ANIMAL MODELS FOR NEUROLOGICAL DISORDERS

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FOREWORD

Drug discovery and drug development nowadays are growing very fast by using new tools and advanced techniques of modern science. Drugs are being synthesized or obtained from natural sources, including marine sources, to get lead molecules. Every such molecule needs to be evaluated in terms of safety and efficacy before it is actually being used for mankind.

Animal models are a basic essential tool to prove the safety and efficacy of any drug molecule in experimental neuroscience. Therefore, the selection of a suitable and reliable experimental model becomes essential. Every experimental model has its own potential advantages and limitations in order to study disease pathologies, and therefore, increasingly, various emerging tools and techniques are being used in addition to it. It becomes imperative for every researcher to be able to select suitable animal models and have an in-depth knowledge of their advantages or limitations while studying disease pathologies.

I am confident that the present book serves the purpose of various researchers working in the areas of neurological problems, in particular Alzheimer's disease, Parkinson's disease, Huntington's disease, depression, autism, *etc*., to understand the various aspects of these experimental models and their potential use to study disease pathologies. The present book has been written with a purpose to provide various key insights related to pathophysiology, experimental (invivo and invitro) techniques, advantages, disadvantages, limitations, including future prospective of various experimental models such as Alzheimer, depression, psychosis, Parkinson, vascular dementia, multiple sclerosis, schizophrenia, autism, Huntington disease and brain tumor model based on researcher's laboratory experience. Biomedical researchers will further benefit from the rich research experiences of the authors working in the areas of neurological problems. Lastly, I would like to commend Prof. Anil Kumar on this brilliant effort.

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PREFACE

The selection of a suitable preclinical, experimental model is one of the challenges for any pharmacologist or biomedical scientist, or researcher. The animal model is one of the essential tools to study any disease conditions and related pathologies. This is also a challenge for a biomedical scientist to choose or develop correct and suitable or reliable animal models to study the respective disease pathology. Every experimental model has its own advantages, limitations, or weaknesses. These limitations and advantages are very important to consider before the selection of any experimental model to study disease conditions.

Authors in the present book made a significant effort to incorporate the details of various experimental models to study different neurological diseases. Details of these models are well mixed up with the researcher's self-experience in the laboratory. This book has been written by a group of active researchers with an aim to cover emerging experimental models of various neurological problems, particularly Alzheimer's disease, psychosis, Parkinson's, Huntington disease, vascular dementia, schizophrenia, neurodegenerative disorders, and brain tumor, *etc*. Authors have made a significant effort to correlate the same with disease conditions. Another feature of the is book is to discuss cognitive dysfunction, which is now very commonly associated with several neurological problems such as diabetes, vascular impairment, and other neurovegetative condition such as Huntington's and Parkinson's disease, *etc*.

This book is intended for all researchers/scientists/ students of biomedical sciences, pharmacology, medical students, pharmacy students, biochemistry, biotechnology, oncologist, neuropsychiatrist, neurologist, *etc*., who are working in the areas of neurodegenerative disease, cognitive dysfunction, and neuropsychiatric or medical sciences or life sciences.

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CHAPTER 1

Accelerating Alzheimer's Disease Research by Pharmacologic, Genetic, and Computational Based Animal Models

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Abstract: Alzheimer's disease (AD) is a type of dementia characterized pathologically by inappropriate neuronal loss in the specific brain regions, mainly in the hippocampus and cerebral cortex, where an accumulation of insoluble plaques of amyloid-beta (Aβ) and tau tangles formation occurs, resulting in progressive memory loss, impaired thinking, deterioration and changes in personality and mood. Alzheimer's disease now possesses a significant health burden and is considered the main source of inability among aged individuals. Recently, Alzheimer's Disease International (ADI) evaluations of 2019 featured that there would be more than 50 million individuals living with dementia around the world, a figure set to increment to 152 million by 2050. Somebody creates dementia-like clockwork, and the current year expense of dementia is assessed at US $1trillion, a figure set to twofold by 2030. AD is the leading cause of dementia and accounts for 60-80% of cases. In spite of the fact that Aβ conglomeration and neurofibrillary tangles (NFTs) development are notable major causative components engaged with AD pathogenesis, the researchers failed to cure or prevent progression of disease effectively by focusing on these pathogenic variables. Thus, tackling AD is a complex job, as we have erudite lately by continuous phase III clinical trial programs failures. Due to the lack of a clear etiology and increased morbidity associated with Alzheimer's disease, there is an immediate need to investigate the underlying causes of the disease and design and develop novel therapeutic agents to slow or reverse disease progression. Animal models mimicking different types of AD-like pathological conditions, which is an essential component in discovering potential therapeutic targets and studying mechanism of action behind that therapeutic agent, as we know, are primary tools in the field of biomedical research including AD. This chapter discusses emerging pathophysiological mechanisms and drug targets, as well as a summary of in-vivo/ex-vivo, *in-vitro*, QSAR, and in-silico models commonly used in Alzheimer's disease research. Moreover, we will also describe how to select suitable

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and valid models and the specifications and relevance of a couple of behavioral assessment methods.

Keywords: Alzheimer's disease, Behavioral animal models, *In-silico* models, Therapeutic strategies, Transgenic animal models.

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disease of the brain involving neuropathological hallmarks such as deposition of plaques of amyloid-beta (Aβ) (outside nerve terminals), the existence of neurofibrillary tangles (NFTs- inside the neurons) produced by aberrantly hyper phosphorylated tau, progressive synaptic loss and neuron degeneration which further leads to decline in memory and cognitive functions as shown in Fig. (**1**) [1, 2]. For quite a long time, AD research has concentrated on the two obsessive neuropathological signs of the disease, *i.e*., amyloid plaques and NFTs. In spite of the fact that amyloid-beta conglomeration and NFTs development are notable major causative components engaged with AD pathogenesis, the researchers failed to cure or prevent the progression of disease effectively by focusing on these pathogenic variables. Tackling AD is a complex job, as we have erudite lately by continuous phase III clinical trial programs failures. The majority of these projects depended on the focusing of Aβ, prompted by amazing research discoveries that Aβ can be cleared from the human mind. Albeit some Aβ-bringing down compounds have applied quantifiable impacts on psychological results, these impacts have commonly been too little to even consider being genuinely critical and clinically significant [3]. Generally, AD is of two types; one is familial Alzheimer's disease (FAD) and other sporadic Alzheimer's disease (SAD), which is also known as early-onset of AD (EOAD) and late-onset AD (LOAD), respectively. Heredity of specific genes is a danger factor for AD, with both familial and sporadic cases happening. Genetic variations in amyloid precursor protein (APP), beta-secretase, and in presinilin-1 (PS1) and presenilin-2 (PS2) genes are thought to be liable for disease production in the FAD. Whereas, in SAD, which is the more normal category, there is a connection with the apolipoprotein 4 (APOE4) allele. The danger is more noteworthy in homozygotic circumstances and metabolic cycle disturbance [4 - 7]. In addition, ecological elements, vascular components, and psychical factors likewise add to the evolution of SAD. As of now, no medications are accessible to end the occurrence of neurodegeneration in AD; the idea of AD treatment is suggestive. For example, acetylcholinesterase enzyme inhibitors, Donepezil (brand name Aricept), Galantamine (Reminyl), Rivastigmine, and Tacrine (Cognex), that advance cholinergic neuronal signaling are utilized in gentle to direct instances of AD [8]. An alternate sort of medication, memantine (Namenda), antagonize N-methyl-D-aspartate (NMDA)

receptor, may likewise be utilized, alone or in the mix with a cholinesterase enzyme inhibitor in moderate to serious cases to forestall excitotoxicity, and antipsychotics and antidepressants are utilized in the treatment of neuropsychiatric side effects [9]. At present, there is no established way to cure AD although research into prevention strategies is ongoing. Due to its complexity, it is far-fetched that any one medication or other intercessions can effectively prompt its legitimate treatment. Recent approaches center around assisting individuals with keeping up mental capacity, overseeing social side effects, and moderating or deferring the manifestations of illness [10, 11]. Scientists desire to create treatments focusing on explicit hereditary, sub-atomic, and cell systems with the goal that the genuine hidden reason for the sickness can be halted or forestalled.

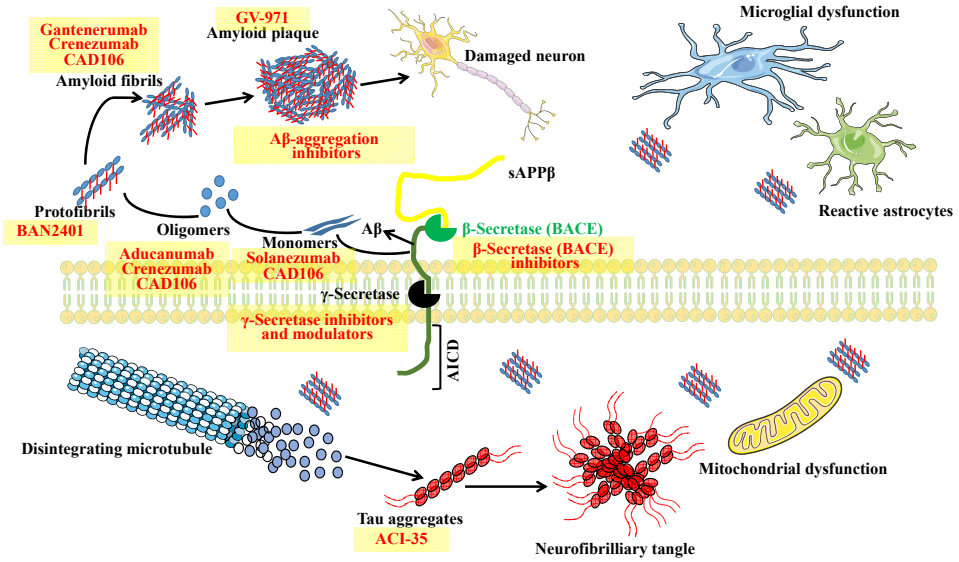

Fig. (1). Molecular mechanism in inhibit A-beta production, clearance, and prevent aggregation. APP: Amyloid precursor protein; AICD: APP intracellular domain; Aβ: Amyloid-beta; BACE: beta-site APP cleaving enzyme 1.

The biggest problem in AD drug development is doubtful mechanisms inherent AD pathogenesis and pathophysiology. Several reported research and existing literature aid the concept that AD is a complex illness. While there is ample manifestation that amyloid plaque is responsible for the pathogenesis of AD, other possible mechanisms have been involved in AD-like tangle formation (tau-tangle) and outspread, neuroinflammation, and altered protein degradation pathways. Therefore, the present-day epitome of AD drug design and development has been modified from a one-on-one target area to a multi-target approach. Here, in this chapter, we will also sum up current techniques and a new way of drug development in the area of AD research, including animal-based (pre-clinical) and

CHAPTER 2

Behavioral and Non-behavioral Models of Depression: Current Scenario And Future Directions

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Abstract: Animal models help understand the possible pathways involved in a disease's pathophysiology, and they offer a significant test to screen the potential of a therapeutic compound. Depression is a chronic mental illness that affects the world's population widely. There are different behavioral and non-behavioral models of depression used in experimental animals to explore and understand the primary mechanism of depression. These models produce different types of depressive symptoms that can correlate with human depressive symptoms. This study highlights the stress and non-stress models by distinguishing the merits and demerits of models used for depression.

