Chapter I

Introduction

INTRODUCTION

Since time immemorial, man has been on look out for new inventions, as a step towards better living. Many discoveries or inventions have found new usage in addition to their existing role. This is true for many drugs, and investigations are being directed towards designing new delivery systems of known drugs to be able to produce a new therapeutic activity. The development cost of a new drug molecule is near to US$250 million (i.e. Rs 1250 Crores) and may take about 12 years to reach the market. Whereas an existing drug molecule can get a second life with a new therapeutic activity by application of newer drug delivery systems that can be developed in much less time and cost.

For many diseases, a substantial number of therapeutically effective compounds already exist but have not found the desired use due to biopharmaceutical limitations. Hence, there exists the necessity to administer the therapeutic molecule through a new route or by a newer delivery system to overcome these limitations and provide a new therapeutic activity.

Diabetes mellitus (DM) is one of the most common non-communicable diseases globally and Type II diabetes (T2DM) accounts for about 90 to 95% percent of total diabetes. Recent findings have proved that T2DM is associated with neuro-degenerative disorder such as Alzheimer's disease resulting in increased disability, reduced life expectancy and enormous health costs for virtually every society.

Alzheimer's disease, the most common cause of dementia in elderly humans, occurs when neurons in the memory and cognition regions of the brain are accompanied by massive accumulation of abnormal fibrous amyloid β-protein (Aβ) at intracellular and extracellular sites, together with widespread

neuronal cell loss (Masters et al., 1985). Currently, there are four FDA-approved drugs (tacrine, donepezil, physostigmine and metrifonate) that treat the symptoms of Alzheimer's disease by inhibiting the active site of acetylcholinesterase (AChE), the enzyme responsible for degradation of acetylcholine, thereby raising the levels of neurotransmitter in the synaptic cleft (Racchi et al., 2004). This symptomatic treatment of Alzheimer's disease is currently unsatisfactory as it shows mild clinical benefits without affecting the fundamental pathology underlying Alzheimer's disease. No treatment has been shown to be disease modifying i.e., to slow the clinical progression of dementia or delay the onset of disease (Galasko, 2005). Thus, reducing Aβ production in the brains or the activation of mechanisms that accelerate its clearance from brains have become major targets for the development of drugs for Alzheimer's disease, since the metabolic imbalance between Aβ anabolic and catabolic activities might be responsible for Alzheimer's disease (Glabe, 2000).

Preliminary results of clinical studies consistently suggested that restoring adequate levels of insulin and glucose by using a thiazolidinedione, facilitates memory in patients with Alzheimer's disease (Watson et al., 2005).

This evidence has led to the notion of metabolic insufficiency or gluco-regulatory impairment in Alzheimer's disease (Gibson et al., 1998; Convit et al., 2003; Hoyer, 2004) and has provided a strong rationale for the therapeutic use of drugs, such as thiazolidinediones, that increase insulin sensitivity and glucose consumption (Galea et al., 2006).

1.1 RESEARCH ENVISAGED

Drugs such as Pioglitazone and Rosiglitazone have proved efficacy in controlling Alzheimer's disease in clinical setting but they are ineffective when administered

through oral route due to their inability to penetrate BBB. Hence it was envisaged to develop targeted nanoparticulate systems in an attempt to target the drug delivery system to the brain and release the drug in brain to have a simultaneous anti-diabetic and anti-alzheimer's activity.

Further the objective of the present study was to develop nanoparticulate drug delivery system and nasal delivery system of anti-diabetic agents (Pioglitazone and Rosiglitazone) for the effective management of diabetes mellitus and related neurodegenerative disorder (i.e. Alzheimer's disease). Thus it was aimed:-

1. To develop and evaluate nanoparticulate drug delivery system of Pioglitazone and Rosiglitazone using PLGA as polymer which is generally regarded as safe (GRAS) as per FDA and attach brain targeting ligand to the developed nanoparticulate system to penetrate BBB and provide the desired therapeutic activity in the brain.
2. To develop and evaluate a nasal solution system of the drug(s) Rosiglitazone and Pioglitazone, to be administered through nasal route to bypass the BBB and hence provide the desired therapeutic activity in the brain.

Therefore, incorporation of the drugs viz. Pioglitazone and Rosiglitazone in nanoparticulate system and actively targeting to the brain as well as passively targeting to the liver would provide the desired therapeutic activity, at the same time, decrease the cardiovascular side effects. The proposed drug delivery systems (targeted nanoparticles and nasal solution) will be safer and more effective as an alternative therapeutic system for highly prevalent and chronic disease like T2DM and associated Alzheimer's disease.

