Introduction

Parkinson’s disease (PD), originally described by British apothecary James Parkinson in “An Essay on the Shaking Palsy” (1817), is a neurodegenerative disease caused by the progressive degeneration of the nigrostriatal dopaminergic pathway. It is the second most common neurodegenerative disorder, with an incidence of 1.5–2% in the population over 60 years of age, which increases significantly with advancing age. As life expectancy is significantly increasing in the modern world, the incidence of PD is steadily escalating. Consequently, the financial and economical burden of the treatment and care of PD patients is substantial and increasing (Toulouse & Sullivan, 2008). Thus, research on the causes of this debilitating disease is critical, as is the development of new treatments.

PD is caused by the progressive degeneration of the nigrostriatal (A9) dopaminergic pathway, which projects from the substantia nigra (SN) in the midbrain to the caudate-putamen (striatum) in the forebrain (Hoehn & Yahr, 1967; Fearnley & Lees, 1991). The resulting loss of dopamine (DA) neurotransmission in the striatum causes the cardinal symptoms of the disease: tremor at rest, cogwheel rigidity, bradykinesia, stooped posture and shuffling gait (Thomas & Beal, 2007; Wu et al., 2011). Although subject to intensive research, the etiology of PD is still enigmatic and the treatment is basically symptomatic. Approximately 5% of PD cases are caused by heritable genetic mutations (Stewart & William, 2008). The remaining cases are sporadic and of unknown origin, although many theories have been proposed to explain the cause of dopaminergic neuronal death which occurs in PD, such as environmental toxins, mitochondrial dysfunction with resulting oxidative stress, disturbances of intracellular calcium homeostasis and inflammatory mechanisms (Dauer & Przedborski, 2003; Lev et al., 2003; Long-Smith et al., 2009). There is no gender preference. Mortality among affected individuals is 2–5 times greater than for their age-matched unaffected peers (Bennet et al., 1996; Morens et al., 1996; Driver et al., 2009).
The loss of dopaminergic cells in the substantia nigra pars compacta (SNpc) results in insufficient DA innervation of the basal ganglia and subsequent increased inhibition of excitatory thalamo-cortical connections. Lewy bodies, intracellular inclusions principally containing α-synuclein, are also found in the remaining nigral neurons of PD patients (Schlossmacher, 2007; Eller & Williams, 2011). The ultimate result of cell loss and cell dysfunction in the SN is the depletion of the neurotransmitter DA in the basal ganglia. This insufficient DA innervation is principally localized to the postcommissural putamen and results in the overdrive of globus pallidus and subthalamic nuclear outputs. The resulting inhibition of thalamocortical function results in the characteristic bradykinesia experienced by PD patients (Soderstrom et al., 2009).

Non-motor symptoms (NMSs) are often an integral part of the disease and some of them, such as depression, anxiety and hyposmia, can precede the onset of Parkinsonism. Other NMSs, such as psychosis, dementia, impulse-control disorders, somnolence and autonomic dysfunctions, are almost invariably present in advanced disease and in various combinations they may represent the principal complaints and therapeutic challenges (Ceravolo et al., 2010; Jellinger, 2012). The dysfunction of striatum results in deficient input to the pre-frontal cortex from the striatum and causes cognitive decline in PD patients (Dubois & Pillon, 1997). These NMSs including cognitive deficits determine the patients’ quality of life and are equally important to the motor deficits occurring in PD (Weerkamp et al., 2012; Wood, 2012).

The therapies presently available for PD are not effective in the long-term and cannot stop the ongoing neurodegeneration. The most commonly used treatment is the DA precursor, levodopa (L-DOPA), which replaces lost DA in the denervated striatum and relieves motor symptoms. However long-term administration of levodopa is associated with the development of dyskinesias (Hollingworth et al., 2011) and does little to treat non-dopaminergic motor and NMSs, which are an important source of morbidity, including dementia, sleep disturbances, depression, orthostatic hypotension and postural instability leading to falls (Henchcliffe & Severt, 2011). It is critical, therefore, to develop a broader
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and more fundamental therapeutic approach to PD and major research efforts have focused upon developing neuroprotective interventions.