Keywords: Anhedonia, Behavioral models, Chronic stress, Depression, Non-behavioral models.

INTRODUCTION

Depression is a common and severe medical illness. Depression causes loss of interest and pleasure, foul mood, low self-esteem, guilt, sleep and appetite changes, and emotional and physical problems. The initial diagnosis usually involves questionnaires based on interests and behavior changes, which measure the possible identification and severity of depression. In 2015, more than 300 million people worldwide, *i.e.*, approximately 4.4% of the world population, were estimated to live with depression. The numbers are rising significantly in lower-income countries due to several factors: a rise in the population, poverty, unempl-

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oyment, physical illnesses, *etc*. Depression is responsible for the most significant number of disabilities and suicides, and the numbers are substantial. According to WHO, 800,000 people commit suicide per year due to depression. Depression affects more than 264 million people worldwide (WHO). According to WHO, the depression rate is higher in women (5.1%) as compared to men (3.6%) [1]. World Health Organization predicts that depression will become the second leading cause of disability in developed and developing countries by 2020 [2]. There are diverse molecular theories on the etiology of depression. After discovering the first antidepressant drug, the monoamine hypothesis of depression came into the picture [3]. According to this theory, the monoamine oxidase enzyme inactivates monoamines (norepinephrine, serotonin, dopamine), leading to a lower monoamines synaptic cleft. Monoamines are mood elevators; therefore, a low level of monoamines ultimately leads to depression [4]. There are currently different antidepressants accessible for clinical practices; major categories comprise tricyclic antidepressants, monoamine oxidase inhibitors, selective serotonin-reuptake inhibitors, and noradrenergic reuptake inhibitors. However, the efficacy of these antidepressants is often inconsistent, and many of them might show adverse effects such as cardiotoxicity, hypertensive crisis, sexual dysfunction, and insomnia [5 - 8]. Apart from monoamines, other evidenced-based pathophysiological reports suggested a considerable involvement of neurotransmitter alterations such as GABA [9], glutamate [10], corticosterone-releasing hormone [11], brain-derived neurotrophic hormone [12], oxidative stress, neuroinflammation [13] involved in the development of depression. However, the exact molecular mechanism of depression remains unclear. None of the above studies has led to a novel satisfactory treatment of the disease. Therefore, to explore the molecular mechanism of depression, various animal models are used in laboratory experiments. The development of animal models of depression has helped identify several drugs' pharmacological mechanisms and potential clinical effects [14, 15].

GENERAL THEORY IN RODENT MODELS OF DEPRESSION

Current depression animal models cannot mimic the disease process, necessitating the use of existing animal models. These manipulations significantly interfere with the outcomes of the depression. Depressive symptoms in humans, such as suicidal thoughts, sad moods, and feelings of guilt, cannot be measured in laboratory animals. Presently, models designed for depression in experimental animals are based on the features that reflect depressive behavior in humans. Models used for experimental animals are based on behaviour measures, hopelessness, helplessness, social withdrawal, motor responses to stress, and changes in sleep and hunger patterns, which are all related to human depression [16].

Animal models are designed based on three criteria:

1. Face validity (phenomenological or morphological appearances) - This validity is attained when the animal shows specific symptoms of depression which are the same as depression symptoms in humans [15], *e.g*., CUMS model offers an excellent face validity as this model reproduces almost all symptoms of depression. Additionally, this model also showed the anhedonia (behavioral nature) as a significant factor that comes under face validity [17].
2. Construct validity (a similar etiology)-. In this validity, symptoms that occurred in the animal model are based on the exact underlying neurobiological mechanisms as in humans [15]. The outcome of this validity is usually less in most currently used animal models for depression because the pathophysiological basis for depression is unknown.
3. Predictive validity (therapeutic similarities) primarily refers to accurately predicting selective and specific responsiveness to antidepressants. *e.g*., in early studies, the CUMS model was shown to respond to chronic stress but not to acute stress. Predictive validity includes the ability of a model to detect treatments that are useful clinically [18] accurately.

The above criteria give a whole management approach in the assessment of an experimental model of depression.

BEHAVIORAL MODELS (DEPRESSION MODELING APPROACH; *BEHAVIORAL TEST)

There is a difference between the behavioral model and the test. Animal models can be defined as a representation of a particular state of an organism that reproduces aspects of human pathology, which provides sufficient predictive, face, and construct validity. On the other hand, behavioral tests should be considered a technical tool and not a model. It gives information only on an end-point behavioral measure to estimate the effect of pharmacological, genetic, or environmental factors [15, 19].

Behavioral Test

1. Forced swim
2. Tail suspension
3. Open field
4. Sucrose preference

CHAPTER 3

Behavioural and Non-behavioural Experimental Models of Psychosis: Current State and Future Aspects

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Abstract: Animal models provide an opportunity to decipher the relationships between the nervous system and animal behaviour as they serve as obligatory tools for screening for new drugs. As psychosis is a chronic and complex mental disorder, therefore, different theories are available. However, the pathophysiology of psychosis is still not fully clear, making it challenging to develop a coherent framework appropriate for animal modeling. Though, limited animal models are available to explore several relevant theories and to evaluate specific mechanistic hypotheses. These animal models have been based on neurotransmitter systems supposed to be involved in psychosis. Now, the emphasis has been shifted to targeting related brain areas to explore possible pathophysiological hypotheses. In the present chapter, the authors have described various behavioural and non-behavioural animal models to test for antipsychotics. Emphasis has been given to the procedure because these models help to shape the direction of future research.

Keywords: Animal model, Behavioural models, Non-behavioural models, Psychosis.

INTRODUCTION

Psychosis is a complex, devastating mental illness, categorized into positive (delusions, hallucinations, and disorganized thought), negative (alogia, social isolation), and cognitive symptoms [1] (Table **1**).

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An individual suffering from psychosis exhibits social withdrawal, emotional detachment, and cognitive-emotional decline [2]. This disorder is multifactorial neurodevelopment, but its etiology is not completely clear yet [1]. Evidenced-based pathophysiological reports suggested a considerable involvement of neurotransmitter alterations such as dopamine, GABA, glutamate, serotonin and oxidative stress, neuroinflammation, mitochondrial dysfunction, extracellular matrix disturbances, hypofunctioning of NMDA receptor in the development of this illness [2, 3]. Currently, available medicines act by D2 and 5-HT receptors for the treatment of psychosis. Still, these medicines only treat one or two symptoms not effective at halting the root of the disorder, and are also associated with withdrawal symptoms and some severe side effects [1, 4]. To overcome these shortcomings, researchers are involved in exploring new compounds. Experimental models are employed to understand the pathophysiological mechanisms involved in psychosis. The animal models also created new theories associated with the human disorder and opened new pathways to investigate new compounds. It is not easy to develop an animal model to test for antipsychotics since their brain is not developed. Models based on neurotransmitters involve altering the neurotransmission dopaminergic, GABAergic, glutamatergic, and serotonergic systems [4]. Lesions produced by chemicals damage the specific region, genetic models (through genetic manipulation), and a neuro-developmental model of brain-damaged induced psychosis in animals [5]. An ideal model must have face (behavioral changes), construct (replicate theoretical pathophysiology), and predictive (disease symptoms reversed by the therapeutic agents) validity. For psychosis, behavioral abnormalities include stereotypy, hyperactivity, stress, abnormal reactions to reward, social isolation, cognitive decline, *etc* [6]. Further, neurobiological abnormalities are mesolimbic and mesocortical dopamine dysregulation, cortical glutamatergic hypofunction, loss of connectivity between cortex and hippocampus, and activity [2]. Recently, animal models of psychosis have been categorized into behavioural and non-behavioural models (Table **2**). Most of the animal models of psychosis imitate the features of the positive symptoms of psychosis.

Table 1. Symptoms of Psychosis.

Positive Symptoms	Negative Symptoms	Cognitive Symptoms
Psychotic episode (inability to separate real and unreal events) Hallucinations (strong perceptions about an object, voice, and event, which are unreal) Delusions (false beliefs/ misconception of perceptions)	Alogia (thought and speech dysfunction) Affective flattening(lack of emotional expression) Anhedonia (decreased capability to feel pleasure) Avolition (decreased motivation) Social withdrawal	Reduced ability to learn things Reduced verbal fluency Impairment in focusing attention, concentrating, and behaviours

Table 2. Pharmacological animal models of psychosis.

Behavioural Model	Non-behavioural Model
Artificial hibernation in rats	Dopaminergic Agonist
Brain self-stimulation	GABA- Antagonists
Catatonia in rodents	NMDAR antagonist induced psychotic symptoms in rodents
Foot-shock induced aggression	Serotonin-Agonists
Golden hamster model	Lesion models
Influence on behaviour of cotton rat	Lesions in limbic structures
Pole climb avoidance	Yawning and penile erection syndrome in rats
	Prepulse inhibition of startle response
	Latent Inhibition
	Genetic model
	Neurodevelopmental models

BEHAVIOURAL MODELS

Artificial Hibernation in Rats

The independent rat is put into ice-cold water with surfactant for 2 minutes to remove the fur's air. Then the rat is put in a hermetically sealed glass jar with a volume of 750 ml and placed in the refrigerator at 2 °C. The jar is opened every 10 minutes for precisely 10 sec per hour, allowing some air exchange and reducing the accumulation of carbon dioxide. The animal is withdrawn from the jar every time and observed for signs of hibernation-like reduced muscle tone and metabolism and evening sleep produced by inhibition of the sympathetic system leading to a diminution of homeostatic reactions. When animals remain on the

CHAPTER 4

Progressive Experimental Screening Tools and Techniques for Parkinson's Disease: An Update

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Abstract: Parkinson's is the 2nd most common neurodegenerative disease in which symptoms range from several motor (rigidity, tremors, and bradykinesia) and non-motor symptoms (cognitive impairment). These symptoms mainly arise due to alterations in dopaminergic pathways that disturb dopamine release, transmission, and storage. Animal models are employed to study human diseases to understand the disease's genetic and pathophysiological aspects. Several pathological conditions, such as the deposition of Lewy bodies, endoplasmic reticulum stress-induced unfolded proteins, and neuroinflammation, result in the degeneration of dopaminergic neurons. These reasons make the screening and evaluation of antiparkinsonian drugs more tedious and difficult. Animal model of Parkinson's includes neurotoxin model (MPTP, 6-OHDA, Paraquat, rotenone] and newer genetic model [α–synuclein, LRKK2, PINK). In this chapter, we have focused on the mode of action, advantages, and disadvantages of animal models of Parkinson's disease.

INTRODUCTION

Parkinson's is the second most common progressive neurodegenerative disease that affects 1% population above the age of 55 [1]. Parkinson's occurs due to selective degeneration of dopaminergic neurons in the nigrostriatal dopaminergic pathway consisting of neuronal extension from substantia nigra pars compacta (SNpc) to striatum or basal ganglia, which is mainly related to motor movement [2]. Hence, Parkinson's disease is characterized by some cardinal motor symptoms, like rigidity, tremor, bradykinesia, postural insufficiency. Apart from this, some non-motor symptoms, like neuro-psychiatric and cognitive impairment, are also seen in patients with Parkinson's disease [3, 4].

