Chapter 1
Introduction

1 Introduction

1.1 Background

Schizophrenia is chronic incapacitating mental disorder represented by number of positive and negative symptoms including delusions, hallucinations, disorganized speech or behavior and impaired cognitive ability [1]. It also results in disintegration of thinking process and emotional responsiveness. Although, intensive research is performed in this area, the precise cause for schizophrenia is unknown so it is accepted that it arises mainly due to interaction among hereditary and ecological factors [2]. Various studies, carried out to estimate etiology of schizophrenia, strongly suggest genetics as predisposing factor for schizophrenia. The statistics also states about 10% risk for first-degree relative while 3% risk for a second-degree relative supporting hereditary cause for schizophrenia. Additionally, schizophrenia risk in monozygotic twins is 48% which reduces to 12% to 14% in dizygotic twins. Furthermore, there is 40% higher risk of schizophrenia in child if both parents have schizophrenia [3, 4].

Currently, schizophrenia is considered to be the foremost cause of disability, worldwide which can be estimated from Figure 1. 1. It has prevalence of 0.6% to 1.9% [5] worldwide diagnosed in about 5.1 per 1000 lives annually [6] and lifetime morbidity of 7.2 per 1000 [7]. The occurrence of schizophrenia is equal both in in men and women but differs in onset time of symptoms i.e. schizophrenic symptoms in men are seen in early 20s while it is seen in late 20s in women [8].

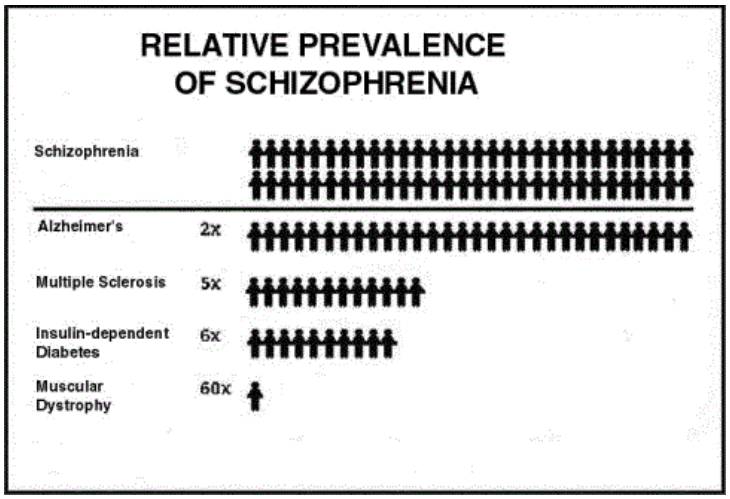

Figure 1. 1: Prevalence of schizophrenia compared to other well-known diseases

Pathophysiology of schizophrenia is described by various theories which depicts abnormalities in neurotransmission. These theories focus mainly on excess or deficiency of neurotransmitters like dopamine, serotonin, and glutamate. Other theories

associate aspartate, glycine and GABA as part of the neurochemical imbalance of schizophrenia [2].

The medications used for schizophrenia treatments are broadly of 2 categories i.e. - typical antipsychotics and atypical antipsychotics.

Table 1. 1: Clinically used antipsychotic drugs with their trade names

Typical Antipsychotics		**Atypical Antipsychotics**	
Drug	**Marketed products**	**Drug**	**Marketed products**
Chlorpromazine	Thorazine®	Aripiprazole	Abilify®
Fluphenazine	Prolixin®	Clozapine	Clozaril®
Haloperidol	Haldol®	Iloperidone	Fanapt®
Loxapine	Loxitane®	Olanzapine	Zyprexa®
Perphenazine	Trilofan®	Paliperidone	Invega®
Thioridazine	Mellaril®	Quetiapine	Seroquel®
Thiothixene	Navane®	Risperidone	Risperdal®
Trifluoperazine	Stelazine®	Ziprasidone	Geodon®

Current medications for schizophrenia are not curative, since people who halt therapy have more than 70% probability to develop full psychotic relapse with within some time. Also, antipsychotics generally do not treat the cognitive deficits (attention, memory) or functional disabilities (social, occupational) that are often most chronic and intractable features of schizophrenia. Furthermore, antipsychotics are accompanied with severe side effects resulting sometimes in discontinuation of therapy. Typical antipsychotics results in neurological and extrapyramidal side effects including tardive dyskinesia, muscle stiffness, temporary paralysis etc. while atypical antipsychotics presents side effects including drowsiness, sexual dysfunction, weight gain etc.