Animal models are an invaluable tool for studying the pathogenesis and progression of human diseases, as well as for testing new therapeutic intervention strategies. Lesions with the neurotoxin, 6-hydroxydopamine (6-OHDA) have provided an important tool to study DA neurons in the brain. The most common version of such lesions is the unilateral one where the toxin is placed in the area of dopaminergic cell bodies in the SN (Schwarting & Huston, 1996; Duty & Jenner, 2011). The 6-OHDA model mimics many of the biochemical features of PD, including reduced levels of striatal DA and tyrosine hydroxylase (TH; rate-limiting step of DA biosynthesis). The DA analog, 6-OHDA, because of its similarity in molecular structure can be taken up into dopaminergic terminals through the DA transporter. Once inside dopaminergic neurons, 6-OHDA initiates degeneration through a combination of oxidative stress and mitochondrial respiratory dysfunction. 6-OHDA readily oxidizes to form reactive oxygen species (ROS) such as hydrogen peroxide (H_{2}O_{2}) (Mazzio et al., 2004), to reduce striatal levels of antioxidant enzymes - total glutathione (GSH) and superoxide dismutase (SOD) (Perumal et al., 1992; Kunikowska & Jenner, 2001), to elevate levels of iron in the SN (Oestreicher et al., 1994) and to interact directly with complexes I and IV of the mitochondrial respiratory chain, leading to subsequent respiratory inhibition and further oxidative stress (Glinka et al., 1997; Soderstrom et al., 2009). 6-OHDA causes apoptotic cell death of dopaminergic neurons with loss of TH immunoreactivity in the SN (He et al., 2000; Zuch et al., 2000). Oxidative stress triggers apoptosis via a signalling pathway initiated by the generation of ROS leading to activation of caspase-9 and caspase-3 (Barzilai et al., 2000; Junn & Mouradian, 2001). Depletion of neurotrophins such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) in the nigrostriatal dopaminergic region trigger the process of apoptosis in PD (Mogi & Nagatsu, 1999; Mogi et al., 1999). All these effects are thought to mirror events occurring in PD brain (Jenner, 1989), thereby supporting a high degree of construct validity for the 6-OHDA model. This lesion model has been used to investigate the
behavioural functions of the basal ganglia and to examine the brain’s ability to compensate for specific neurochemical depletions. 6-OHDA lesion model have served as an experimental basis to develop new antiparkinsonian drugs and treatment strategies, or surgical approaches, including transplantation of neural tissue.

Brain serotonergic and monoamine dopaminergic systems are closely related (Alex & Pehek, 2007). The dopaminergic disturbance in the brain leads to serotonergic changes. *In vivo* microdialysis studies in the prefrontal cortex and corpus striatum have showed that local infusion of the DAD2 receptor antagonist raclopride significantly inhibited the tail pinch induced increases in serotonin (5-HT) (Mendlin et al., 1999). Similar results also indicated that local administration of the nonselective DA receptor agonist apomorphine into the hippocampus increased 5-HT release in a concentration-dependent manner and this increase was abolished by pre-treatment with the selective DAD2 receptor antagonist, S(-)-sulpiride (Matsumoto et al., 1996). Reciprocally, 5-HT afferents are able to facilitate the release of DA. It has been shown that DA release is induced in different brain regions following local cerebral application with 5-HT (West & Galloway, 1991; Parsons & Justice, 1993).