There are many pathological conditions, like the deposition of Lewy bodies and endoplasmic reticulum stress-induced unfolded proteins and neuroinflammation resulting in the degeneration of dopaminergic neurons, that make screening and

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evaluation of antiparkinsonian drugs more tedious and challenging [5 - 7]. In this chapter, we will discuss the various models for Parkinson's disease ranging from the classical neurotoxic model (MPTP, OHDA, parquet, rotenone) that produces oxidative stress and eventually cell death of dopaminergic neurons to a new genetic model that causes mutations in genes (like α-synuclein, Parkin, LRKK2, PINK-1, DJ-1) as a potential cause for Parkinson's disease [8]. We also try to discuss the advantages and disadvantages and mechanisms behind the various models.

PREVALENCE OF PARKINSON'S DISEASE

Parkinson's disease (PD) is a common neurological disorder. Epidemiological studies have shown the risk factors involved in this disease. Meta-analysis of international studies has depicted the rising incidence with age in both men and women in PD. Males with all age groups had a higher incidence of PD, mostly between 60-69 and 70-79 years of age [9]. Poisson regression adjusted by age is used to analyze each year's trend of PD's prevalence and incidence. Therefore, epidemiological data helps in guiding us towards the effective planning of medical services. Many studies expressed the possible reasons for the divergence between men and women and its increased incidence in men. They were found to be caused by estrogen's neuroprotective effects, a higher frequency of exposure to toxins and minor head trauma in men, and recessive susceptibility genes on the X chromosome [10]. Lifestyle changes, like the increased use of tap water instead of well water, environmental changes like decreased population working in agriculture, dietary changes, excessive consumption of caffeine-containing drinks, also increase PD incidence.

A published report estimated that the positive predictive value of the clinical diagnosis for the whole group was 85.3%, with 122 cases correctly clinically diagnosed, 98.6% (72 out of 73) for idiopathic PD, and 71.4% (50 out of 70) for other parkinsonian syndromes [11]. Positron tomography (PET) and single-photon emission computed tomography (SPECT) are imaging techniques that detect and display radiolabeled tracers' distribution within the body. Patients with PD showed a reduced tracer accumulation in the striatum using markers of the presynaptic dopaminergic system in the very early stages of the disease. Although these methods are promising, they have not been developed to the extent that they can be used outside the research setting. Their broad application is difficult because they are not widely available [12].

Globally Parkinson’s disease caused 211296 deaths (95% 167771 – 265160; 95% 73702-118421 in women and 117784, 93729-147607 in men) and 3.2 million daily 1.1- 1.7 in women and 1.8 in men in 2016. The number of daily deaths was

2.6 times higher in 2016 than in 1990 because of increased age-standardization rates from 1990- 2016. It has been reported that many health risks are associated with smoking tobacco, drinking alcohol, cigarette, coffee, but these are inversely associated with the risk of developing PD. A meta-analysis indicated a 40% reduced risk of PD in smokers. This may be due to its neuroprotective action. One report suggested that inverse relation to smoking with PD is only present in those with a specific monoamine oxidase B allele and many other interactions of genes with smoking. Among the potential responses available, the disease can be prevented by increasing physical activity earlier in adulthood, decreased exposure to pesticides, improving worldwide access to healthcare, and effective treatments already available like levodopa. Also, increasing the funding for research can help us develop new therapies for these kinds of diseases [13].

1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine (MPTP) Model

MPTP mimics most of the chemical features of Parkinson's disease and induces the main biochemical and pathophysiological hallmarks akin to Parkinson's disease. In 1982, a short parkinsonian epidemic was reported in patients with drug addiction. After the investigation, it was reported that all of them were injected through IV, an illicit Street drug called super Demerol, that has a significant amount of MPTP due to which they developed movement disorder similar to Parkinson and also had a low concentration of HBA in the CSF [14].

Mechanism of MPTP Neurotoxicity

Selective neurotoxicity induced by MPTP drastically increased the research perspective on Parkinson's disease and rejuvenated it. MPTP is a lipid-soluble, amphiphilic compound that rapidly penetrates the blood-brain barrier (BBB) and enters the acidic organelles (mostly lysosomes) of astrocytes in the brain. MPTP itself does not produce neurotoxicity, but its oxidized metabolite 1-methyl 4-phenyl pyridinium (MPP^+) is toxic. MAO-B (monoamine oxidase-B) present in the serotonergic neurons and astrocytes is responsible for the conversion of MPTP to MPP^+. The oxidized product of MPTP (*i.e.*, MPP^+) reaches the extracellular fluid and then is transported to dopaminergic neuron terminals by dopaminergic transporter (DAT) and vascular monoamine Transporter-2 (VMAT-2). Although the exact mechanism underlying MPP^+ toxicity is not clear, the following mechanisms of toxicity are proposed [15].

Interference with Mitochondrial Respiration

MPP^+ energetically concentrates into the mitochondria and blocks the complex-I involved in the mitochondrial oxidation. This blockade of mitochondrial oxidation leads to impairment of ATP formation and inhibits energy-dependent ion

CHAPTER 5

Overview on Experimental Models of Vascular Dementia and Vascular Cognitive Impairment

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Abstract: Vascular cognitive impairment (VCI) encompasses vascular dementia (VaD) and is the second leading form of dementia after Alzheimer's disease (AD) plaguing the elderly population. VaD is a progressive disease that affects cognitive abilities, especially executive functioning. At present VaD lacks suitable animal models, which constrain the progress of identification of molecular and cellular mechanisms of the disease and developing suitable interventions. In this chapter, we will present and discuss the experimental animal models that have been used for VaD studies. The limitations and strengths of these models, along with important research findings, are also addressed.

Keywords: CADASIL, Cognition, 2-VO, Vascular Dementia, Vascular Cognitive Impairment, White Matter.

INTRODUCTION

Vascular cognitive impairment (VCI) is a term that includes a continuum of cognitive deficits with cerebrovascular pathology contribution, ranging from mild cognitive impairment to vascular dementia (VaD). Vascular dementia (VaD) is the leading form of dementia after Alzheimer's disease (AD), accounting for about 17–20% of all dementia patients [1]. Patients suffer from loss of executive functions like problem-solving, thinking, judgment, working memory, reasoning, planning, and execution of tasks, with increasing task complexity leading to a decline in performance. Common etiologies of VCI and VaD include cardiovascular disease, stroke (atherosclerosis, small vessel disease, myogenic stroke disorders, *etc*.), and various other mechanisms (*e.g*., oxidative stress, metabolic disorders, amyloid angiopathies, metabolic disorders, *etc*.) [2]. VaD has been classified as small vessel dementia [subcortical ischaemic VaD (SIVD)],

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large vessel dementia (multi-infarct dementia and strategic infarct dementia), mixed [usually with Alzheimer's (AD-VaD)] and hemorrhagic, ischaemichypoperfusion, and dementias resulting from specific arteriopathies [3]. At present, there are no particular criteria for the diagnosis of VaD due to the complexity and diversity in cerebrovascular pathologic conditions, progression, risk factors, and severity of disease [4]. As such, it is essential to get a better understanding of VaD. Several animal models are currently available to study how vascular disease contributes to dementia. In this chapter, we aim to discuss various animal models and highlight the vascular pathologies present in each model.

Etiopathogenesis

Vascular factors (myocardial infarction, arterial hypertension, atrial fibrillation, coronary heart disease, diabetes, lipid abnormalities, generalized atherosclerosis, smoking), genetic factors (*e.g*., family history, individual genetic features), demographic factors (*e.g*., age, education), and stroke-related factors are risk factors associated with vascular dementia [5]. The pathology and mechanisms underlying VaD are yet to be fully understood. An increase in inflammation and oxidative stress (due to β-amyloid and the enzyme nicotinamide adenine dinucleotide phosphate (NADPH) oxidase pathway) was observed in blood vessels due to various pathogenic mechanisms such as AD, amyloid deposition, atherosclerosis, aging, and hypertension [6]. Microvascular dysfunction along with inflammatory factors such as Matrix Metalloproteinases (MMPs) are known to degrade the BBB, increasing BBB permeability to infiltration of inflammatory factors like TNFα (tumor necrosis factor), interleukins (IL-1, IL-6), TLR4 (toll-like receptor 4), MMPs (MMP 2,9), and C-reactive protein. Thus, cerebrovascular dysfunction and BBB alterations may contribute to neuronal dysfunction and cognitive deficit alterations by compromising the cerebral microenvironment and increasing the vulnerability of regions critical for cognition to ischemic-hypoxic brain damage [6]. Furthermore, hypoxia-induced oligodendrocyte damage may also contribute to demyelination, which is associated with delays in neural signal transmission and, ultimately, cognitive loss.

CLASSIFICATION AND CLINICAL CRITERIA

Vascular dementia has been divided into subtypes based on clinical, neuropathological, and radiological features. Various subtypes of vascular dementia include multi-infarct dementia (cortical lesions), small-vessel dementia or subcortical ischaemic vascular disease and dementia (SIVD) (subcortical deep lesions), and strategic infarct dementia. While the NINDS-AIREN criteria have differentiated VaD into cortical vascular dementia, subcortical vascular dementia,

Binswanger's disease, and thalamic dementia, the ICD-10 includes six subtypes (acute onset, multi-infarct, subcortical, mixed cortical and subcortical, other, and unspecified) [7]. Regarding diagnostic criteria, the DSM-IV and ICD-10 require evidence of focal neurological damage and significant cerebrovascular disease [5]. The NINDS-AIREN research criteria, which is still widely used in clinical drug trials for vascular dementia, include dementia syndrome, cerebrovascular disease, and a relationship between the two [8]. The shortcoming of these criteria includes a lack of aetiological criteria, detailed guidelines (*e.g.*, of unequal cognitive deficits), and changes in neuroimaging and heterogeneity.

Vascular Cognitive Impairment (VCI)

Animal Models of VaD

At present, there are no FDA-approved treatment options for VaD. Therefore, a suitable animal model is the first step for defining disease-related mechanisms and developing novel therapeutic approaches. In this chapter, we will discuss various animal models relevant to vascular dementia and the extent to which these models reflect different characteristics of the disease (Fig. **1**).

