efficacy noted in non-pain formulations from anti-epileptics and depressants. Unfortunately, the level of efficacy reaches only 5-30% for any particular drug. Advance pain treatments, such as, regional or local nerve blocks, epidural steroid injections, spinal cord stimulators, and acupuncture have no rational basis in terms of efficacy or outcome studies and are associated with serious complications. (Kavar B et al., 2000; Smith LA et al., 2000)

Tramadol is a central acting analgesic which has been shown to be effective and well tolerated, and likely to be of value for treating several pain conditions (step II of the World Health Organization ladder) where treatment with strong opioids is not recommended. Due to absence of clinically relevant effects on respiratory or cardiovascular functions and negligible tendency of abuse, Tramadol, is a drug of choice for alleviation of post operative/ moderate-to-severe pain (Scott LJ and Perry CM, 2000; Bamigbade TA and Langford RM, 1998). However high frequency of drug administration (4-6 hourly), short half-life (5-6 h), low bioavailability of 68% and dose dependent side effects like GI disorders, pruritus poses challenge for its clinical use. Adverse effects are dose-dependent and therefore considerably more likely to appear if the loading dose is high. (Dayer P et al., 1997) Transporting tramadol in enhanced concentration to the brain would result in enhanced bioavailability for effective pain management and reduction in drug exposure to other organs and thereby reducing the side effects.

Anticonvulsant drugs gain importance in neuropathic pain treatment because of advantages of lesser side effects as compared to opioids and anti-depressants. Lamotrigine, a sodium and calcium channel blocker, has demonstrated efficacy for the treatment of neuropathic pain in multiple, randomized, controlled trials (Eisenberg E et al., 2005). However there is a risk of dose dependent severe rashes, as well as Stevens-Johnson syndrome, a potentially fatal epidermal necrosis associated with high dose and prompt dose escalation (Sach B et al., 1996). High dose, variable brain permeability, dose dependent side effects and severe skin rashes associated with poses challenge for their effective clinical use in neuropathic pain.

With IR formulations, the drug peak serum concentration may be associated with considerable adverse effects. Enhancing the drug concentration to the brain would there by lead to reduction in systemic exposure and resulting in reduced side effects. Moreover, such concentration curve helps to avoid a drug trough level, which makes pain control more efficient.

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 DJ, 1996; Pardridge, 1999; Schlossauer B and Steuer H, 2002) Several approaches are employed to enhance drug delivery across BBB. (Su Y and Sinko PJ, 2006; Neuwelt E et al., 2008)

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. The transport of essential nutrients across the BBB using interaction of ligand with the receptors located at the luminal membrane is known as Receptor mediated transport (RMT). The movement of free iron into cells is essentially mediated via a family of non-heme iron binding glycoproteins termed transferrin, lactotransferrin and melanotransferrin, and their respective cognate membrane receptors (Mathew W et al., 2006).

Tf and Tf family receptors are expressed on the luminal membrane of brain endothelial cells and mediate the internalization of iron-saturated Tf through RMT. The Tf receptors are of particular interest because their substantial expression in brain capillaries (Jefferies et al. 1984). On the other hand they suffer shortcoming that Tf receptors are almost saturated under physiologic conditions because of high endogenous plasma Tf concentration (Pardridge WM, 1987). Nevertheless, the receptor-mediated endocytosis of Tf from blood to brain is well documented (Visser CC et al., 2004; Hatakeyama H et al., 2004; Ulbrich K et al., 2009; Changa J et al., 2009).

Lf is a multifunctional protein to which several physiological roles have been attributed (Ward P et al. 2005) which are mediated by Lf receptors (Suzuki YA and Lonnerdal B, 2002). Lf has been demonstrated to cross the BBB via receptor-mediated transcytosis (Fillebeen C et al., 1999). However there are only few citations from different authors signifying the role of Lf as brain delivery vector (Hu K et al., 2009; Huang RQ et al. 2010). Therefore, it was also of interest to determine whether which is better ligand among Tf or Lf for brain delivery.

Amongst the various strategies proposed for improving drug delivery to brain, the research on exploitation of nanoparticles as vectors is gaining impetus (Misra A 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 A et. al, 2003, Kreuter J, 2001; Christophe JO, 2005). PLGA nanoparticles were chosen as carrier system for its main advantages viz biocompatibility, ease of preparation, high physical stability, and the possibility of modulating the drug release for sustained delivery by controlling the polymer degradation (Anderson JM and Shive MS, 1997; Olivier JC, 2005). In addition, FDA has already approved several formulations comprising of PLGA which are currently being marketed. (Bala I et al., 2004) Moreover, safety of PLGA for parenteral administration is also established. (Semete B et al., 2010).

Surface engineering of nanoparticles with ligand like transferrin and lactoferrin offers promising tool for brain delivery of otherwise inaccessible drugs. Several researchers across the globe have successfully targeted drugs across BBB by incorporation into the nanocarrier and surface modifying the nanoparticles with transferrin ligand. Therefore, it was of interest to determine whether Lf conjugated drug loaded PLGA can be transported to the extent of Tf conjugated drug loaded PLGA, into the brain across the BBB *in vivo.*

By incorporating Tramadol and Lamotrigine in ligand conjugated NPs the higher amount of drug can be delivered to the site of action with lesser systemic exposure leading to reduced side effects. There is possibility of dose reduction which will minimize dose dependent side effects. The drug delivered form PLGA nanoparticles in sustained manner provide improved patient compliance. The proposed delivery system is also useful for maintaining therapeutic effect for prolonged period of time.

