Introduction

As described by the English physician James Parkinson in “An Essay on the Shaking Palsy,” Parkinsonism is clinically characterized by the triad of tremor, rigidity and bradykinesia. Parkinsonism is defined as any combination of six specific, independent motoric features: tremor-at-rest, bradykinesia, rigidity, loss of postural reflexes, flexed posture and the freezing phenomenon (Thomas & Beal 2007; Varanese S et al., 2011). Dopamine (DA) and non-DA neurotransmitter systems within the basal ganglia are intimately connected by complex and not totally well understood relationships (Linazasoro et al., 2008). Parkinson's disease (PD) is currently regarded as the most common degenerative disorder of the aging brain after the Alzheimer's dementia. Most epidemiological studies estimate that over five million individuals in the world are carrying the diagnosis of PD and that roughly one lakh new cases arise each year (Fahn & Przedborski, 2010). PD is a neurodegenerative disease and these early motoric symptoms appear to be related to striatal dopamine deficiency due to loss of dopaminergic neurons in the substantia nigra pars compacta, which sends axons to the striatum.

Parkinson’s disease is an age-related disorder, more common in senior citizens than in younger ones. The proper cause of this disease still remains a mystery, despite the role of oxidative stress, free radical formation, genetic susceptibility, programmed cell death and some unknown factor, which is endogenous or exogenous. The disease progresses slowly and ultimately produce complete akinesia. The neuropathology of the disease is based on the depigmentation and cell loss in the dopaminergic nigrostriatal tract of the brain with the corresponding decrease in the striatal dopamine concentration and the presence of eosinophilic inclusions called Lewy bodies (Marley, 2010).

Parkinson’s disease is a progressive neurodegenerative disease marked by motor and non-motor abnormalities. The hallmark pathological features of PD are selective nigrostriatal dopaminergic degeneration and formation of filamentous,
cytoplasmic inclusions called Lewy bodies, containing α-synuclein and ubiquitin. Brains of PD patients show evidence of extensive oxidative damage and microglial activation. Additionally, PD patients are characterized by systemic mitochondrial dysfunction, marked by inhibition of complex I of the mitochondrial electron transport chain (Sherer et al., 2003). The pathogenesis of idiopathic PD is believed to involve an interaction between genetic and environmental factors. Specifically, PD has been associated with pesticide exposure and rural living. Most insights into PD pathogenesis come from investigations performed in experimental models of PD, especially those produced by neurotoxins.

Increasing evidence has suggested an important role for environmental toxins such as pesticides in the pathogenesis of PD. Chronic exposure to rotenone, a common herbicide, reproduces features of Parkinsonism in rats. Rotenone-induced dopaminergic neurodegeneration has been associated with both its inhibition of neuronal mitochondrial complex I and the enhancement of activated microglia (Hui-Ming et al., 2010).

Parkinson’s disease appears essentially as a sporadic condition, without any other family members being affected. PD etiology remains mysterious, whereas its pathogenesis begins to be understood as a multifactorial cascade of deleterious factors. Although PD develop at any age, it is most common in older adults, with a peak age at onset around 60 years. The prevalence and incidence increases with age, with a lifetime risk of about 2%.

The early symptoms and signs are rest tremor, bradykinesia and rigidity. Bradykinesia is slowness and reduced amplitude of movement. Features of limb bradykinesia are a smaller and slower of handwriting, difficulty shaving and brushing teeth. Walking becomes slow, with decreased arm swing and with a tendency to shuffle feet. Difficulties arising from a deep chair, getting out of automobiles and turning in bed are symptoms of truncal bradykinesia (Saravanan 2005). Rigidity of muscles is detected by the examiner when he/she moves the
patient’s limbs, neck or shoulders and experiences increased resistance. There is
often a ratchet-like feel to the muscles, so-called cogwheel rigidity. These early
motoric symptoms appear to be related to striatal dopamine deficiency due to loss
of dopaminergic neurons in the substantia nigra pars compacta, which sends axons
to the striatum. The early features of PD usually respond to medication that
activates striatal dopamine receptors, such as levodopa and dopamine agonists,
whereas three later motoric symptoms of flexed posture, loss of postural reflexes
and freezing of gait do not. This lack of response suggests that these late features
of PD are the result of nondopaminergic effects (Nicholson 2002). The
neuropathology of PD is far from being restricted to the nigrostriatal pathway and
histological abnormalities are also found in many other dopaminergic and
nondopaminergic cell groups. Moreover, increasing bradykinesia that is not
responsive to levodopa also appears as the disease worsens.

While the motor symptoms of PD dominate the clinical picture – and even
define the parkinsonian syndrome – most patients with PD have other features that
have been classified as nonmotor. These include bradyphrenia, slowness in mental
function, decreased motivation and apathy, dementia, fatigue, depression, anxiety,
sleep disturbances, fragmented sleep and REM sleep behaviour disorder,
constipation and other autonomic disturbances of sexual and gastrointestinal
complaints. Sensory symptoms include pain, numbness, tingling and burning in
the affected limbs occurs in about 40% of patients. Dementia is associated with
age and has been reported to occur in over 70% of patients with PD eventually.