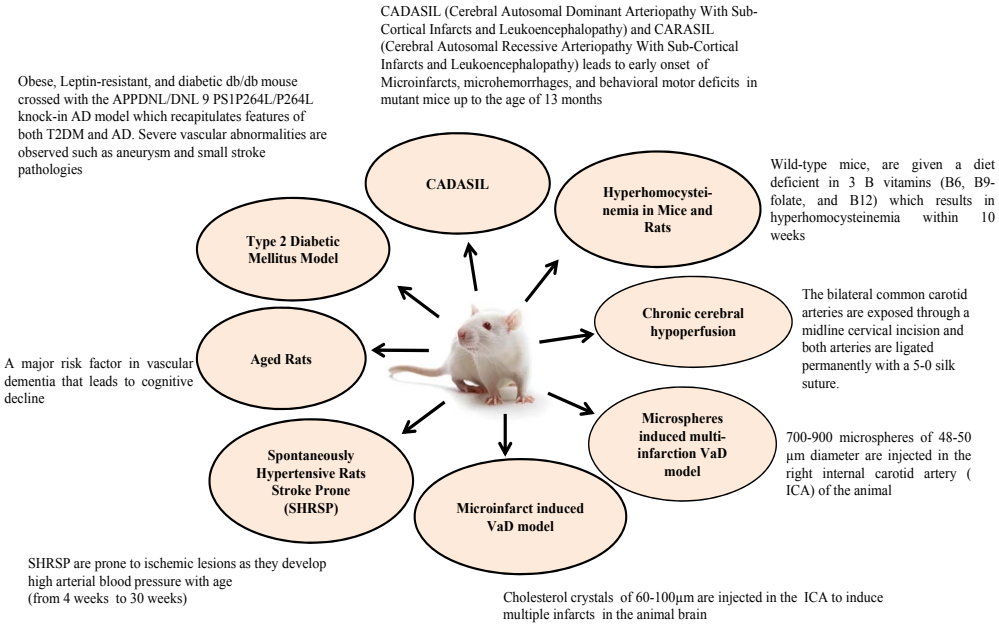

Figure 1: An Overview of common animal models for vascular dementia

Fig. (1). An overview of common animal models for vascular dementia.

CHAPTER 6

Recent Updates in the Animal Models of Multiple Sclerosis

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Abstract: Multiple sclerosis (MS) is a chronic inflammatory, autoimmune disease characterized by neuronal demyelination of the Central Nervous System (CNS). It affects more than 2 million people worldwide. Animal models are of great importance in elucidating immune-pathological mechanisms of MS. The three most commonly studied categories of MS animal models are (1) the Experimental Autoimmune Encephalomyelitis (EAE); (2) chronic demyelinating disease models through virus inoculation known as Theiler's Murine Encephalomyelitis Virus (TMEV) infection and (3) toxin-induced models of demyelination, comprising the focal toxin-induced demyelination by lysolecithin (lysophosphatidylcholine), ethidium bromide, anti-galactocerebroside (GaIC) antibody) and systemic toxin-induced demyelination by cuprizone. EAE is a widely accepted animal model that reflects the pathological mechanisms of MS, making it highly useful to analyze new therapeutic approaches. However, TMEV infection and toxin-induced models are most suitable for studying the role of de and remyelination processes and axonal injury or repair in MS. Furthermore, Zebrafish models have also emerged in recent years as novel animal models for MS because of their swift development and controllable genetic manipulations. In a nutshell, despite their limitations, animal models remain the most useful research tools to answer specific research questions related to pathological mechanisms and to validate potential experimental therapies for MS.

Keywords: Animal models, Demyelination, Experimental autoimmune encephalomyelitis, Remyelination, Theiler's Murine Encephalomyelitis Virus, Zebrafish.

INTRODUCTION

Multiple sclerosis (MS) is an autoimmune neurodegenerative disorder characterized by chronic neuroinflammation and demyelinated neurons in the

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Central Nervous System (CNS). It is the foremost cause of non-traumatic infirmity among young adults and has pronounced socioeconomic influence in developed countries. MS is a complex disease with varying signs and symptoms, which depends mainly on the region or area (sensory, visual, and cognitive functions) of the CNS (brain and spinal cord) affected besides autonomic infirmities. In the 1800s, features of MS were described for the first time and recognized as a disease that causes function disabilities. According to a recent estimate, around 2 million people worldwide are affected by this autoimmune disorder. MS is more prevalent in females (3:1) than males. The US alone has 300,000 to 400,000 individuals affected by MS [1 - 3]. MS is classified into various subclasses based on the disease pathology, such as Relapsing-Remitting (RR) MS, the most common (85%) and characterized by exacerbations followed by subsequent remission periods. The Primary Progressive (PP) MS is the second major subclass, observed in 10-15% of patients. Generally, the 20-40 years old age group is most affected by MS; however, 2-10% of adults above 50 years are also affected by MS [4 - 6].

The three major classes of neural cells of vertebrate CNS include neurons, astrocytes, and oligodendrocytes. Neuron’s function is to rapidly transmit the information in the form of action potentials through the brain and spinal cord with a high level of conformity. Different types of neurons arise from various CNS parts characterized by specific neurotransmitters, connectivity, and morphology determined by intrinsic factors and surroundings in which the neuroblasts grow [7]. Astrocytes constitute the second major class of neural cells of the CNS. These heterogeneous cells perform multiple and diverse functions that upkeep neuronal development and maintain the neural microenvironment in CNS [8]. During neuronal development, astrocytes and their precursors guide neuronal cell bodies and act as a substrate to grow axons towards the final destination. Furthermore, astrocytes in the adult brain remove excess neurotransmitters, maintain an ionic environment and stabilize the blood-brain barrier (BBB). CNS insults resulting in disruption of BBB cause hypertrophy and proliferation of astrocytes in insult vicinity, leading to dense glial scar or gliosis that inhibit axonal regeneration and myelin repair, a less known function of astrocytes. The third main class of neural cells of adult CNS is oligodendrocytes originating from progenitors and spreading throughout the CNS. Oligodendrocytes secrete myelin sheath. Myelin sheath offers insulation and shield to neuronal axons. Myelin sheath consists of several plasma membrane layers that intensify the action potential's conduction velocity and decrease the firing threshold of an action potential. Myelin sheath is discontinuous along the axon, and the region between the two-myelin sheaths is called the Node of Ranvier. It is composed of a high concentration of ion channels essential for the conduction of electrical signals. This region is most prone to injury, and any injury to the Node of Ranvier disrupts the axonal conduction.

PATHOGENESIS OF MULTIPLE SCLEROSIS

MS is a multifaceted autoimmune demyelinating disease. The pathogenesis of MS is obscure, but several immunes, environmental, and genetic factors have a pivotal role in its pathophysiology. Until recently, its cause was considered as the focal demyelination of white matter. However, this concept has been shifting, with growing research reporting frequent demyelination in the grey matter [9]. Pathological studies revealed that CNS tissue taken from MS patients shows discrete lesions, inflammation (due to infiltration of immune cells and cytokines), focal demyelination, gliosis or scar formation, and considerable axonal damage [10, 11]. Previous studies have proved that autoimmune reactions started by pathogen-specific molecule's interaction with antigen-presenting cells (APCs) release specific cytokines, such as IL-12, IL-23, and IL-4, which in turn induce T helper (T_H) cells (also known as $CD4^+$ T cells) T_H1, T_H2, or T_H17 phenotypes to release particular cytokines such as Interferon-gamma (IFN-γ) or type II interferon and tumor necrosis factor-alpha (TNF-α) which further promote inflammation [12, 14]. In addition, $CD8^+$ T cells (or cytotoxic T cells) via the production of cytolytic proteins (e.g., perforin) facilitate suppression and inactivation of $CD4^+$ T cells. Further, these cells increase vascular permeability, terminate glial cells, and stimulate oligodendrocyte death [13, 15]. B cells promote inflammation via the secretion of TNF-α and lymphotoxin or TGF-β. In addition, B cells also secrete IL-10, an antiinflammatory cytokine showing mixed effects in disease development [16]. Furthermore, Fas ligand (FasL) is produced by lymphocytes, which binds to Fas receptors on oligodendrocyte cells and initiates the apoptosis process. In addition to CNS inflammation, the myelin repair process due to oligodendrocyte death is also damaged [17].

ENVIRONMENTAL FACTORS

Exposure to viral agents such as Epstein Barr Virus (EBV), human herpesvirus type-6, and bacterial agents such as *Mycoplasma pneumonia* along with smoking, vitamin D and vitamin B12 deficiency, diet, and UV radiation exposure also play a crucial role in the onset of multiple sclerosis [18].

GENETIC FACTORS

Genetic predisposition may also be involved in the pathogenesis of MS. Studies have shown that in monozygotic twins with 100% similar genetic makeup, the risk rate is almost 25%. Dizygotic twins and first-degree relatives with 50% genetic similarities are at higher risk [19 - 21]. Furthermore, the Human Leukocyte Antigen (HLA) region on chromosome 6 containing genes such as HLA-DR2+, HLA-DQ6, DQA 0102, and DQB1 0602, HLA-DRB1, DR15, DRB1*1501, DRB1*1503, and IL-7, IL-2 genes are associated with the onset of

CHAPTER 7

Animal Models of Schizophrenia and Associated Cognitive Dysfunction

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Abstract: Schizophrenia, a chronic debilitating brain disorder, affects about 1% of the world's population and is one of the most complex diseases in psychiatry. Despite intensive research, the molecular etiology and pathophysiology of the disease remain ambiguous and limited. Modeling aspects of schizophrenia in animals is critical for understanding the pathophysiology of the disease and may play a pivotal role in the development of novel treatments. This chapter aims to review various animal and in-vitro models relevant to schizophrenia and discuss various aspects to comprehend the pathophysiology, mimic the symptoms and utility in novel target identification and development. The clinical symptoms of schizophrenia are broadly classified as positive, negative, and cognitive, with the current treatments focusing mainly on positive symptoms and a limited focus on negative and cognitive symptoms. We further focus on the models to evaluate various cognitive deficits associated with schizophrenia which tend to be long-lasting, like working memory, visual memory, attention, and social cognition.

Keywords: Animal models, Genetic model, *In-vitro* model, Lesion model, MAM model, Pharmacological model, Post-weaning social isolation model, Schizophrenia.

INTRODUCTION

Schizophrenia, a chronic debilitating brain disorder that has been mentioned as one of the top ten causes of disease-related disabilities in the world, affects about 1% of the world's population [1]. This complex psychiatric disorder manifests in late adolescence or young adulthood [2]. In the ten-year follow-up study, the prevalence and predictors of suicide in schizophrenic patients were examined, which was 16 times higher than that in the general population [3]. It is a major mental disorder that is characterized by positive (hallucinations, delusions, thou-

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ght disorder), negative (alogia, anhedonia, avolition), and cognitive symptoms. Dysregulation of brain dopaminergic level, as evident from the dopamine hypothesis, is the dominant theory of schizophrenia, and patients experience relief from positive symptoms by responding to first-generation antipsychotic drugs (D2 blockers) [4]. It has been evaluated that the drug which increases dopaminergic transmission, like amphetamine, can worsen the psychotic symptoms in a schizophrenic patient [5]. Glutamate, an excitatory neuro-transmitter, acts on inotropic receptors, *i.e*., NMDA (N-methyl-D-aspartate receptor) and AMPA receptors, which play a pivotal role in learning and memory (synaptic plasticity) [6]. Various experimental observations have demonstrated that the glutamate system plays an important role in schizophrenia as the drugs ketamine and PCP, being the NMDA antagonists, produce positive, negative, and cognitive symptoms [7]. In the postmortem studies as well as animal models, glutamate levels were found to be high in the basal ganglia region of the brain [8]. On the other hand, a wide array of treatments are available for this mental disorder, but all of them have some limitations [9]. Treatment with typical antipsychotic drugs leads to various extrapyramidal side effects due to the blockage of a substantial number of D2 receptors in the nigrostriatal dopaminergic pathway. Antipsychotic medications also fail to treat various cognitive and negative symptoms, which is the leading cause of morbidity occurring in schizophrenia [10].