Although, medical science has evolved greatly, mortality rate in schizophrenics has increased which may be accredited to lifestyle (i.e. irregular diet pattern, minimum physical activity, smoking and alcohol intake), adverse effects and suicide [9]. Additionally, pathophysiology of schizophrenia still remains vague which is primarily the main cause for failure of currently available therapies [10]. Even though, progresses on existing drugs and drugs acting at novel neurotransmitters will provide improved treatment, this approach is not as logically satisfying as efforts to find causes. Thus, researchers have sidetracked their focus on the root cause of disease and have concentrated on developing gene therapy. With these efforts numerous genes involved

in pathophysiology of schizophrenia were discovered some of which may be listed as RGS4, DISC1, NRG1, DAOA, CHRNA7, COMT, PRODH, AKT1, DRD3, DTNBP1, G30/G72, HTR2A, SLC6A4, ZDHHC8 etc.

Overexpression of NRG1 gene was considered to play important role in causing schizophrenia [12-17]. It was speculated to control NMDA receptor expression and thus was considered as a vital therapeutic target for schizophrenia [18]. Thus, this research aims to target the aforementioned gene using RNA interference (RNAi) to reduce its overexpression and thus offer anticipated curative approach for schizophrenia.

RNA interference (RNAi) is novel technique which employs antisense therapeutics to knock down overexpressed genes post-transcriptionally [19]. RNAi mechanisms may include short interfering RNA (siRNA) causing degradation of target RNA, or micro RNA (miRNA) which may halt translation of target RNA. RNAi-based therapeutics are mainly classified as: DNA-based RNAi and RNA-based RNAi. In DNA-based RNAi, short hairpin RNA (shRNA) is encoded by plasmid DNA. While, a siRNA duplex is synthesized without a DNA intermediate in RNA-based RNAi.

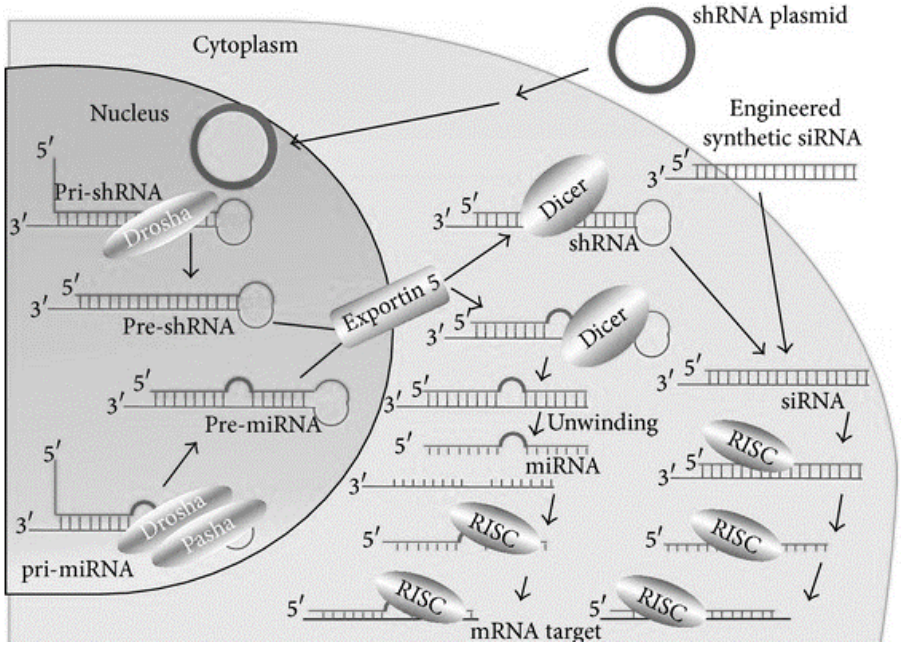

Figure 1. 2: RNAi mechanism

Amongst all RNAi therapeutics, miRNA is non-selective and numerous gene expression can be regulated by single miRNA while shRNA is technologically challenging and also poses several problems regarding delivery and safety. Thus, siRNA proves to be simple and effective strategy for RNAi therapy as it can be

transfected efficiently in target cells, rapidly and economically generated, and can be modified to minimize off target effects [21-23].