In addition to the correlation between 5-HT and DA transmission, there is tight functional interaction between these receptors. 5-HT receptors modulate dopaminergic function. 5-HT, by entering DA terminals, may elicit a carrier mediated release of DA or inhibit enhanced DA release (Navailles & De Deurwaerdère, 2011). 5-HT generally facilitates dopaminergic release via the 5-HT1A, 5-HT1B, 5-HT2A, 5-HT3 and 5-HT4 receptors whereas 5-HT2C receptors tend to inhibit DA release (Alex & Pehek, 2007; Fox et al., 2009). Intracortical infusion of the 5-HT2A receptor antagonist M100907 profoundly attenuated DA release induced by systemic administration of the 5-HT agonist, suggesting that stimulation of cortical 5-HT2A receptors increased DA release from the mesocortical system (Pehek et al., 2006). Similar DA modulation was also observed by systemic or local administration of the 5-HT2C receptor agonist mCPP or SB 206553 (Alex et al., 2005). The 5-HT system directly and indirectly
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participate in the mechanisms of action of L-3,4-dihydroxyphenylalanine (L-DOPA), the metabolic precursor of DA and the gold standard medication of PD. Numerous data have shown that 5-HT drugs ameliorate the motor and psychotic side effects induced by L-DOPA (Jenner et al., 1983; Carta et al., 2007; Navailles & De Deurwaerdère, 2011). Thus, the pharmacological treatments that modulate 5-HT receptors activity limit the extent of nigro-striatal damage, improve motor and NMS’s of PD (Zoldan et al., 1995; Pact & Giduz 1999) and prevent or reduce levodopa-induced dyskinesia (LIDs) (Melamed et al., 1996; Carta et al., 2008) in animal models of PD.

Cell transplantation to replace lost neurons is a promising approach for the treatment of progressive neurodegenerative diseases. Replacement of dopaminergic neurons in patients with PD has spearheaded the development of this approach and was the first transplantation therapy to be tested in the clinic (Björklund et al., 2003). 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. The fetal brain tissue used in clinical transplantation studies is ethically challenging to obtain (Lindvall, 2001). Also the Central Nervous System (CNS) immune response can mount a well-organized innate immune reaction in response to allogeneic antigens (Boulanger & Shatz, 2004; Arias-Carrión & Yuan, 2009). Number of reports claim that Bone marrow cells (BMC) can generate endoderm and ectoderm derivates including neural cells (Jiang et al., 2002; Kim et al., 2002). Hematopoietic system can be used as a source of progenitor cells for the CNS and it also has the property to differentiate into both microglia and macroglia when injected directly to the brain of adult mice (Martin & ‘Eva, 1997). Intrastriatal grafts of mesenchymal stem cells derived from bone marrow protects against 6-OHDA (Blandini et al., 2010), elevates TH expression and DA levels in adult rats and differentiates into neurons, astrocytes and oligodendrocytes (Jin et al., 2009). BMC can differentiate into dopaminergic neurons (Chai et al., 2007), exert neuroprotection on dopaminergic neurons (Park et al., 2008; Kim et al., 2009; Wang et al., 2010) and holds potential as a readily available autologous
cellular therapy for ameliorating the degeneration of DA and 5-HT neurons in PD (Glavaski-Joksimovic et al., 2009). Autologous BMC to treat neurological disorders offers several unique advantages over other cell replacement therapies. Immunological reactions are avoided and it also bypasses ethical issues in the use of embryonic cells.

Alterations in the brain monoamines DA, 5-HT and gamma amino butyric acid (GABA) have been implicated in the etiology and/or pharmacotherapy of PD. Most of the effects of 5-HT and GABA on DA neurons are indirect, mediated through actions on complex neuronal circuitry, rather than direct effects on DA terminals. Since the different 5-HT receptor subtypes are differently distributed in dopaminergic brain regions, it is possible to specifically “target” individual brain regions with serotonergic ligands and thereby affect dopaminergic function selectively in these areas (Alex & Pehek, 2007). As GABA helps "quiet" excessive neuronal firing and has been deficient in patients in the advanced stages of PD, directly targeting GABA production rather than DA replacement is an effective way of improving brain function in late-stage PD which also avoids the known therapeutic limitations and complications associated with the over-production of DA. 5-HT and GABA can be also used as agents for cell proliferation and differentiation. 5-HT plays an important trophic role during neurogenesis (Lauder et al., 1981; Hernández Rodriguez, 1994). 5-HT can influence both biochemical and morphological differentiation of neurons and have an organizing function in the developing nervous system which involves effects on neurite outgrowth and other aspects of neuronal differentiation, including synaptogenesis (Lauder, 1990). GABA, the main inhibitory neurotransmitter in the mature CNS, was recently implicated in playing a complex role during neurogenesis. GABA acts as a chemoattractant and involves in the regulation of neural progenitor proliferation. GABA induces migration and motility of embryonic cortical neurons (Behar et al., 1996; Haydar et al., 2000). GABA acts as a trophic factor not solely during prenatal neurogenesis but also postnatally and promotes cell proliferation and NGF secretion (Ben-Yaakov & Golan, 2003). Our earlier studies showed that 5HT and GABA acting through specific receptor
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subtypes 5HT₂ (Sudha & Paulose, 1998) and GABA_B (Biju et al., 2002) respectively, control cell proliferation and act as co-mitogens.