Cognitive defects, which are one of the integral features of schizophrenia, results in impairment in working memory, visual learning and memory, attention, and social cognition [11]. Based on the above hypothesis, various animal models have been developed to recapitulate clinical symptoms of schizophrenia. It also draws the focus on the role of various neurotransmitters in psychiatric diseases [12]. The development of good and validated animal models will allow a better understanding of this psychiatric disease and will also help test various novel targets.

ANIMAL MODELS

Lesion Model

In neonatal lesion model (PND7) of ventral hippocampus (vhip) of the rat, local injection of ibotenic acid (0.3microlitre) or artificial cerebrospinal fluid bilaterally infused for a 3 min period by a 1 microlitre Hamilton syringe aiming that the tip of injection needle should reach the ventral hippocampus(anteroposterior-3.0mm,medio-lateral+3.5mm and ventrodorsal-5.0mm relative to bregma) [13]. There is a decrease in the number of dendritic spines in the prefrontal cortex, basolateral amygdala, and nucleus accumbens [14], as well as a decrease in the expression of NRP/B, which is a nuclear matrix protein that regulates gene

expression, cell cycle, and nuclear structural integrity in the late developmental phase. It is expressed in the neuroectodermal area of the epiblast and is the insoluble framework of the nucleus [15]. The lesion in rats on day 7 [16] produces an increase in the locomotion, which on further stimulating by the novel environment and social isolation, aggravate the locomotion effect of NVH lesion validated by open field test hypothesized as an increase in dopamine content in the hippocampus region [17]. Selective spatial learning deficits have also been demonstrated by using two radial arm maze tasks [18]. Because of its proficient features, it is one of the experiential models of schizophrenia as it mimics the positive symptoms of schizophrenia which are verified by behavioral models.

MAM Model

Methylazoxymethanolacetate, a DNA alkylating agent, is one of the neurodevelopmental models, given at gestational day 17 in rodents, will result in a change in mRNA or protein expression of D2 and D3 receptors of specific brain regions like the hippocampus, prefrontal cortex, and nucleus accumbens which will result in cognitive, social and affective impairments [19]. This model measures both electrophysiological and behavioral parameters, which can be ascribed to disrupted GABA-mediated inhibition within the ventral hippocampus due to increases in dopamine levels [20]. There is a study that tells that this model in mice identifies sex differences in which both male and female mice show thinning of the cortex and hippocampus, which is an indication of positive symptoms, but the PV (parvalbumin) reduction is only seen in male mice [21].

Post-Weaning Social Isolation Model

To study the role of early life experiences in the change in the expression of normal brain and neural brain development, the most valuable model is the post-weaning social isolation model [22]. As it is understood that early stress in life may lead to the risk of the development of psychiatric disorders, and this model will cause long-lasting changes in the development of brain function and behavior. Social deprivation of rat pups from the age of weaning (by placing them in separate cages from littermates) for 4-8 weeks [23] alters brain development and causes behavioral deficits in adulthood, which includes a decrease in adaption to a novel environment, increased or alle*via*ted anxiety, related behavioral parameters evaluated in elevated plus maze and impaired learning and memory [24].

CHAPTER 8

Neuropsychopathology And Neurobehavioral Characteristics of PPA-Induced Autism Like Rat Model and Its Correlation with Gut-Brain Dysbiosis Occurring in Autism Spectrum Disorder

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Abstract: Abstract: Autism Spectrum Disorder (ASD) involves social interaction deficit, impaired communication skills, and pervasive and stereotypic behavior. It also involves co-morbidities such as anxiety, aggressive nature, and epilepsy. Apart from the above, this disorder also affects physiological co-morbidities that co-exist with behavioral symptoms, such as immune system and mitochondrial dysfunction, and gastrointestinal complications, leading to oxidative stress neuroinflammation, further worsening the behavioral complications. It has been reported that 23%-70% of patients who have ASD account for gastrointestinal complications, which correlate with behaviors relevant to autistic endophenotype. A strong gut-brain dysbiosis occurs in ASD patients due to the enormous production of short-chain fatty acids such as propanoic acid (PPA) by abnormal gut-flora, worsening the behavioral neurochemical and mitochondrial dysfunction. This further leads to the generation of free radical species responsible for synthesizing pro-inflammatory cytokines, which cause microglia activation. There are various animal models of autism, such as the induced animal model and transgenic animal model, which could give valuable hints toward understanding the molecular, cellular, and pathomorphological processes involved in this neurodevelopmental disorder heterogeneous and has a multifactorial origin. However, though all animal models focus on establishing the face validity of ASD, very few focus on construct validity and predictive validity about gut-brain dysbiosis in ASD patients because of the abnormal gut-flora leaky-gut phenomenon. Thus, in this chapter, our focus would be to understand the phenomenon of gut-brain cross-talk in ASD, the role of short-chain fatty acids, and to bring forth the neuropsychopathology of propanoic acid (PPA)-induced rat model of ASD, which can help in establishing construct as well as predictive validity with the gut-brain cross-talk and the neuroimmune as well as behavioral complications occurring as a result of short-chain fatty acids and abnormal gut flora.

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Keywords: Autism, Gut-microbiota, Inflammatory cytokines, Neurobehavior, Propanoic acid.

INTRODUCTION

Autism Spectrum Disorder (ASD) is an interlaced heterogeneous disorder appertaining to various categories of neuropsychiatric disorders affecting brain development and behavior. Although the etiology is not well defined, scientific evidence reveals hereditary and environmental factors at the root cause. Behavioral characterization of ASD can be done by observing social deficits, restricted and repetitive behavior in children with the usual age of diagnosis being three years. Diagnosis can be made as per DSM-5 guidelines, which defines ASD as a complex of Asperger syndrome, pervasive developmental disorder, and autistic disorder. Significant symptoms include obstinate social interaction deficits, stereotypic or repetitive movements, and hindered development (lower IQ than normal children) and are the third most common developmental disorder. According to WHO reports of 2019, one in 160 children has ASD [1]. A recent report has revealed Hong Kong to be the country with the highest prevalence rate of autism in 2020, with about 372 in 10,000 children. According to a study conducted in 2018, around 3 million people have autism in India, with every one in 10 children below the age of 10 is affected by autism [2]. Centers for Disease Control and Prevention (CDC) in 2020 reported, based on a 2016 study, that 1 in 54 children in the US is affected by ASD, with boys being 4 times more vulnerable than girls [3, 4]. ASD can bound a person's routine activities, affects their employment opportunities, and subject them to discrimination. It also poses a huge social and economic burden on the person and their families as some people are incapable of living alone due to improper brain development. Due to the unavailability of the cure for the fundamental symptoms associated with ASD, associated symptoms such as irritability, depression, anxiety, epilepsy, and some mood disturbances can, however, be diminished with pharmacological agents like antipsychotics, antidepressants, mood stabilizers, medications for ADHD, NMDA receptor antagonists, melatonin, oxytocin, and omega-3 fatty acids.

Mainstream research in ASD focuses on genetic causes and other neurological and synaptic transmission abnormalities. However, genetic causes account for merely 6-15% of ASD [5] cases indicating the involvement of other causes or co-morbidities in the pathogenesis of ASD. Research nowadays is trying to examine ASD as a whole-body ailment, including various other factors such as immune deficiencies, oxidative stress, metabolic abnormalities, and gastrointestinal dysfunction. Therefore, more profound insights into different mechanisms of ASD pathology also need to be discussed. There are various animal models of autism, such as the induced animal model and transgenic animal model, which could give

valuable hints toward understanding the molecular, cellular, and pathomorphological processes involved in this neurodevelopmental disorder heterogeneous and has a multifactorial origin. However, though all the animal models focus on establishing the face validity of ASD, very few focus on construct validity and predictive validity about gut-brain dysbiosis in ASD patients because of the abnormal gut-flora leaky-gut phenomenon. Thus, in this chapter, our focus would be to understand the phenomenon of gut-brain cross-talk in ASD, the role of short-chain fatty acids, and to bring forth the neuropsychopathology of propanoic acid (PPA)-induced rat model of ASD, which can help in establishing construct as well as predictive validity with the gut-brain cross-talk and the neuroimmune as well as behavioral complications occurring as a result of short-chain fatty acids and abnormal gut flora.

GUT-BRAIN DYSBIOSIS IN ASD

The human body receives numerous benefits from the microbiotic population present in the gut, verified that great interfaces are occurring between them during development, and any interference might manifest multiple harmful consequences. The Gut-brain axis is a vital pathway that is quite closely linked to ASD. Gastrointestinal complications are highly prevalent in ASD, with around 9%-70% prevalence [6]. It is connected with behaviors predictable with the endophenotype of autism, demonstrating that these are significant ASD-related co-morbidities. A spectacular metabolic capacity comparable to that of the liver is envisioned by the microbiota's collective metabolic capacity, depicting their role as a supplementary human organ and enlightening the representative microbiota-host mutualism in the gut [7]. Their ultimate role in the upkeep of host health comprises of synthesis of short-chain fatty acids (SCFAs), which are readily absorbable from dietary fibers which are indigestible otherwise, boosting and regulating the immune system, synthesis of vitamins, decontamination of detrimental substances, and improved resistance against pathogenic microorganisms colonization [8]. ASD patients, when exposed to antibiotics, develop altered microflora, which affects the normal homeostasis and leads to the development of gastrointestinal diseases, systemic inflammation, and ASD. Laboratories working on germ-free mice have reported extensive immune deficiencies on structural and cellular levels, such as reduced CD8+ and CD4+ T helper cells, reduced IgA production, and reduced Peyer's patches [9]. Peripheral injuries lead to systemic inflammation and ultimately central inflammation by activating microglia, staffing immune cells in the CNS, and cerebral inflammation upsetting neuronal function [10].

CHAPTER 9

Animal Models of Huntington's Disease

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Abstract: Huntington's disease (HD) is a neurological disorder caused due to mutation in the dominant IT15 gene. It is a "trinucleotide repeat" disorder caused by an increase in the number of CAG repeats in the HD gene. Progressive cell death in the cortex and striatum accompanied by a decline in cognitive, motor, and psychiatric functions are the disease's characteristics. Various animal models for HD have been developed to provide insight into disease pathology and study therapeutic strategies outcomes. Previous HD studies are primarily based on toxin-induced models to learn mitochondrial dysfunction and cell death induced by excitotoxicity seen in the HD brain. The discovery of the huntingtin mutation in 1993 resulted in creating new models that introduce a similar genetic defect in animals and then studied the disease's pathophysiology. Various Genetic models are developed to date. Invertebrate models of HD: *Caenorhabditis elegans* and Drosophila melanogaster models; rodent models, and some transgenic large animal models are discussed here. Each model has its advantages and limitations. The researcher must decide which model to use depending on the study's type and requirements.

Keywords: Excitotoxicity, Genetic models, Huntington's disease, Mitochondrial dysfunction.