Patients with PD live 20 or more years, depending on the age at onset. The
mortality rate is about 1.5 times that of normal individuals of the same age. Death
in PD is usually due to some concurrent unrelated illness or due to the effects of
decreased mobility, aspiration, or increased falling with subsequent physical injury.

To model the systemic defect in complex I reported in PD, researchers
have used rotenone exposure. Rotenone is a commonly used pesticide and potent,
specific inhibitor of mitochondrial complex I. Rotenone because of its lipophilic nature, crosses biological membranes easily and independent of transporters. As a result, systemic rotenone exposure inhibits complex I uniformly throughout brain (Betarbet et al., 2000).

Most studies using the rotenone model of PD use chronic treatment regimens. Rotenone gains access to the brain whether given intravenously, subcutaneously, or intraperitoneally (Alam & Schmidt 2002; Betarbet et al., 2000; Sherer et al., 2003).

Rotenone infused animals demonstrated reduced locomotor activity, hunched posture and in some cases rigidity and freezing behavior (Betarbet et al., 2000; Sherer et al., 2003). Specifically, rotenone-treated animals show decreased rearing, line crossing and head dips in open field tests and increased catalepsy. Behavioral deficits in rotenone treated animals correlated with striatal dopamine loss (Alam & Schmidt 2002). An initial study examining the effects of rotenone on the nigrostriatal dopaminergic system demonstrated that direct stereotaxic injection of rotenone into the medial forebrain bundle damaged the nigrostriatal dopaminergic system, marked by reduced dopamine levels in the striatum (Heikkila et al., 1985). While chronic exposure to rotenone at high doses (12 mg/kg/day) failed to cause selective dopaminergic neurodegeneration (Ferrante et al., 1997), chronic systemic low dose (2–3 mg/kg/day) rotenone exposure caused highly selective nigrostriatal dopaminergic degeneration.

Despite causing uniform mitochondrial inhibition throughout the brain, rotenone treatment reproduces many features of PD including motor abnormalities, selective nigrostriatal dopaminergic degeneration and formation of α-synuclein, ubiquitin-positive aggregates in nigral neurons.

Dopamine, a major neurotransmitter in central nervous system is involved in the control of motor and cognitive programmes. Dopaminergic neurons appear early during development, 6-8 weeks in humans. DA is synthesised from tyrosine, stored in vesicles in axon terminals and released when the neuron is depolarised.
DA interacts with specific membrane receptors to produce its effects. These effects are terminated by reuptake of dopamine into the presynaptic neuron by a dopamine transporter or by metabolic inactivation by monoamine oxidase B (MAO-B) or catechol-O-methyltransferase (COMT). DA plays an important role both centrally and peripherally. The recent identification of five dopamine receptor subtypes provides a basis for understanding dopamine's central and peripheral actions. DA receptors are classified into two major groups: DA D\textsubscript{1} like and DA D\textsubscript{2} like. DA D\textsubscript{1} like receptors consists of DA D\textsubscript{1} and DA D\textsubscript{5} receptors. DA D\textsubscript{2} like receptors consists of DA D\textsubscript{2}, DA D\textsubscript{3} and DA D\textsubscript{4} receptors. Stimulation of the DA D\textsubscript{1} receptor gives rise to increased production of cAMP. DA D\textsubscript{2} receptors inhibit cAMP production, but activate the inositol phosphate-second messenger system (Seeman, 1980). Disturbances of the development of the dopaminergic system lead to dyskinesia, dystonia, tics and abnormal eye movements. An imbalance between dopaminergic neurotransmission and DA receptors is known to be associated with the symptomatology of numerous neuropsychiatric disorders, like schizophrenia, psychosis, mania and depression as well as neuropathological disorders, like Parkinson's disease and Huntington's disease (Carlsson, 1988, 1993; Bermanzohn & Siris, 1992; Brown & Gershon, 1993; Jakel & Maragos, 2000; Kostrzewa & Segura-Aguilar, 2003). The dopaminergic cells in particular are highly sensitive to excitotoxicity and oxidative stress when the energy metabolism is impaired (Callahan \textit{et al.}, 1998). Of all the neurotransmitter systems, DA is of particular interest in relation to the development of cognitive abilities subserved by the prefrontal cortex. The most postsynaptic markers of the DA system are its receptors.

Non-dopaminergic neurotransmission is also affected in PD. The dysfunction of non-dopaminergic systems explains the principal non-dopaminergic symptoms, such as ‘axial’ signs and cognitive impairment.

The non-dopaminergic neurotransmitters affected in PD are noradrenaline (norepinephrine), serotonin (5-hydroxytryptamine; 5-HT), glutamate, gamma-
aminobutyric acid (GABA), acetylcholine and neuropeptides (Bonnet, 2000). Dysfunction of these systems lead to some of the motor symptoms of the disease and provide targets for pharmacological interventions to treat these symptoms. For example, antagonists of certain glutamate receptors have been found to improve Parkinsonian symptoms when given with levodopa, although adverse effects limit their use.