In the present study, a detailed investigation on the alterations of 5-HT and its receptors in the brain regions of unilateral 6-OHDA infused rats were carried out. 5-HT receptor subtypes - 5-HT₂A, 5-HT₂C and 5-HT transporter (5-HTT) gene expression were also studied in the 6-OHDA lesioned Parkinsonian rats. Oxidative stress induced neuronal damage was studied by assessing the activities of antioxidant enzymes - SOD and Catalase (CAT), gene expression of SOD and glutathione peroxidase (GPx) and the extent of lipid peroxidation. In addition to that the possible linkage between the 6-OHDA induced oxidative stress and subsequent apoptosis was studied by the gene expression of Akt, nuclear factor-kappa B (NF-κB) and Caspase-8. Expression of Neurotrophins – BDNF and Glial cell line-derived neurotrophic factor (GDNF) were also studied. Behavioural studies were conducted to evaluate motor deficits in Parkinson’s rats and functional recovery by 5-HT and GABA in combination with BMC. We also demonstrated the autologous differentiation of BMC to neurons using comitogenic 5-HT and GABA by confocal studies with BrdU labelling and NeuN expression. Our present study on 5-HT, GABA and BMC dependent regulation of serotonergic receptors and oxidative stress in the brain will certainly enlighten novel therapeutic possibilities for PD management.
OBJECTIVES OF THE PRESENT STUDY

1. To induce Parkinson's disease in rats by unilateral infusion of 6-OHDA and to study the effects of 5-HT, GABA and BMC in combinations.

2. To study the behavioural changes in control and experimental rats using apomorphine induced rotational analysis, elevated body swing test (EBST), stepping test, footprint analysis test and beam-walk test.

3. To examine the dopaminergic neuronal regulation in SNpc by measuring DA content and studying TH gene expression and immunohistochemistry using specific antibody.

4. To measure 5-HT content in the brain regions – SNpc, corpus striatum, cerebral cortex, hippocampus, cerebellum and brain stem of control and experimental rats using High Performance Liquid Chromatography (HPLC).

5. To study the total 5-HT, 5-HT\textsubscript{2A} and 5-HT\textsubscript{2C} receptor subtypes binding parameters in corpus striatum, cerebral cortex, hippocampus, cerebellum and brain stem of control and experimental rats.

6. To study the 5-HT\textsubscript{2A}, 5-HT\textsubscript{2C} receptor subtypes and 5-HTT gene expression in the brain regions of control and experimental rats using Real-Time PCR.

7. To examine the oxidative stress by assessing the activities of antioxidant enzymes – SOD and CAT, gene expression status of SOD and GPx and the extent of lipid peroxidation in SNpc and corpus striatum of control and experimental rats.
8. To study the oxidative stress mediated apoptosis by examining the gene expression status of Akt, NF-κB and Caspase-8 in the brain regions of control and experimental rats.

9. To study the gene expression of Neurotrophins – BDNF in SNpc, corpus striatum, cerebral cortex, hippocampus, cerebellum and brain stem and GDNF in SNpc and corpus striatum of control and experimental rats.

10. To study the localisation and expression status of 5-HT$_2A$, 5-HT$_2C$, 5-HTT, BDNF, GDNF and TH by immunofluorescent specific antibodies in the brain slices of control and experimental rats using Confocal microscope.

11. To examine the BMC differentiation by BrdU and NeuN co-localisation studies.