INTRODUCTION

Huntington's disease (HD) is a progressive neurodegenerative condition that is characterized by cognitive, psychiatric, and motor impairments, as well as peripheral phenotypes such as weight loss and muscle wasting [1]. The disorder is caused by the expansion of polymorphic trinucleotide CAG repeats (>60 repeats) in exon 1 of the HD gene. Therefore, the repetitive CAG units result in an expansion of the polyglutamine (poly Q) tract in the N terminal region of htt, a macromolecular protein (3,144 amino acids) that is abundantly expressed in different cellular organelles. It interferes with disease protein huntingtin (Htt) [2 - 5], and the extension of polyQ tract forms indissoluble aggregates or inclusion

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mutant htt (mHtt) in the brains of patients with mutant htt (mHtt). The number of HD patients contains extended polyQ repeats in the range of 38–55 glutamines and experience late-onset neurological symptoms in their early fifties, typically between the ages of 30 and 50 [6]. Longer CAG repeat tracts accelerate the onset of the disease, which appears to be influenced, albeit to a lesser extent, by environmental and genetic modifiers [7]. A prominent neuronal loss in the striatum, particularly in the caudate-putamen region, is a pathological feature in patients' brains post mortem, typically followed by cell loss in the cerebral cortex and widespread brain atrophy in patients with grade HD based on severity. Grade 0 exhibits no gross changes and neuropathological abnormalities. Grade 2 shows early pathological changes in the caudate nucleus. Neuronal count of caudate nucleus shows 50% loss in grade 1 and up to 95% loss by grade 4. Astrocytosis is seen from grades 2 to 4. Grade 4 exhibits up to 60% of loss in total brain weight majorly in the caudate nucleus of basal ganglia and about 20% is contributed by atrophy in the cerebral cortex and thalamus [8 - 10].

It is crucial to establish animal models of HD that mimic the striatum's and its connections' symptoms. A mutation in the htt gene is the most common cause of HD. The insinuation of this mutation in the rodent genome is used in most existing models. The increased polyglutamine stretch in mutant htt causes the protein to achieve a toxic gain of function. These genetically engineered species result in neurological defects and complex pathologies that resemble the human version of the disorder. Many features of the human condition are replicated in mouse models of HD. They are significant methods for examining disease pathogenesis and assessing the outcomes of possible new drugs.

ANIMAL MODELS OF HUNTINGTON'S DISEASE

Genetic and nongenetic HD models are the two broad types of HD models. The area of HD research has traditionally been dominated by nongenetic models. Even though George Huntington first identified HD in 1872, it was not until 1993 that researchers discovered the actual genetic mutation that causes the disease, delaying the creation of suitable genetic models until the last decade. Excitotoxic pathways or degradation of mitochondrial functionality are commonly used in nongenetic models to cause cell death. In both rodent and primate HD models, quinolinic acid (QA) and kainic acid (KA) has become the most widely used neurotoxic agents. By binding to their cognate receptors, N-methyl-D-aspartic acid (NMDA1) and non-NMDA, respectively, these amino acids (QA and KA) cause cell death. In both rodents and nonhuman primates, the mitochondrial contaminants 3-nitropropionic acid (3-NP) and malonic acid (MA) have been used to trigger cell death in striatal neurons by inhibiting Complex II (succinate dehydrogenase) of the tricarboxylic acid cycle and the electron transport chain in

mitochondria, effectively limiting the development of adenosine triphosphate (ATP).

Toxins Induced Models

Excitotoxic Model: Quinolinic Acid

To identify the potential pathways involved in HD's abnormalities, excitotoxic animal models have been used. The excitotoxic model of HD was suggested after it was shown that kainic acid, a kelp-derived excitotoxin, could trigger neuronal death and gliosis when injected into the striatum [11, 12]. Other excitatory amino acids, like ibotenic acid, had been reported to penetrate striatal lesions that cause locomotor hyperactivity and impairment in learning [13, 14]. IA lesions are also relatively non-selective, rendering this excitotoxin ineffective for the HD analysis [15, 16]. In rats or the human brain, KA and IA are not intracellular compounds and thus may not be involved in neurodegenerative diseases' pathophysiology. However, it is conceivable that HD may be the outcome of a genetic sensitivity to an exogenous or endogenous agent that is common but potentially excitotoxic.

The chosen neurotoxin for excitotoxicity use in HD studies was Quinolinic acid (QA; 2,3-pyridine dicarboxylic acid). In initial QA studies, one of two products produced *via* the kynurenine pathway from the metabolism of tryptophan noted its elimination in the urine of rats that received a high tryptophan-containing diet [17, 18]. QA is an endogenous tryptophan metabolite in human brains and has been present in both normal rats and human beings [14, 19, 20]. Using transporters shared by other neutral protein units, tryptophan crosses the blood-brain barrier [21]. In the brain, astrocytes, macrophages, microglia, and dendritic cells take up tryptophan and convert it to kynurenine [22]. In the presence of the enzyme 3-hydroxyanthranilic acid oxygenase, a series of enzymatic reactions transforms kynurenine to QA. Normal QA levels do not cause harm, but slight changes in QA levels can cause toxicity. Schwarcz and colleagues have shown an increase in the enzyme 3-hydroxyanthranilic acid oxygenase in the postmortem brains of HD patients compared to the level in the control brain [23]. Since QA cannot cross the blood-brain barrier, it is administered directly to the striatum in experiments [24]. Schwartz and colleagues reported that when injected into rat striatum stereotaxically, quinolinic acid exerted an excitotoxic function [14, 16]. It causes neurodegeneration in striatal rats [25] and primates [26] in a similar pattern that can be seen in human HD. Symptoms also resemble deficits found in early (but not later) stages of HD due to QA lesions. For example, animal models of hyperactivity caused by lesions without toxin show hypoactivity later in the disease.

CHAPTER 10

Intracerebroventricular-Streptozotocin Induced Insulin Resistant *In Vivo* Model of Sporadic Alzheimer's Disease: Pathophysiological Aspects and Potential Therapeutic Targets

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Abstract: Streptozotocin (STZ) through the intraperitoneal route is used as a diabetic model, while intracerebroventricular (ICV) STZ administration in rodents is a model for sporadic Alzheimer's disease (AD). It majorly induces insulin resistance along with oxidative stress, mitochondrial dysfunction, and neuroinflammation in prime brain regions of the cortex and hippocampus. The significant pathological hallmarks in AD are phosphorylated tau protein generated neurofibrillary tangles and amyloid plaques which are also observed in this model. The ICV-STZ model can be validated through various behavioral, biochemical, mitochondrial, molecular, and histopathological analyses. The potential target molecules in the insulin signaling pathway could include IR, IRS-1, PI3K, AKT, GSK-3β, *etc*. In a nutshell, we can say that the ICV-STZ model is quite robust for insulin-resistant sporadic AD; however, there are a few limitations like mortality and the requirement of sophisticated procedure.

Keywords: Alzheimer's disease, Amyloid plaques, ICV-STZ, Insulin resistance, Oxidative stress, Tau protein.

INTRODUCTION

Sporadic Alzheimer's disease (SAD) is a late-onset, and progressive neurodegenerative disease accounting for more than 80% of dementia cases and generally occurs in the elderly population above 65 years of age [1]. Contrarily, the familial type of AD is early-onset, less prevalent involving genetic mutations [2]. With the advent of the considerable progress made to unveil the molecular pathways involved in the pathogenesis of AD, potential therapeutic approaches

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are being developed using different animal models. SAD is a multifactorial disease caused by factors like genetic, epigenetic, environmental, and metabolic factors. Among metabolic factors, impaired glucose metabolism and energy utilization are observed in the disease's initial stages [3, 4]. This fuelled researchers' interest in conducting studies that confirmed insulin's presence, its receptors in the central nervous system and further explored the origin of central insulin and its physiological roles. Studies demonstrated that insulin has diverse roles, like inducing neuronal network formation that is implicated in the functions of learning and memory in the central nervous system [5]. In patients with early-stage Alzheimer's disease, a reduction of insulin levels was observed in the hippocampal region. Accumulating studies suggest that brain insulin resistance exists in the brains of AD and type 2 diabetes cases [6 - 7]. Accordingly, AD has been proposed to be a brain-specific form of diabetes mellitus called "type 3 diabetes".

Inspired by the potential of streptozocin (STZ) to induce peripheral insulin resistance, intracerebroventricular (ICV) administration of STZ in rodents has widely been employed to cause brain insulin-resistant state to generate an animal model of SAD [8].

Streptozotocin (STZ) is glucosamine-nitrosourea in chemical nature, obtained from soil bacteria [9]. It has earlier been used as an antibiotic and is no longer used for the same. STZ has been extensively used as an experimental tool in preclinical studies. It is mainly used to establish a diabetic model of rat which produces insulin resistance and hyperglycemia upon intraperitoneal administration in a dose of 55-60 mg/kg [10]. Moreover, STZ in a sub diabetogenic dose (3 mg/kg) through the intracerebroventricular (ICV) route bilaterally in rodents gives rise to pathological signs and symptoms closely resembling sporadic AD [10, 11]. The brain regions of the cerebral cortex and hippocampus are principally influenced by ICV-STZ administration. The prime pathological hallmarks associated with ICV-STZ induced rodent model are the deposition of amyloid plaques and intracellular neurofibrillary tangles [12].

A few decades back, the presence of insulin receptors was reported in brain regions like the cortex, hippocampus, hypothalamus, olfactory bulb, *etc*. Furthermore, there have been reports of downregulated insulin, insulin receptors, and insulin receptor substrates under the influence of ICV-STZ mediated insulin resistance [13, 14]. Other phenomena associated with the ICV-STZ model of AD include oxidative stress, mitochondrial dysfunction, cholinergic dysfunction, and neuroinflammation. These factors are considered the triggering points of neurodegeneration and are adjacently interlinked with insulin resistance [15, 16]. Finally, these advances in the neurobiology of ICV-STZ, leading to the cognitive

deficit and memory impairment, signify a strong reason to validate the sporadic AD model. For the confirmation and validation of the ICV-STZ induced SAD model and its potential to generate brain insulin resistance as seen in SAD patients, various studies have been carried out in the past and recently. This review aims to provide insights into the molecular mechanisms involved in causing brain IR-induced AD by ICV-STZ and further its advantages and limitations.

STREPTOZOTOCIN: PHYSICAL AND CHEMICAL PROPERTIES

Streptozotocin (STZ) is 2-deoxy-2-(3-methyl-3-nitrosourea)-1-D-glucopyranose in terms of chemical origin and was first derived from the soil bacterium *Streptomyces achromogenes* in 1960 [17]. The molecular weight of STZ is almost 265 g/mol and is a blend of α & β-stereoisomers. The pale yellow and solid powder in crystalline form has been observed [18]. More importantly, STZ exists as a hydrophilic compound and is practically well soluble in water and non-polar organic solvents [19]. Earlier, it has been used as an antimicrobial and chemotherapeutic agent, but due to its toxic characteristic of DNA alkylation, cellular damage, teratogenicity, and carcinogenic nature, it was later withdrawn [20, 21]. It has been studied to destroy insulin-producing β-cells of the pancreas, thereby inducing an insulin-resistant state and diabetic complications [22, 23]. Its elimination half-life is around 20-30 minutes and is preferentially metabolized in the liver and kidney. Moreover, STZ is photosensitive and can be degraded upon exposure to light. Hence, STZ is recommended to be stored at 2-8°C, away from the light that remains stable for up to 3 months. However, its stability persists only for one hour at 37°C with a pH of 7.4 [24]. Additional toxicities associated with STZ could be in the form of irritation, headache, nausea, and vomiting, *etc*. So utmost care must be taken while handling this chemical [17, 18].