The dysfunction of non-dopaminergic neurotransmitter systems in PD is also important because it leads to non-motor symptoms that are not responsive to dopaminergic therapy and can be a major cause of disability during disease evolution (Zhang Y, 2010). Dysautonomia is not infrequent in individuals with PD and is characterised by constipation, urinary disorders and orthostatic hypotension, the latter resulting from deficits in adrenergic and noradrenergic neurotransmission. Postural instability is caused by abnormalities in both dopaminergic and non-dopaminergic pathways. Depression is partially a result of dopaminergic denervation, but also of a decrease of serotonergic transmission. Cognitive impairment with frontal lobe-like symptomatology is a result of the dopaminergic deficit but also, at least in part, a cholinergic and noradrenergic deficit.

Long term use of levodopa is associated with complications such as dyskinesias. Although these are treated initially with other dopaminergic treatments, including changes in the levodopa administration schedule and dopamine receptor agonists, there have been attempts to treat dyskinesias with non-dopaminergic drugs (Phylinda 2010). Agents such as glutamate antagonists and opioid antagonists have been found to be useful.

Stem cell study is one of the most fascinating areas of research. Stem cells have the ability to differentiate under appropriate conditions to the required mature cell types (Kaplitt 2001). Bone marrow has stem cells, mesenchymal and hematopoetic, which can be used for therapeutic applications (Bjorklund, 1999). This promising area of science led scientists to investigate the possibility of cell-
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Based therapies to treat diseases like Parkinson's, Alzheimer's, spinal cord injury, epilepsy, stroke, heart disease and diabetes. In the present study, we administrated bone marrow derived stem cells in combination with Serotonin and GABA for Parkinson’s disease management and the brain dopaminergic functional regulation by Serotonin, GABA and bone marrow cells at the molecular level.

5-HT receptor subtypes and their putative role in the control of movement suggest possible novel intervention strategies for modulating dopaminergic and non-dopaminergic systems in PD patients based on the distribution, localization and function in the basal ganglia (Barnes 1999). 5-HT receptor subtypes and serotonergic modulation of dyskinesia syndrome and psychosis in PD has made significant progress with the availability of these selective serotonergic agents. Serotonin has been recognized to cause proliferation of a variety of cells in culture and the activation of tyrosine kinase as done by many of the novel mitogens (Di Matteo et al., 2010)

GABA, the main inhibitory neurotransmitter in the mature CNS is implicated in playing a complex role during neurogenesis. Through embryonic development, GABA was demonstrated as acting as a chemo-attractant and being involved in the regulation of progenitor cell proliferation (Behar et al., 2000; Haydar et al., 2000). GABA acts as a trophic factor, being involved in neurogenesis, neuron development and migration. GABA and its receptors play a key role in neuroblast proliferation, migration and differentiation in nervous system development (Owens 2002).

Cell transplantation to replace lost neurons is a promising approach for the treatment of progressive neurodegenerative diseases. Induced pluripotent stem cells derived from somatic cells of PD patients used for mechanistic studies of PD pathogenesis and drug screening (Soldner et al, 2009). Hematopoietic system is used as a source of progenitor cells for the central nervous system (CNS) and it also has the property to differentiate into both microglia and macroglia when injected directly to the brain of adult rats (Martin & Eva 1997). The success of the
cell transplantation will depend on the ability of the cells to replace those neurons lost as a result of the disease process in the DA-deficient striatum and reverse, at least in part, the major symptoms of the disease. Serotonin (5HT) and Gamma aminobutyric acid (GABA) as therapeutic agents for cell proliferation and differentiation is a novel approach.

The signaling from the neurotransmitters is carried to the cell nucleus by second messengers like IP3, cAMP and cGMP. Their expression and changes play a major role in the signaling cascade (Hajnoczky et al., 2010). Different transcriptions factors, α-synuclein, ubiquitin and Bax are modulated during Parkinson’s disease to overcome the neurodegeneration. Cyclic AMP responsive element binding protein (CREB) plays an important role in a variety of cellular processes, including proliferation, differentiation and adaptive responses. Increased CREB phosphorylation during Parkinson’s disease recovery is reported to be associated with neuronal survival. (Walton & Dragunow 2000, Finkbeiner 2000; Shimamura et al., 2000).

In the present work, the effects of serotonin, GABA and bone marrow cells supplementation intranigrally to substantia nigra as treatment individually and in combination on rotenone induced Hemiparkinson’s disease in rats were analyzed. Dopaminergic binding parameters investigated its role in the regulation of dopamine receptor subtypes in the brain regions of the experimental rats. Gene expression analysis of receptor specific probes for Dopamine D1, D2, pro-apoptotic protein bax, transcription factor CREB, regulatory protein ubiquitin and neural protein of α-synuclein in the brain regions of control and experimental groups of rats were studied. Immunohistochemistry of brain slices were done to confirm the binding studies and gene expression analysis using specific antibodies. Behavioural responses in Rotarod, Social interaction, Elevated plus maze, Grid walking and Narrow beam tests were carried out to assess the motor learning deficit in rotenone induced PD rats.