There are various hindrances in transport of naked siRNA in-vivo depending on target and route of administration. Degradation by nucleases was primary cause of concern which would render the therapy ineffective. Additionally, there are challenges in delivering siRNA to target site effectively in required doses and also minimize off target effects [24]. All these hurdles can be overcome by conjugating siRNA to suitable vector and then delivering it to target site. Additionally, delivery of gene therapeutics through viral vector could be effective but poses problems like immunogenicity, carcinogenicity and mutagenicity [25]. Thus, effective delivery of siRNA can be sought by conjugating it to non-viral vector and then administer it for in-vivo application.

In case of non-viral vectors, PEI is broadly explored for nucleotide-based therapies like DNA, siRNA and oligonucleotides [26, 27]. It has been also used for administering siRNA by local routes including intrathecal, subcutaneous, intraperitoneal etc. Although its widespread use, there is huge concern regarding its toxicity and thus researchers have diverted their focus to modify PEI resulting in polymer having desired transfection with less toxicity [28-30].

Conclusively, current treatments available for schizophrenia are ineffective providing symptomatic relief and also results in severe side effects worsening the quality lifestyle of the patients. This has provoked the need to develop innovative therapy with reduced side effects and thus the project focuses on developing RNAi therapy (i.e. siRNA) which would target main cause of disease. The use of non-viral vectors was rationalized for efficient delivery of gene therapeutics to the targeted site.

1.2 Objective of the proposed work

The aim of current project was to develop a novel non-viral gene delivery approach for sufficient siRNA entrapment which could transfect brain cells and result in effective treatment of schizophrenia. Furthermore, entrapment of siRNA would prevent its degradation by nucleases and thus would efficiently deliver therapeutic dose to desired site.

1.3 Hypothesis

It is hypothesized that nose to brain delivery of non-viral vector conjugated gene therapeutics would reach to effective site and would give better and safe therapeutic approach for treatment of schizophrenia.

1.4 Research design and Methods

1. Development and characterization of novel non-viral vectors for administration of siRNA.
2. Conjugation of siRNA with formulated vectors to form polyplexes and lipopolyplexes.
3. Formulation and characterization of nasal spray for nose to brain delivery of siRNA conjugated formulations.
4. In-vitro characterization of the formulations to assess its toxicity, membrane permeation and cell uptake.
5. In-vivo studies to determine toxicity and efficacy of the developed formulations.

1.5 Expected results

The literature review proposed that the developed siRNA conjugated formulation, if delivered efficiently to brain cells would effectively result in cure of schizophrenia without any severe adverse effects. The proposed treatment would also result in removal of root cause of disease by targeting genes and thus would result in prolonged efficacy through single dose.

Additionally, the developed formulations would serve as model formulation to deliver any gene to brain cells even for the other psychiatric disease than schizophrenia which would boost the research in field of neurological disease.

1.6 Work plan

1. Literature review
2. Selection and procurement of siRNA and other reagents necessary for the synthesis of non-viral vectors.
3. Synthesis of copolymers and polymer conjugates using PEI.
4. Characterization of synthesized copolymers and conjugates by FT-IR, NMR and light scattering technique to determine molecular weight.
5. Analytical method development of siRNA by UV and agarose gel electrophoresis.
6. Development and optimization of polyplexes.
7. Development and optimization of liposomes and later formulation of lipopolyplexes using the optimized liposomes.

8. Physicochemical characterization of formulations including particle size, zeta potential, conjugation efficiency etc.
9. Formulation and optimization of nasal spray for nose to brain delivery of the prepared polyplexes and lipopolyplexes.
10. Characterization of nasal spray for
    a. pH
    b. Osmolality
    c. Viscosity
    d. Pump delivery volume
    e. Spray pattern
    f. Droplet size distribution
    g. Plume geometry
    h. Weight loss
    i. Tail-off characteristics
    j. Priming and repriming
    k. Microbial limit and preservative efficacy testing
11. In-vitro cell line studies including
    a. Cell uptake and cytotoxicity study
    b. In-vitro permeation study
    c. Gene expression study
12. In-vivo study including
    a. Acute toxicity study
    b. Efficacy study
    c. Nasal ciliotoxicity study
    d. Brain distribution study
13. Stability study

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