Intraperitoneal-Streptozotocin Induced Diabetic Model

Administration of STZ dissolved in 0.1 M sodium citrate buffer (pH 4.5) in rats or mice in a dose of 55-60 mg/kg through intraperitoneal (*i.p*) route gives rise to hyperglycemic condition reaching fasting glucose level in serum or plasma above 150 mg/dl after 24-72 hours. Here, glucose is measured either by a glucometer or glucose assay kit. Hence, a type 1 DM model is generated [25, 26]. However, for type 2 DM, STZ can be administered parallel, feeding a high-fat diet for a few weeks. This model gives rise to peripheral insulin resistance as STZ invades insulin-releasing β-cells of islets of Langerhans. In addition to impairment in glucose metabolism, *i.p* injection of STZ also leads to dyslipidemia or imbalance in lipid metabolism leading to weight loss [27, 28].

CHAPTER 11

Brain Tumor: An Insight into *In-vitro* and *In-vivo* Experimental Models

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Abstract: Despite intensive research, brain tumor remains one of the deadliest forms of cancer with rapid progression and poor prognosis. A brain tumor is physically, emotionally, socially, and financially challenging not only for the patient but also for the caregiver. Morbid conditions like seizures, paralysis, cognitive impairment, and permanent neurological damage are the potential impacts of either the disease or therapy. Poor long-term survival with the 5-year and 10-year survival rates of almost 36% and 31%, respectively, adds to the burden. Animal models have undergone constant development with time and remain an indispensable tool for exploring the underlying pathophysiological mechanism and evaluating potential therapeutic strategies. Initial brain tumor models used chemical carcinogens to induce brain tumors, with nitrosourea derivatives being the favorable choice. These tumors could be maintained easily under *in vitro* conditions as cell lines and grafted in suitable syngeneic or xenogeneic hosts to study the cellular and physiological features of different types of brain tumors. The advent of transgenic technology has revolutionized animal modeling by allowing the manipulation of the host genome. Transgenic animals with gain/loss of function (knock-in/knockout) can be produced to investigate the role of any specific protein/gene involved in the cell cycle, metabolism, and signal transduction. Since the first oncomice in the 1980s, the transgenic technique and the subsequent expression of the transgene have been carefully worked out in mice. The role of different mutations, tumor suppressors, and oncogenes has also been studied. 2D and 3D *in vitro* techniques for faster evaluation and pre-screening of drugs have been established to mimic the brain microenvironment by manipulation of the culture conditions. Furthermore, a brief summary of non-rodent models and their potential applications has been discussed.

Keywords: Animal models, Brain tumor, Chemical models, Genetic models, *in-vitro* models, Non-rodent models, Oncogenic virus, Syngeneic models, Xenograft models.

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INTRODUCTION

Tumors of the brain and spinal cord are grouped together as central nervous system (CNS) tumors and identified according to the cell type and origin. On May 9, 2016, the World Health Organization (WHO) published an official reclassification of tumor types of the central nervous system, classified by integrating molecular data into traditional histopathology to provide a better prognosis, diagnosis, and treatment recognizing over 120 CNS tumors [1]. In 2020, estimated new cases in the United States were 23,890, accounting for 1.3% of all new cancer cases, with estimated deaths of 18,020 accounting for 3.0% of all cancer-related deaths [2]. Although the occurrence is low, the high morbidity and mortality rate makes them a serious concern for researchers. As per the Central Brain Tumor Registry of the United States (CBTRUS) Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2013-2017, the average annual age-adjusted incidence rate (AAAIR) of the brain and other CNS tumors was 23.79 (Malignant=7.08, non-Malignant=16.71) with a higher occurrence rate in females than males, blacks than whites and non-Hispanics than Hispanics, accounting for 81,246 deaths. For this period of 5 years (2013-2017), glioblastoma was the most commonly occurring malignant brain and other CNS tumor (14.5% of all tumors) and was more common in males, whereas meningioma, the most common non-malignant tumor (38.3% of all tumors) was more common in females. Non-malignant tumors were found to be more than twice as common as malignant tumors (70.3%and 29.7%, respectively). An estimated 24,970 malignant and 58,860 non-malignant new cases of brain and other CNS tumors were expected to be diagnosed in the US in 2020 [3].

Late detection, common and non-specific symptoms like headache, nausea, seizure, and confusion with fast progression contribute to a high rate of mortality. The requirement of highly specialized medical and surgical equipment for diagnosis, therapy, and management, along with high cost and lack of accessibility, poses a great health challenge on a global level. Despite advancements in technology, brain tumor treatment is challenging due to resistance to chemotherapy and radiation, and thus, the development of animal models is essential to understand the mechanisms of occurrence and development of brain tumors and promote clinical research for better detection and treatment.

TYPES OF BRAIN TUMOR

Brain tumors are formed by the abnormal growth of cells in brain and may begin in different parts of the brain. They are mainly classified based on their origin and malignancy (Fig. **1**). Tumors that start in the brain are called primary brain tumors

that may spread to other parts of the brain or the spine but rarely spread to other parts of the body. A tumor that starts in another part of the body and spreads to the brain is called a metastatic or secondary brain tumor and is more common than primary brain tumors. Metastatic brain tumors are mainly (50%) from lung cancer. The tumors may be either benign or malignant. Benign tumors may grow and press on nearby areas of the brain, but rarely spread into other tissues and may recur, whereas, malignant are likely to grow quickly and spread into other brain tissue. Primary tumors may be glial or non-glial (developed on or in the structures of the brain, including blood vessels and glands). Gliomas are the most prevalent type of adult brain tumors, accounting for 78% of malignant brain tumors, arising from supporting cells of the brain glial cells (astrocytes, ependymal cells, and oligodendroglial cells). Tumors are named based on the origin like glioma from glial cells, astrocytoma from astrocytes, *etc*. According to the Pediatric Brain Tumor Foundation, approximately 4,200 children are diagnosed with a brain tumor in the U.S. with the most commonly occurring are medulloblastomas, low-grade astrocytomas (pilocytic), ependymomas, craniopharyngiomas, and brainstem gliomas [4].

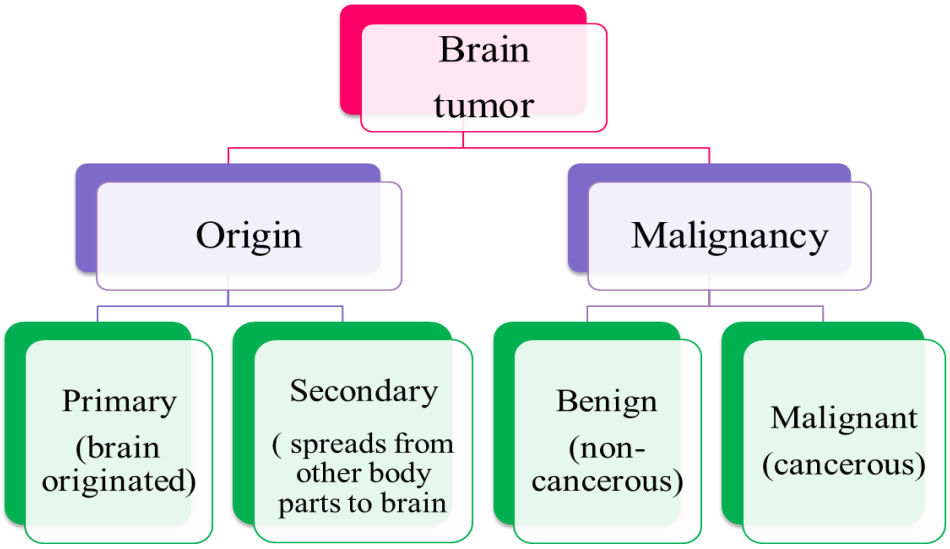

Fig. (1). Basic classification of brain tumor.

Based on histological features, infiltration, and progression rate, tumors are graded into four (I-IV) grades by World Health Organization (WHO), described in Table **1**. Genetic alterations like the mutational status of IDH (isocitrate dehydrogenase) with better prognosis in IDH mutant than IDH wild types, 1p/19q co-deletion (balanced translocation of the p arm of chromosome 1 with the q arm of chromosome 19), *ATRX* (alpha thalassemia syndrome X-linked) loss and, TP53

CHAPTER 12

In vitro Models of Age-Related Neurodegenerative Diseases

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Abstract: Neurodegenerative diseases are associated with progressive degeneration of neurons or death of nerve cells. This chapter emphasizes mainly on age-related neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and Prion disease. Abnormal fibrous tangles, β sheet plaques, cholinergic deficits, chronic neuroinflammation, nerve cell death, oxidative stress, and inflammatory cascade are the common molecular and biochemical changes of Alzheimer's disease. Aggregated neurofibrillary tangles and accumulated amyloid-beta(Aβ) are the defining pathological hallmarks of Alzheimer's disease. Parkinson's disease is a composite and multifactorial disease in which different factors concur with pathogenic factors. Prion disease is an infectious neurodegenerative disease characterized by misfolded prion protein accumulation in the brain and leads to nerve cell loss. Currently, different models have been established to understand the pathophysiology of these diseases and are also used to investigate new therapeutic compounds. Although various *in-vivo* models are used to study neurodegenerative diseases, *in vitro* models provide more insights on various pharmacological targets and mechanisms of disease during neurodegeneration. Human and animal cells derived cell cultures are used to study neurodegenerative diseases in order to accurately mimic brain environment and neuronal cell interactions. *In vitro* models show the reliable effect of compounds on various targets in the brain to study pathophysiological characteristics of the disease, and it also provides a controlled environment favourable to explore single pathogenic mechanisms. In this chapter, we discuss different *in-vitro* models used to study age-related neurodegenerative diseases.

Keywords: Alzheimer's disease, *in vitro* models, Neurodegeneration, Parkinson's disease, Prions disease.

INTRODUCTION

Neurodegenerative diseases are debilitating pathological conditions that result in

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progressive degeneration of nerve cells. Alzheimer's disease contributes to 60-70% of dementia, and worldwide 50 million people have dementia, with 10 million new cases every year [1]. Alzheimer's is the leading cause of disability, and the fifth leading cause of death among those older than 65, one in ten people of age 65 has Alzheimer's disease [2]. Parkinson's disease with 2 to 1 male to female ratio for an age group 60 or more have prevalence between 0.3 and 3% worldwide [3]. Neurodegenerative diseases are a group of disorders characterized by degeneration of structure and function of the central nervous system showing pernicious effects on speech, memory, movement, and memory of the patient [4]. The common examples of neurodegenerative diseases are Parkinsonism, Amyloidoses, Dementia, and Tauopathies. Abnormal conformation of proteins such as Tau in neurofibrillary tangles, α-synuclein in Lewy bodies leads to a cellular diagnosis of neurodegeneration. Alzheimer's disease due to mutation in presenilin genes (PSEN1 and PSEN2), alteration in amyloid metabolism, and amyloid precursor protein is considered as a prevalent secondary tauopathy. Parkinson's disease occurs due to neuronal death in the part of the brain called substantia nigra that releases dopamine which communicates with the movement-producing brain part, *i.e*., Frontal lobe and Basal ganglia [5]. Prion disease is an infectious disease caused by the accumulation of pathological PrP protein that leads to neuronal and synaptic loss [6]. The prevention and treatment of neurodegenerative disease is a new challenge for the pharma industry, for patients, and their families. With the advent of technology, approaches to studying neurodegenerative diseases have changed. *In vitro* cell culture systems are preferred to study neurodegenerative disease, induced pluripotent stem cells, astrocytes, oligodendrocytes, and microglia cultivated *in vitro* are becoming useful tools in drug discovery [7].