Transferrin was chosen as the targeting ligand due to over expression of transferrin receptor on brain capillary endothelial cells in addition to its expression on liver and muscles. Hence transferrin conjugated nanoparticles were prepared which would bound to transferrin receptors and specifically deliver the encapsulated drug only to brain in addition to liver and muscle cells, which will provide the required anti-alzheimer's activity with reduced cardiovascular side effects of these drugs.

These brain targeted drug delivery systems are expected to fulfill the following objectives:-

1. Targeted delivery to penetrate the BBB and provide new therapeutic activity (anti-alzheimer's) in addition to the established anti-diabetic activity.
2. Sustained delivery with reduced frequency of dosing (with NP's system).
3. Reduced incidence of hypoglycaemic attack and cardiovascular risk's during treatment.
4. Reduction in other side effects of drug(s) due to reduction in normal recommended dose(s).
5. Optimized therapy and improved patient compliance by the use of nasal drug delivery system with nasal drops in the form of solution.

1.2 PLAN OF WORK

The present work was planned as follows:

1. Literature survey and selection of drugs
2. Preformulation studies of selected drugs
   i. Identification and characterization of drug(s) sample by UV and IR spectroscopy
   ii. Melting point determination
   iii. Solubility determination

iv. Partition coefficient determination.

3. Development of analytical methods for estimation of drug(s) by UV spectrophotometery
4. Preparation of PLGA nanoparticles encapsulating drug (Pioglitazone/ Rosigliazone) and optimization of variables like amount of polymer, concentration of drug, concentration of surfactant, concentration of stabilizer, sonication time, stirring rate, stirring time, etc.
5. Conjugation of ligand (transferrin) to the optimized PLGA nanoparticles bearing drug (Pioglitazone/Rosiglitazone).
6. Characterization of developed formulations for
   i. Particle size and Polydispersity Index
   ii. Zeta Potential
   iii. Percent drug entrapment
   iv. *In vitro* drug release study in phosphate buffer saline (pH 7.4)
   v. Confirmation of ligand conjugation by FTIR and NMR
   vi. Morphological study by TEM
   vii. Determination of physicochemical interaction by DSC and XRD studies
7. Stability studies of optimized formulations in accordance with ICH guidelines
8. *Ex vivo* study on selected cell lines to study the neuro-protective effect of developed formulations
9. *In vivo* biodistribution study of optimized formulations by gamma scintigraphy
   i. Radiolabeling and its optimization of selected formulations
   ii. *In vivo* organ distribution study
10. Summary and conclusion.

REFERENCES:

- Convit, A., Wolf, O.T., Tarshish, C., de Leon, M.J. Reduced glucose tolerance is associated with poor memory performance and hippocampal atrophy among normal elderly. *Proc. Natl. Acad. Sci. USA.* 2003. 100, 2019–2022.
- Galasko, D. Biomarkers for Alzheimer's disease – Clinical needs and application. *J. Alzheimer's Dis.* 2005. 8, 339–346.
- Galea E., Feinstein D.L., Lacombe, P. Pioglitazone does not increase cerebral glucose utilization in a murine model of Alzheimer's disease and decreases it in wild-type mice. *Diabetologia.* 2006. 49, 2153–2161.
- Gibson, G.E, Sheu, K.F., Blass, J.P. Abnormalities of mitochondrial enzymes in Alzheimer disease. *J. Neural. Trans.* 1998. 105, 855–870.
- Glabe, C. Does Alzheimer disease tilt the scales of amyloid degradation versus accumulation? *Nat. Med.* 2000. 6, 133–134.
- Hoyer, S. Glucose metabolism and insulin receptor signal transduction in Alzheimer's disease. *Eur. J. Pharmacol.* 2004. 490, 115–125.
- Masters, C.L., Simms, G., Weinman, N.A., Multhaup, G., Mcdonald, B.L., Beyreuther, K. Amyloid plaque core protein in Alzheimer disease and Down syndrome. *Proc. Natl. Acad. Sci. USA.* 1985. 82, 4245–4249.
- Racchi, M., Mazzucchelli, M., Porrello, E., Lanni, C., Govoni, S. Acetylcholinesterase inhibitors: Novel activities of old molecules. *Pharmacol. Res.* 2004. *50,* 441-451.
- Watson, G.S., Cholerton, B.A., Reger, M.A., Baker, L.D., Plymate, S.R., Asthana, S., Fishel, M.A., Kulstad, J.J., Green, P.S., Cook, D.G., Kahn, S.E., Keeling, M.L., Craft, S. Preserved cognition in patients with early Alzheimer disease and amnestic mild cognitive impairment during treatment with rosiglitazone: a preliminary study. *Am. J. Geriatr. Psychiatry.* 2005. 13, 950–958.