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 route is noninvasive mode of drug administration in comparison to the other routes of administration. Intranasal drug delivery delivers the drug directly to the brain by circumventing BBB and reduces drug delivery to non targeted sites. Direct transport of drugs to the brain may lead to the administration of lower doses and in turn can reduce toxicity. Systemic dilution effect and first pass metabolism are also avoided (Illum L, 2003). Direct transport could result rapid and/or higher uptake in brain, which provides an alternative option of self-medication in management of emergencies. However, the development of nasal drug products for brain targeting is facing enormous challenges. High lipophilicity and preferably low molecular weight of drug are the prerequisites as it could influence the uptake across nasal mucosa (Chein YW and Chang S, 1987). 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. Drug compounds devoid of offensive/pungent odor/aroma and non-irritant nature are highly desirable to facilitate dosage form design for intranasal drug delivery systems. The other practical difficulties that have to be overcome include active degradation or alteration by enzyme, low pH of nasal epithelium, the possibility of mucosal irritation or the possibility of large variability caused by nasal pathology, such as common cold.

Among the novel systems for brain delivery through intranasal route, microemulsions gained considerable interest for their simple formulation with more stability and optical clarity and efficient to across the biological membranes, biocompatibility, biodegradability, easy to prepare and handle and most importantly solubilization capacity for both water and oil soluble drugs. Microemulsion demonstrates a possible alternative to i.v. administration and a promising approach for rapid onset delivery of CNS medications (Lianli L et al., 2002).

Microemulsions or micellar emulsions are defined as single optically isotropic and thermodynamically stable multicomponent fluids composed of oil, water and surfactant (usually in conjunction with a co-surfactant). The droplets in a micro emulsion are in the range of 1nm-100nm in diameter. The dispersal of drug as a solution in nano meter-sized droplets enhances the rate of dissolution into contacting aqueous phase and *in vivo* generally results in increase in drug bioavailability. In addition, the presence of surfactant and in some cases co-surfactant, for example medium chain triglycerides in many cases serve to increase membrane permeability thereby increasing the drug uptake.

MEs are equilibrium systems (i.e. thermodynamically stable), while nanoemulsions (NEs) are non-equilibrium systems with a spontaneous tendency to separate into the constituent phases.

Nevertheless, NEs may possess a relatively high kinetic stability, even for several years (Solans C et al., 2003). NEs can be obtained by high shear methods, ultrasonication and condensation method and globule size (GS) of NEs is in nanometer range. Evidently, the preparation method influences emulsion properties (e.g. droplet size, stability, etc.), but the nature of the final dispersion (the constituent phases) is the same whether the method of preparation uses high shear (external energy, dispersion methods) or the chemical energy stored in the system (condensation methods). The foremost advantage of nanoemulsion system is it consists of lesser amount of surfactants suggesting suitability for multiple dosing without affecting nasal mucosal epiltelium. Also, use of lecithin as surfactant ensures biocompatibility and safety for chronic treatment. However, drug loading is lesser compared to microemulsion.

By formulating Tramadol and Lamotrigine in microemulsion and nanoemulsion formulation, delivers drug directly to brain by passing BBB which is useful for episodic and emergency pain treatment. The drug will be delivered to brain circumventing BBB in brisk manner establishing immediately, minimum effective concentration required for therapeutic response. Direct transport of drugs to the brain may lead to the administration of lower doses, reduce the toxicity and avoids systemic dilution effect and first pass metabolism.

RESEARCH ENVISAGED

The objective of the study is to incorporate therapeutic pain alleviating drugs with variable or little BBB permeability into nanoconstructs for their enhanced & selective brain uptake after parenteral or nasal administration for prolonged and rapid drug delivery respectively in pain treatment. For parenteral administration, nanoconstructs formulated as nanoparticles were surface modified with surfactants for long circulation and attached with brain selective ligand such as Tf/ Lf for enhancing brain bioavailability and reducing systemic toxicity. It is also the objective to compare the targeting capability of NPs after conjugation with Lf and Tf. For intranasal administration, nanocostructs formulated as microemulsion and nanoemulsion could result rapid and higher uptake in brain, which provides an alternative option of self-medication in management of emergencies.

The objectives were based on the hypothesis that, ligand attached and surface modified (hydrophilic surfactant coated) long circulating nanoconstructs formulated as nanoparticles will selectively target the brain capillary endothelial cell receptors for enhanced and prolonged brain uptake for the effective treatment of pain and will reduce the systemic side effects. It is also hypothesized that, the intranasal microemulsion and nanoemulsion may provide comparatively faster and higher uptake via direct nose to brain transport of drug by brain by circumventing BBB and reducing drug delivery to non targeted sites, avoiding systemic dilution effect and first pass metabolism.

The proposed plan of research includes:

I. 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, *in vivo* models for evaluation of brain targeting, suitable methods for analysis of drug for biodistribution studies.

II. Preparation and optimization of nanoparticles of selected drugs using suitable statistical design, surface modification with ligand for selective brain delivery Preparation and optimization of microemulsion and nanoemulsion.

III. Characterization of nanoparticles for particle size, drug entrapment efficiency, *in vitro* release, surface morphology. Characterization of drug microemulsions and nanoemulsions for their globule size, zeta potential, % transmittance, drug content, pH, viscosity, nasal mucosa tissue compatability, *in vitro* diffusion studies across nasal mucosa.

IV. Stability studies of the prepared nanoparticles, microemulsion and nanoemulsion in accordance with ICH guidelines.

V. Radiolabeling of the selected formulations and optimization of radiolabeled complex for its suitability for *in vivo* studies.

VI. Pharmacokinetics and biodistribution studies of nanoparticles and emulsions after intravenous and intranasal administration respectively.

VII. Pharmacodynamic studies of the drugs on suitable animal models.

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