PARKINSON's DISEASE

Parkinson's disease, a movement disorder, is characterized by damage or death of neurons in the brain part of called substantia nigra. As of today, there has been no complete cure for Parkinson's disease, but ongoing research on therapeutic compounds and surgery provides substantial improvement. Parkinson's disease affects both sides of the body, but symptoms are prevalent to one side [8]. *In vitro* cell lines, human brain organoids, and animal models are used to study several molecular events of parkinsonism, among which oxidative stress, neuroinflammation, mitochondrial dysfunction, and α-synuclein misfolding and aggregation are major molecular and cellular hallmarks of the disease. Neuronal excitotoxicity, loss of microtubular integrity, disruption of vesicular transport, dysregulation of iron metabolic pathways, impaired endoplasmic reticulum, and other enzymatic regulation also leads to disease severity. Dysfunction of Axonal mitochondria particularly contributes to impaired axonal transport, which leads to

neurodegeneration in PD [9].

Pathophysiology

Dopaminergic neurons are destroyed by abnormal aggregation of α-synuclein, and the decreased mitochondrial complex I activity leads to potential mitochondrial activation of caspase cascade and ultimately causes cell death. Mitochondrial dysfunction is the result of reduced glucocerebrosidase, accumulation of oxidized dopamine, and the deleterious effect of certain Parkinson's disease-related genes such as Parkin, PINK 1, and DJ1 [10]. Dopaminergic functions are lost by progressive degeneration of dopaminergic neurons in substantial nigra pars compacta (SNPs). Gamma-aminobutyric acid (GABA) dysfunction is caused by the loss of dopamine in the striatum of PD's patient. It causes inhibition of the thalamus, which further leads to the activation of the frontal cortex, resulting in decreased motor activity. Lewy bodies (LBs) are the hallmark of neurodegenerative disease and are found in dopaminergic neurons of substantia nigra as round bodies with radiating fibrils. Gene mutations involving the alpha-synuclein (αSyn) protein aggregate and form insoluble fibrils associated with LBs. The formation of LBs leads to increased production of misfolded forms of ubiquitin proteins, which are an important part of protein cycling [11].

In Vitro Models of Parkinson's Disease

In vitro models are useful in carrying out molecular and pathophysiological studies with high credibility. They can be used for the development of therapeutic approaches to Parkinson's disease focusing on one particular characteristic of the disease. The Two most prevalent changes seen in Parkinson's disease are the degeneration of dopaminergic neurons in substantia nigra pars compacta (SNPs) and the presence of α-synuclein containing protein aggregates.

1. Dopaminergic Neuron Degeneration Model

By the selective loss of dopaminergic neurons in the brain, neurodegenerative diseases develop and cause neurotoxicity. Brain dopaminergic neurons are vulnerable to toxic events produced by MPTP, rotenone, and paraquatx [12]. SH-SY5Y neuroblastoma cell lines and PC12 pheochromocytoma cell lines are easier to maintain than primary neurons, release catecholamine, develop neuron-like properties, and are used to study degeneration of dopaminergic neurons. Lund human Mesencephalic (LUHMES) is one of the commonly used immortalized cell lines, stable dopaminergic phenotype having electrical properties similar to dopaminergic neurons. These cell lines are subclones of MESC2 10 cells, which are generated from 8-week-old human ventral mesencephalic tissue [13].

CHAPTER 13

Conclusion

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This book provided information about animal models of human neurodegenerative disease (Alzheimer's disease, Parkinson's disease, Huntington's disease, Multiple sclerosis, Prions disease, Vascular dementia) and other CNS disorders (Autism, Depression, Psychosis, Schizophrenia) and additionally brain tumor. As we all are aware that CNS disorders are now possessing a significant health burden and are considered as the main source of inability among individuals and has no clear-cut defined etiologic and increased morbidity. Thus, there is an imperative requirement to make more and more efforts to look into the underlying reason of the illness and design and create a new therapeutic agent to halt or inverse the illness advancement. Therefore, this book chapters are about emerging pathophysiological mechanisms along with drug targets and also summarized discussion about *in-vivo/ex-vivo, in-vitro,* QSAR and *in-silico* models. Moreover, we will also describe how to select suitable and valid models and the qualities and pertinence of some behavioral appraisal strategies. The fundamental problem we all face when selecting an animal model to research an underlying mechanism of a disease, for example, various models of depression involve different-2 procedures, each with its own set of benefits and drawbacks. So, it is tough to select the therapeutic result of drug-using only one animal model. Moreover, the lack of biomarkers or genes-based animal models is currently in progress and has been highlighted by many papers; they can be used as a rationale to target the early stage of the disease.

Despite depending on a single parameter in animal models, it should be better to make strategies that would merge behavioral and non-behavioral parameters based on performing several tests. *e.g.*, the OBX model, for example, causes stress in animals, therefore completing the TST/sucrose preference test/open field test after this model will give better results. This development can also minimize

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the risk of behavioral models and provide available information on non-behavioral animal models of research.

As we know, in the case of psychosis and schizophrenia, the laboratory models are used to clarify the neurochemical and structural alterations in the brain that contributes to the pathophysiology of psychosis rather than an emphasis on the treatment of symptoms is a requirement to facilitate more potential therapeutic drugs to be developed. The biggest challenge for attaining this role is to define how we can mimic the symptoms of schizophrenia in animal models for the investigation of novel drug therapy. Various cognitive impairments like working, attention, visual learning, and memory can be measured objectively and quantitatively in animal models. But the question arises that the most effective treatment (olanzapine, risperidone, and clozapine) produces only marginal benefits from cognitive symptoms, although the symptoms-based models have proven useful in mimicking drug effects that impair cognition. With that in mind, the possibilities for developing good animal models need to be focused on the treatment of symptoms seen in schizophrenic patients. So, conclusively, it is challenging to choose an effective animal model to test new compounds. Furthermore, the pathophysiology of psychosis is not fully understood. Animal models based on biomarkers are currently being designed to help understand the pathophysiological pathways involved in the development of psychosis.

Neurodegenerative diseases are a group of disorders characterized by the degeneration of nerve cells. Several models are now available to study neurodegenerative diseases, including *in-vivo/ex-vivo, in-vitro* animal models, QSAR, *in-silico*, and *in-vivo*/*in-vitro* cell culture-based models. *In vitro* models are undoubtedly advancing our knowledge of neurodegenerative diseases and are providing important insight for the screening of potential pharmacological agents. To this end, the book chapters summarize *in-vitro, in-vivo, in silico,* and cell-based models used in studying age-related neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and Prions disease. The extracellular aggregation of Aβ and neurofibrillary tangles (NFT) can be easily recapitulated by the cell culture system. The aggregation of α-synuclein demonstrated in neurons differentiated from iPSCs derived from sporadic and familial PD patients can reproduce the hallmark of Parkinson's disease. Cell culture models are also widely used for prion propagation and the study of different drugs.

When we focused on vascular cognitive impairment (VCI), there are several useful animal models, but none of them can mimic all of the pathological changes seen observed in VaD and VCID. An ideal model would aid in identifying key biomarkers linked to vascular processes and identify various cellular and

molecular mechanisms and targets underlying dementia. This means the need to develop more reliable predictive animal models of this disorder will continue in the future.

In the case of HD, transgenic as well as other non-genetic models are of vital importance as they mimic either the pathogenesis or other downstream mechanisms that are altered because of mutations in the htt protein. Although none of the animal models can be described as best, using these to understand the disease could prove to be a stepping stone for future development of pharmacotherapeutic treatment options to cure or prevent HD. Researchers are now putting in tremendous effort to develop new knockin and transgenic models that can be used in the future to explore detailed HD research.

We found that no single animal model can capture the whole spectrum of disease heterogeneity in Multiple Sclerosis (MS), a demyelinating disease of the CNS involving complex immune-pathological mechanisms. Although, over the years, different animal models have been developed that mimic specific aspects of human MS. Animal models of EAE are well established and, most widely accepted, and formed the basis of the development of novel therapeutic approaches. Toxin-induced demyelinating models such as lysolecithin (LPC) or ethidium bromide have been effective in understanding cellular and molecular mechanisms involved in remyelination and demyelination, and EAE models have been useful in developing insights into the immunological mechanism involved, specifically the role of T cells in demyelination. Furthermore, Zebrafish models developed in recent years are novel tools for understanding complex mechanisms involved in disease and screening novel therapeutic approaches.

In the case of Autism, an ideal animal model must demonstrate at least three diagnostic symptoms characteristic of clinical patients, including impaired social interaction and deficits in communication skills. This book also gives a piece of information about the neuropsychopathology and neurobehavioral characteristics of the PPA-induced autism-like rat model and its correlation with gut-brain dysbiosis occurring in autism spectrum disorder (ASD). We strongly believe that the animal model with high predictive validity must understand the diet-related mechanisms, gastrointestinal complications, dysbiosis, abnormal gut-flora, and short-chain fatty acids in patients with ASD. PPA-induced autism-like rat model can help us in understanding the central effects of gut-brain dysbiosis. However, more pre-clinical and clinical studies are required in the scientific exploration of specific biomarkers of gut-brain dysbiosis and targets such as GPR41 receptors of PPA. This would help us deeply understand the gut-brain cross-talk so that such targeted neurotherapeutics can be developed for autistic patients, which can help mitigate gastrointestinal complications responsible for biochemical alterations

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Dr. Anil Kumar (M. Pharm, PhD, MBA, LLB) is presently working as a Professor of Pharmacology, and has more than 20 years of experience in the areas of neurological problems. Dr. Kumar's work has been cited extensively in International journals of repute and recently recognized among top 2% Scientists in Pharmacy and Pharmacology category. Prof. Kumar has guided several doctoral (15) and post graduate's (38) students. Dr. Kumar has received ICMR Award for Biomedical Scientist, APTI's Young Pharmacy Teacher Award, AICTE Career Award.

Kanwaljit Chopra

Prof. Kanwaljit Chopra, Former Dean & Chairperson of UIPS, has 30 years' prolific research, teaching experience and expertise in the field of neuronal and metabolic disorders. With more than 300 international and national publications to her credit, eleven prestigious national and international awards. Prof. Chopra has supervised 30 M. Pharm students and 22 PhD till now. She has been recognised as among the top 2% of the world scientists in the Pharmacy and Pharmacology category.

Anurag Kuhad

Dr. Anurag Kuhad is among world top 2% scientists in the field of Pharmacology. He is working in the area of Neuropsychopharmacology and Metabolic Pharmacology. His research work has 4471 citations with h-index - 36 and i10 index - 62. Dr. Kuhad was awarded with highly prestigious "Yuva Vigyan Ratan Award" by DST, Government of Haryana; AICTE Career Award for Young Teachers (CAYT); UGC Research Award; Rafaelsen Young Investigators Award 2012 at CINP, Stockholm, Sweden.

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